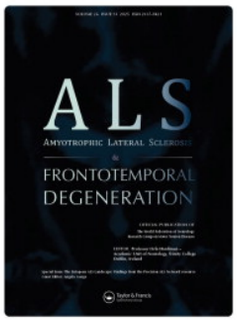
Article:
McFarlane, R. orcid.org/0000-0001-7380-4219, Opie-Martin, S., Caravaca Puchades, A. et al. (26 more authors) (2025) Clinical trajectories of genetic variants in ALS: a European observational study within PRECISION-ALS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 26 (sup 1). pp. 41-49. ISSN 2167-8421

https://doi.org/10.1080/21678421.2025.2450805



Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

ISSN: 2167-8421 (Print) 2167-9223 (Online) Journal homepage: www.tandfonline.com/journals/iafd20

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To cite this article: Robert McFarlane, Sarah Opie-Martin, Alejandro Caravaca Puchades, Adriano Chiò, Philippe Corcia, Miriam Galvin, Mark Heverin, Frederik Hobin, Oskar Holmdahl, Caroline Ingre, Nikita Lamaire, Éanna Mac Domhnaill, Umberto Manera, Christopher J. Mcdermott, Harry McDonough, Mohammed Mouzouri, Fouke Ombelet, Mónica Povedano Panadés, Stefan Sennfält, Pamela Shaw, Cristina Terrafeta Pastor, Jan H. Veldink, Philip Van Damme, Leonard van den Berg, Ruben P. A. Van Eijk, Rosario Vasta, Daphne N. Weemering, Ammar Al-Chalabi & Orla Hardiman (2025) Clinical trajectories of genetic variants in ALS: a European observational study within PRECISION-ALS, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 26:sup1, 41-49, DOI: 10.1080/21678421.2025.2450805

To link to this article: https://doi.org/10.1080/21678421.2025.2450805

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2025; 26: 41–49

RESEARCH ARTICLE

Clinical trajectories of genetic variants in ALS: a European observational study within PRECISION-ALS

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Abstract

Objective: To investigate the association between *C9orf72*, *SOD1*, *FUS* and *TARDBP* variants on the clinical trajectory of ALS patients in Europe. *Methods*: Nine ALS centers with population-based registries provided data on demographic and disease characteristics – at diagnosis and longitudinally – as part of PRECISION ALS. These data were harmonized and collated for analysis. *Results*: 21,820 ALS patients were identified, 9,887 underwent genetic testing for at least one of the 4 genes of interest. 9.8% of patients carried a hexanucleotide expansion in *C9orf72;* 2.9% carried a pathogenic variant in *SOD1*; 1.4% carried a pathogenic variant in *TARDBP;* and 0.8% carried a pathogenic variant in *FUS.* Only one p.A5V variant was identified in this dataset. The most frequently identified *SOD1* variant was p.D91A, with evidence of other variant clusters in Belgium, Italy and the United Kingdom. *TARDBP* variants were clustered in the Netherlands and Italy. Earlier ages of onset were demonstrated compared to wild-type populations; *C9orf72* 59.58 (IQR 62.5, $p < 2.2e\text{-}16$), *SOD1* 54.19 (IQR 19.4, $p = 6.304e\text{-}14$), *TARDBP* 58.30 (IQR 16.23, $p = 0.00024$) and *FUS* 51.16 (IQR 25.08, $p = 1.58e\text{-}06$). *C9orf72* was more bulbar ($p < 0.0001$) in onset and *SOD1* more spinal ($p < 0.0001$). Those carrying variants spent distinctly different periods in each of the King's stages. *Conclusions*: Genetic forms of ALS have an earlier age of onset, have distinct patterns in their sites of disease onset, and progress differently as compared to populations without such major-effect genes. There is also evidence of disease clusters across Europe suggestive of founder effects.

Keywords: *Genetics, c9orf72, sod1, fus, tardbp*

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/21678421.2025.2450805.

(Received 30 July 2024; revised 11 November 2024; accepted 4 January 2025)

ISSN 2167-8421 print/ISSN 2167-9223 online
DOI: 10.1080/21678421.2025.2450805

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a heterogeneous neurodegenerative condition affecting movement and cognition. The disease exhibits considerable heterogeneity which is incompletely understood. Population-based studies have confirmed that heritability accounts for up to 50% of disease risk (1–3) and up to 40 at-risk genes have been associated with the disease (4). Additionally, a Mendelian inheritance pattern can be identified in up to 15% of patients with ALS (1).

Within the framework of PRECISION-ALS (5), data from 21,820 patients from nine European sites have been analyzed to determine the clinical phenotype, disease trajectory and genomic profile of ALS. Here we describe the demographics and clinical features of the four genes of major effect, which account for up to 70% of familial ALS (6); namely pathogenic *SOD1* variants; a hexanucleotide expansion in *C9orf72*; pathogenic variants in *FUS*; and pathogenic variants in *TARDBP*.

Methods

Under GDPR-compliant data sharing agreements, data collected from previously funded collaborations, and large-scale genomic projects, have been harmonized and collated for analysis, as described within PRECISION-ALS (5). In brief, nine European specialized ALS centers, with active population-based registries provided de-identified data on demographic and disease characteristics, both at diagnosis and during longitudinal follow-up. All patients diagnosed with either 'possible,' 'probable (± laboratory supported)' or 'definite' ALS according to the revised El Escorial criteria were eligible.

All genetic testing was performed in accredited diagnostic laboratories or within genomic research laboratories associated with each clinical site. For *C9orf72*, a cut off of 30 hexanucleotide repeats was used. Due to the length of the longitudinal collection of data at each of the sites, only data from patients for whom a definitive positive or negative genomic result could be verified were used. Each genomic variant provided was manually searched on ClinVar, ALSoD (https://alsod.ac.uk/) and Pubmed to determine pathogenicity; those classified as 'benign' or as a 'variant of uncertain significance' were excluded, variants with conflicting pathogenic reports have been included. Variant amino acid residue position is described inclusive of the initial methionine (i.e., p.A5V rather than A4V).

All sites use the form of longitudinal follow-up best suited to their healthcare system. This commonly takes the form of clinic visits, community clinician outreach, or both. Symptom onset was determined by clinical interview.

Statistical analysis

Non-normally distributed groups are reported with medians and interquartile ranges (IQR). Normally distributed groups are compared with means and standard deviations. Continuous variables are compared using a pairwise Wilcoxon rank sum test with a Bonferroni correction, and categorical variables are compared using a chi-squared test for dependence. Statistical analysis was performed using R software version 4.2.2.

Average post-diagnosis point reduction in ALSFRS-R is used to aid comparison in the rate of progression between groups. This was calculated for the whole cohort using 7,030 patients and their ALSFRS-R data; individual progression rates and change from baseline score were calculated. Quartiles were then applied to categorize patients as "slow," "average," or "fast "progressors. The slowest progressing quartile were designated "slow" progressors, the middle two quartiles were designed as "average" progressors and the fastest quartile as "fast" progressors. This produced the following values: Slow −0.54 points/month or higher, average −0.55 to −1.24 points/month and fast −1.25 points/month or less. The full statistical modeling of ALSFRS-R is explored in the sister paper of this series.

King's staging has been calculated using the ALSFRS-R. It has been shown there is a 92% concordance when converted in this manner (7). King's staging was then verified with NIV usage and requirement based on forced vital capacity, presence of gastrostomy, and weight loss.

Results

Data from 21,820 extant patients were available, the median age at symptom onset was 63.9 years (95% CI: 63.7 – 64.0), median time from onset to death was 2.81 years (95% CI: 2.77 – 2.85).

The total number of patients who were identified as having undergone genetic testing was 9,887. However, not all patients were tested for variants within all four genes. 8816 were tested for *C9orf72*, 5061 for *SOD1*, 4461 for *TARDBP* and 4324 for *FUS*. Of these, 1,122 had a pathogenic variant identified. These included 866 (9.8% of 8816) patients who carried a hexanucleotide expansion in *C9orf72;* 149 (2.9% of 5061) carried a pathogenic variant in *SOD1*; 64 (1.4% of 4461) carried a pathogenic variant in *TARDBP;* and 33 (0.8% of 4324) carried a pathogenic variant in

Table 1. Geographic distribution of variants.

	C9orf72	*FUS*	*SOD1*	*TARDBP*
Barcelona	89.36%	0%	10.64%	0%
Stockholm	86.84%	0%	13.16%	0%
Leuven	62.66%	3.80%	26.58%	6.96%
Sheffield	70.59%	0.84%	24.37%	4.20%
Tours	85.07%	0%	11.94%	2.99%
Dublin	97.43%	0.64%	1.28%	0.64%
Turin	58.03%	7.14%	21.88%	12.95%
Utrecht	88.47%	3.43%	3.43%	4.67%

This table shows the difference in the proportion of identified variants at each site as a percentage. The prevalence of variants varies significantly between the sites. *C9orf72* remains the most common at all sites, *FUS* is a rare variant at all sites, *SOD1* shows significant variation with three sites contributing most of the variants and *TARDBP* is rare at almost all sites—with the notable exception of Turin –which has a significantly different variant profile to other sites.

FUS. The female:male ratio of those with genetic testing information was 1:1.34.

Some variants were more common in specific geographic regions (Table 1); *C9orf72* was the most common variant at all sites; *SOD1* showed significant variation with three sites contributing 79% of the variants (Turin $n=49$, Leuven $n=42$, and Sheffield $n=29$); *FUS* is a rare variant at all sites; and *TARDBP* was rare at almost all sites, with the notable exception of Turin, where this mutation represented 12.95% of all cases.

Specific genomic variants were also examined in the three genes where this is possible. A full list of the variants provided for analysis, separated by site of origin, is available in Supplementary Table 1.

Geographic distribution of variants

SOD1. There were 44 pathogenic variants recorded across 8 sites in 149 patients. One p.A5V variant was identified in this dataset, in Turin. The most frequently identified variants were p.D91A (14.76% $n=22$, of which only one person was specifically identified as homozygous), p.G94C (10.06%, $n=15$, all in Leuven), p.I114T (6.04%, $n=9$; of which, Sheffield $n=6$; Utrecht $n=2$; Leuven $n=1$), p.L145F (6.04%, $n=9$, all in Turin). p.I114T was the most common variant in Sheffield, p.G94C was the most common variant in Belgium, p.L145F was the most common variant in Turin. Unlike other sites, Turin identified a wide range of different *SOD1* variants ($n=19$).

FUS. 12 pathogenic variants were identified across 4 sites in 33 patients. The most common variant was p.R521C (27.27%, $n=9$), identified in Utrecht and Turin only. Turin again identified a wider range of *FUS* variants than other sites ($n=6$).

TARDBP. 17 pathogenic variants were identified across 5 sites in 64 patients, there was evidence of significant clustering. p.A382T was the most common variant (26.56%, $n=17$) and this was only present in Turin ($n=16$) and Leuven ($n=1$). p.N353S was the second most common (18.75%, $n=12$) and was only present in Utrecht ($n=11$) and Leuven ($n=1$).

Site of onset

The breakdowns of site of onset according to genetic variant were as follows (Figure 1); *SOD1*, spinal 86.11%, bulbar 8.33%, respiratory 5.56% and cognitive 0%; *C9orf72*, spinal 62.47%, bulbar 35.14%, respiratory 0.92% and cognitive 0.46%; *TARDBP*, spinal 74.65%, bulbar 23.94%, respiratory 1.41% and cognitive 0.13%; *FUS*, spinal 77.78%, bulbar 19.44%, respiratory 2.78% and cognitive 0%. Cognitive onset was assessed as cognitive symptoms preceding the development of motor symptoms and has been reported here only when there was no other identifiable motor site of onset within the dataset.

Each genetic variant can present at any site of onset and spinal remains the most common site of onset in every group. However bulbar onset was more common in those carrying a repeat expansion in *C9orf72* ($p<0.0001$) and spinal onset was significantly more common in those carrying a *SOD1* variant ($p<0.0001$). Comparison between other groups did not reach statistical significance.

Age of onset

The age of onset was 63.85 (IQR 15.33) for the cohort without a known genomic variant. By contrast, median age of onset for *C9orf72* was 59.58 (IQR 62.5, $p<2.2e\text{-}16$), for *SOD1* 54.19 (IQR 19.4, $p=6.304e\text{-}14$), *TARDBP* 58.30 (IQR 16.23, $p=0.00024$) and *FUS* 51.16 (IQR 25.08, $p=1.58e\text{-}06$) (Figure 2).

FUS variant populations showed a distinct bimodal pattern in their age of onset. A younger peak between 30-40 years and another peak between 60 and 70 years (Supplementary Figure 1). Patients younger than 50 had a disease duration median of 18.24 months (IQR 8.52) compared to those over 50 who had a disease duration median of 23.69 months (IQR 8.67), this difference however did not reach statistical significance ($p=0.37$) and there was no significant difference in the variants identified in those below and over 50. However, it should be remembered that the absolute numbers are small.

Diagnostic delay

The diagnostic delay for the cohort with no known genomic variants was 11.76 months (IQR 11.48). Diagnostic delay was shorter for those carrying a

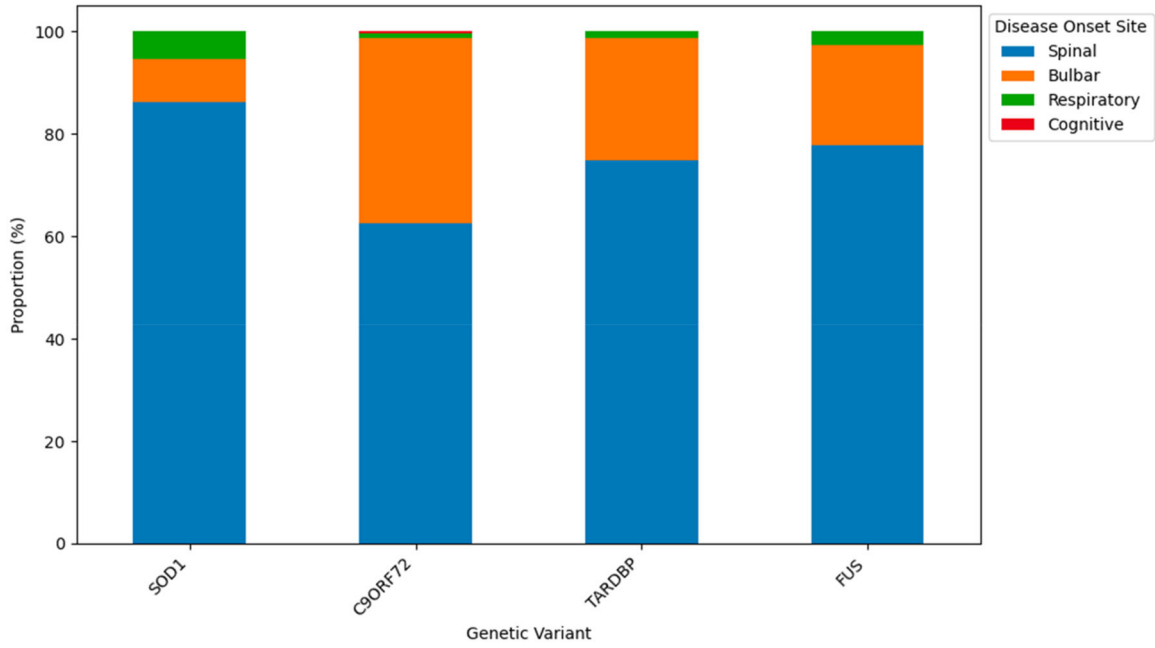

Figure 1. Site of onset differences across four genes. A bar chart showing the differences in site of onset of ALS in each of the four genes of major effects There was a significant difference when positive and negative populations of *C9orf72* ($p < 0.0001$) – significantly more bulbar – and *SOD1* ($p < 0.0001$) – significantly more spinal – were compared, however there was no significant difference between *FUS* and *TARDBP* positive and negative populations ($p = 0.5302$ and $p = 0.5651$ respectively).

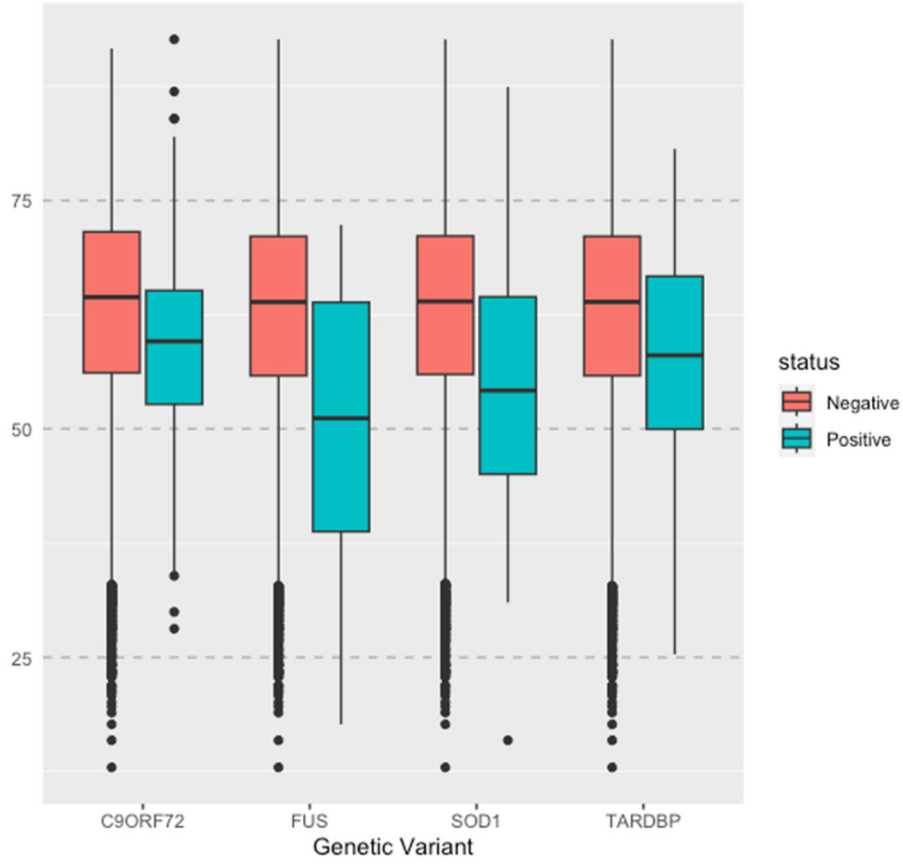

Figure 2. Age of onset comparisons across four genes. Paired box plots comparing the age of onset of ALS in each of the four genetic variants. In every case, those with an identified genetic variant (positive) presented significantly earlier than those who did not have an identifiable genetic cause of their ALS (negative). The age of onset was 63.85 (IQR 15.33) for the negative group. Median age of onset for *C9orf72* was 59.58 (IQR 62.5, $p < 0.0001$), for *SOD1* 54.19 (IQR 19.4, $P < 0.0001$), *TARDBP* 58.30 (IQR 16.23, $P < 0.001$) and *FUS* 51.16 (IQR 25.08, $P < 0.0001$).

repeat expansion in *C9orf72* 9.88 months (IQR 10.08, $p = 0.00001$). There was no significant difference in diagnostic delay for other genomic variants when compared with the cohort with no known variants. (*SOD1* 10.25 months (IQR 14.21, $p = 0.81$), *TARDBP* 11.96 (IQR 14.76, $p = 0.84$), *FUS* 11.20 (IQR 8.78, $p = 0.81$)).

ALSFRS-R

When compared using average post-diagnosis ALSFRS-R decline, the *C9orf72* ($p < 0.0001$) population progressed significantly faster than the negative group, and *SOD1* ($p < 0.0001$) positive populations progressed significantly more slowly than those with no known variant. There was no significant difference between *TARDBP* ($p = 0.09$) and *FUS* ($p = 0.28$) positive and negative groups.

When defined by category, 30.60% of the negative cohort were classified as slow progressors, 35.93% were average and 33.46% as fast. Those carrying a repeat expansion in *C9orf72* had a higher proportion with fast progression (19.33% slow, 38.43% average, and 42.25% fast). By contrast, those carrying variants in *SOD1* contained a higher proportion of slow progressors (61.11% slow, 17.78% average, 21.11% fast). Similarly, those carrying a *TARDBP* variant were more likely to progress slowly (40.43% slow, 34.04% average, 25.53% fast). For those with variants in FUS, the largest proportion progressed at an average rate (22.22% slow, 44.44% average, 33.33% fast).

King's staging

Time at onset of symptoms was taken as the baseline for the calculation of staging and the time spent within each stage in months (Figure 3). Wild-type populations spent a median of 4.29 (IQR 2.53-8.95) months in stage one, 7.82 (IQR 3.71-14.34) months in stage two, 11.17 (IQR 4.86-23.58) months in stage three and 9.53 (IQR 4.42-18.55) months in stage four.

Patients carrying a repeat expansion in *C9orf72* spent a median of 4.14 (IQR 1.77-18.19, p = ns) months in stage one, 8.05 (IQR 3.44-13.53, p = ns) in stage two, 8.95 (IQR 4.60-16.26, $p = 0.006$) in stage three and 8.75 (IQR 4.36-15.43, p = ns) months in stage four. No *SOD1* patients were recorded as stage one, however they spent a median of 5.93 (IQR 3.94-11.42, p = ns) months in stage two, 26.74 (IQR 5.74-67.18, $p = 0.0001$) in stage three and 22.01 (IQR 9.61-46.34, $p = 0.02$) in stage four. No *FUS* positive patients were recorded as stage one; however, they spent a median of 2.86 (IQR 2.11-3.20, $p = 0.02$) in stage two, 7.79 (IQR 4.26-17.31, p = ns) in stage three and 6.30 (IQR 2.83-19.02, p = ns) months in stage four. No *TARDBP* patients were recorded as stage one, they spent a median of 12.09 (IQR 8.40-20.27, p = ns) in stage two, 21.56 (IQR 9.33-38.57, $p = 0.02$) months in stage three and 12.12 (IQR 2.94-18.46, p = ns) months in stage four.

Survival

A multivariable Cox proportional hazards regression analysis was conducted to investigate the effect variants had on survival time (onset to death) including age, sex, site of onset and diagnostic delay in the model. An increasing age of onset was associated with an increased hazard ratio (HR1.03, CI1.03-1.03, $p < 0.001$), as was bulbar onset (HR 1.24 CI 1.15-1.33, $p < 0.001$) and detection of a *C9orf72* expansion (HR 1.45, CI 1.29-1.62, $p < 0.001$). Detection of a *SOD1* variant was associated with a decreased hazard ratio (HR 0.72, CI 0.55-0.94, $p = 0.016$). Other genetic variants did not significantly affect the hazard ratio and neither did any of the other variables included.

Discussion

We have shown the effect that four genes of major effect can have on commonly measured clinical variables and how the distribution of specific variants can vary throughout Europe. Clinical indicators are consistent across sites providing insights into the patient journey of these European ALS sub-populations. We have also shown how, at the variant level, there is significant evidence of geographical variation and clustering which requires further investigation.

C9orf72

We we have shown that *C9orf72* variant populations are the most common, younger at presentation, with more bulbar onset disease, progress more quickly in terms of ALSFRS-R decline. Cox modeling also shows an increased hazard ratio compared to wild-type populations. Delineation of this distinct genetic-phenotypic relationship is useful at an individual patient and clinician level but also more generally in the investigation of underlying pathogenic mechanisms.

SOD1

The frequency of each *SOD1* variant varies between populations and countries with over 180 pathogenic variants identified thus far (4). In some cases, classification of the specific *SOD1* variant allows the identification of distinct survival trajectories (8). Most patients with *SOD1* ALS have been shown to have spinal onset and our work aligns with this (9). However, the variants identified here are markedly different to previously published work (10), including within North America (11), where the p.A5V variant remains the most common. Similarly, where European and Chinese

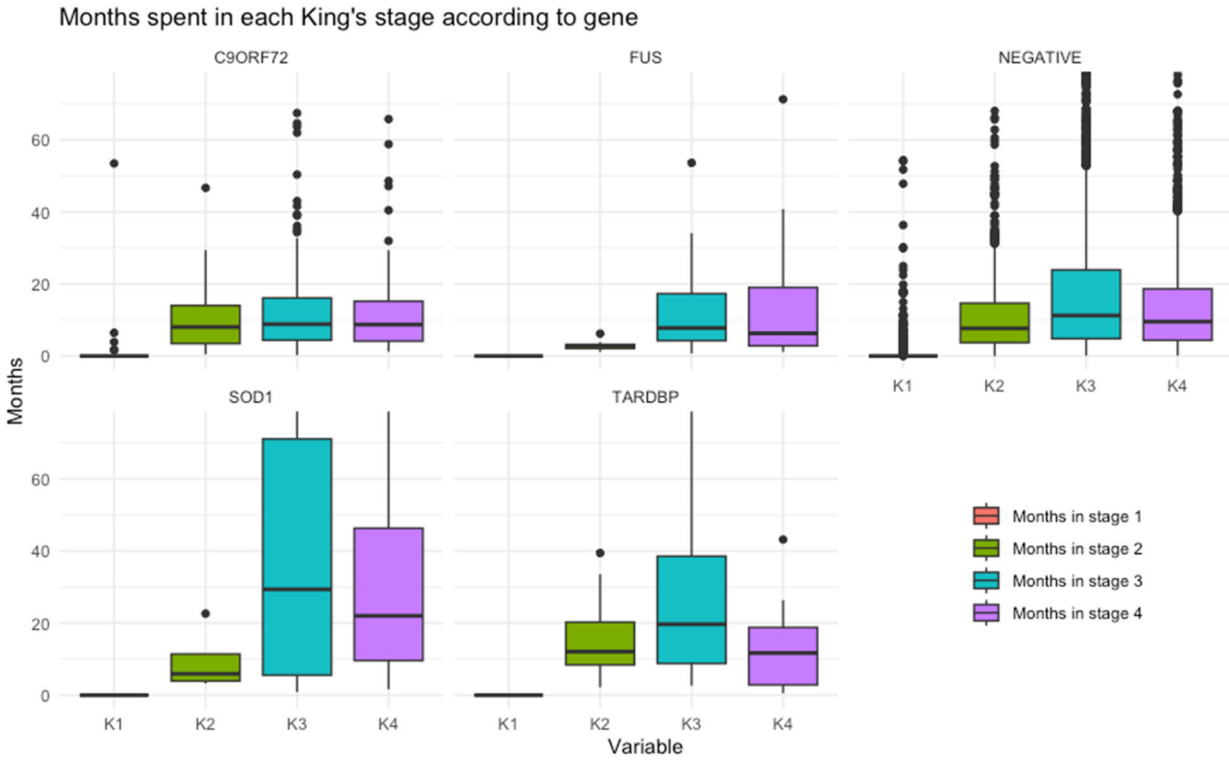

Figure 3. Length of time spent in each King's stage across the four genes. box plots representing the time spent in each of the King's stages, as calculated from the ALSFRS-R, according to genetic variants and compared to negative populations. The Y-axis has been truncated at 75 months for visual comparison, however all values reported are inclusive of outliers. Distinct patterns can be seen between the variants. *C9orf72* populations spent a significantly shorter time in stage three than negative populations (8.95 months, IQR 4.6-16.26 months, p = 0.006). *SOD1* patients spent a significantly longer time in stages three (26.74 months, IQR 5.74-67.18 months, p = 0.0001) and four (22.01 months, IQR 9.61-46.34, p = 0.02). TARDBP patients spent a significantly longer period in stage three (21.56 months, IQR 9.33-38.57, p = 0.02). FUS patients spent a significantly shorter period in stage two (2.86 months, IQR2.11-3.20, p = 0.02).

populations are compared, *SOD1* mutation location has been shown to exhibit positional variation (12). Indeed, *SOD1* is the most common familial ALS diagnosed in China, which is in contrast to the work presented here (13).

The most common *SOD1* variant here was p.D91A, only one person was specifically identified as homozygous. Generally, this variant has been shown to have a slower than average disease progression profile (14) and this explains why in this analysis variant *SOD1* populations progressed more slowly, resulting in a significantly different ALSFRS-R post-diagnosis decline and a reduction in the hazard ratio on cox regression.

The p.I114T variant was the most common variant in Sheffield. This variant has previously been linked to a founder effect in Scotland (15, 16) suggesting geographic clustering on the island of Great Britain. Similarly, certain variants clustered in specific sites, p.G94C was only identified in Leuven and p.L145F was only identified in Turin. These three clusters in different geographical areas are all suggestive of founder effects, however further validation is needed.

Our results highlight the genetic differences that exist both within and between continents of those living with *SOD1* ALS, once again showing the importance of a precision-medicine based approach in ALS, where complex genetic clusters and ancestry are likely to play a crucial role in the phenotype a patient develops.

FUS

Variants in *FUS* are rare, and the clinical phenotype and progression rates of those with variation in this gene are not well characterized. As expected from clinical experience, people carrying these variants presented significantly younger than those carrying other variants and all other investigated ALS sub-groups.

There was evidence of a bimodal distribution in the ages of onset. Two groups emerged, those with an early age of onset with rapid progression and another older group with a longer disease duration. Whilst this difference was not significant, it suggests differing mechanisms of pathogenicity within the same gene.

TARDBP

TARDBP variants were infrequently identified in this analysis, which corresponds to previous global estimates of frequency (17). However, Turin has a notably different genetic profile from that of other

sites, where more *TARDBP* variation was identified and one variant in particular emerged as the most common. The p.A382T variant has previously been associated with up to a third of all ALS cases on Sardinia (18) and was the most common variant identified in this work. Extended pedigree characterization using haplotype data suggests that these cases have a common ancestor, again providing evidence of a founder effect.

Apart from showing that patients identified as having a variant in *TARDBP* present at a significantly younger age, all other comparisons showed that *TARDBP* positive and negative populations did not significantly differ alongside a non-significant change in hazard ratio in survival modeling. It is possible however that this is the result of the scarcity of variation in this gene.

Limitations

This dataset is representative of European populations and therefore generalizability should be considered. The countries that participate in PRECISION ALS provide public, universal healthcare which aims to mitigate the confounding factor of differing healthcare systems. It should also be considered that those more able to participate in testing may skew from the true ALS population average. The availability of genetic testing also varies and the practical decision of whom to test may have acted as a source of bias, especially as the access to commercial or research lab genetic testing is likely to vary both between sites and over time, shown here by the fact that not all patients were tested for every gene and that different proportions of patients were tested at each research center.

Conclusion

The aim of this study was to investigate the clinical features of the four most common genomic variants associated with ALS within a European population. Genetic forms have an earlier age of onset consistent with other studies (19), have distinct patterns in their sites of disease onset, and progress differently as compared to populations without such major-effect genes.

This study shows significant geographical variation in variant and gene frequency. Variant level information is suggestive of founder effects in Great Britain, Italy, and Belgium. With the advent of genomic based therapeutics, there is renewed interest in further understanding the natural history of genetic ALS. Our observations emphasize the need for ongoing surveillance, deep phenotyping, and multimodal analysis to understand the factors that drive heterogeneity. With large, high-quality databases and growing international collaboration, this becomes increasingly possible.

Ethics

All procedures and methodologies were in accordance with the ethical guidelines and standards of the institutional and national ethics committees of each of the sites involved. Informed consent was obtained from all participants, ensuring their autonomy and understanding of the study's objectives.

Ethical approvals were obtained from the local Institutional Review Board (IRB) at each participating site for use of the data in this study and for the central storage required to facilitate the cleaning and harmonization of the data. Personal data were transferred and stored securely to ensure that the privacy of these data was maintained, and relevant steps were taken to minimize any potential harm to participants.

Acknowledgements

We would like to thank the people with MND who provided their data for this study by consenting to their inclusion.

Disclosure statement

Alejandro Caravaca Puchades reports no competing interests to declare. Cristina Terrafeta Pastor reports no competing interests to declare. Mònica Povedano Panadés reports consultancies/advisory boards for Amylyx Pharmaceuticals, Biogen, Ferrer, Grifols, Italfarmaco, Mitsubishi Tanabe Pharma and Roche. Stefan Sennfält reports no competing interests to declare. Oskar Holmdahl reports no competing interests to declare. Caroline Ingre has consulted for Cytokinetics, Pfizer, BioArctic, Novartis, Tikomed, Ferrer, Amylyx, Prilenia and Mitsubishi. She is also a board member of Tobii Dynavox; all outside the submitted work. Sarah Opie-Martin reports no competing interests to declare. Ammar Al-Chalabi reports consultancies or advisory boards for Amylyx, Apellis, Biogen, Brainstorm, Clene Therapeutics, Cytokinetics, GenieUs, GSK, Lilly, Mitsubishi Tanabe Pharma, Novartis, OrionPharma, Quralis, Sano, Sanofi, and Wave Pharmaceuticals. Frederik Hobin reports no competing interests to declare. Fouke Ombelet reports no competing interests to declare. Philip Van Damme reports advisory boards for Biogen, CSL Behring, Alexion Pharmaceuticals, Ferrer, QurAlis, Cytokinetics, Argenx, UCB, Muna Therapeutics, Alector, Augustine Therapeutics, VectorY, Zambon, Amylyx (paid to institution). He has received speaker fees from Biogen,

Zambon and Amylyx (paid to institution). He is supported by the E. von Behring Chair for Neuromuscular and Neurodegenerative Disorders (from CSL Behring, paid to institution). Harry E. McDonough reports no competing interests to declare. Cristopher J. McDermott reports consultancies or advisory boards for Amylyx, Ferrer, Novartis, PTC therapeutics, Verge Therapeutics. Pamela J. Shaw reports consultancies or advisory boards for Biogen, Aclipse Therapeutics, Quell Therapeutics, BenevolentAI, QurAlis, Astex, GeniUS, Lilly, Novartis, Samsara, Eikinoklastes, Maat Pharma and AL-S Pharma and collaborates with and has received research funding from Quell Therapeutics, Aclipse Therapeutics, Pfizer SwanBio, and Takeda. Mohammed Mouzouri reports no competing interests to declare. Philippe Corcia reports consultancies or advisory boards for Amylyx, Biogen, Coave Therapeutics, Cytokinetics, Ferrer, Mitsubishi Tanabe, QurAlis, Vectory, Zambon. He is member of the Board of the Journal Amytrophic Lateral Sclerosis and the Frontotemporal Dementias and of the Revue Neurologique. Robert McFarlane reports no competing interests to declare. Miriam Galvin reports no competing interests to declare. Mark Heverin reports no competing interests to declare. Éanna Mac Domhnaill reports no competing interests to declare. Orla Hardiman reports consultancies/advisory boards for Biogen, Takeda, Ferrer, Novartis, Alchemab and Medici Nova. She is Editor in Chief of the Journal Amyotrophic Lateral Sclerosis and the Frontotemporal Dementias. Rosario Vasta reports no competing interests to declare. Umberto Manera reports no competing interests to declare. Adriano Chiò serves on the editorial advisory board of Amyotrophic Lateral Sclerosis and Neurological Sciences. Adriano Chiò serves on scientific advisory boards for Mitsubishi Tanabe, Biogen, Roche, Denali Pharma, Cytokinetics, Lilly, Ferrer, Zambon Biotech, and Amylyx Pharmaceuticals, has received a research grant from Biogen and serve on Drug Safety Monitoring Board for AB Science, Corcept, and Eli Lilly. He has received research support from the Italian Ministry of Health (Ricerca Finalizzata), Regione Piemonte (Ricerca Finalizzata), Italian Ministry of University and Research (PRIN projects), University of Turin, and the European Commission (Health Seventh Framework Programme, Horizon 2020 and Horizon Europe). Ruben van Eijk reports no competing interests to declare. Daphne Weemering reports no competing interests to declare. Jan Veldink reports no competing interests to declare. Leonard van den Berg reports no competing interests to declare.

Funding

This paper was supported by the PRECISION ALS Programme, a Science Foundation Ireland-funded academic/industry research collaboration between TRICALS, Trinity College Dublin and Biogen. This research was conducted, in part, with the financial support of Science Foundation Ireland under Grant Agreement No. 20/SP/8953 and 13/RC/2106_P2 at the ADAPT SFI Research Centre at Trinity College Dublin. ADAPT, the SFI Research Centre for AI-Driven Digital Content Technology, is funded by Science Foundation Ireland through the SFI Research Centres Programme.

Data were generated from funded projects including Euromotor (259867), the JPND-supported ALSCarE, SOPHIA, and BRAIN-MEND programme and MNDA AMBRoSIA. Additional support was from ALS Stichting Nederland (grant no. NMZ Biobank/PAN Studie).

Harry E. McDonough, Cristopher J. McDermott, and Pamela J. Shaw are supported by the NIHR Sheffield Biomedical Research Center (IS-BRC-1215-20017). Pamela J. Shaw is supported as an NIHR Senior Investigator (NF-SI-0617-10077). Cristopher J. McDermott is supported by an NIHR Professor Award (NIHR301648).

Philip Van Damme declares grants from TBM from FWO-Vlaanderen (n° T003519N), holds a senior clinical investigatorship of FWO-Vlaanderen (G077121N) and is supported by the E. von Behring Chair for Neuromuscular and Neurodegenerative Disorders, the ALS Liga België, the KU Leuven funds "Een Hart voor ALS", "Laeversfonds voor ALS Onderzoek" and the "Valéry Perrier Race against ALS Fund". This work was also supported by the Horizon 2020 Programme (project Brainteaser under grant agreement 101017598; project Hereditary under grant agreement 101137074), the Italian Ministry of Education, University and Research (Progetti di Ricerca di Rilevante Interesse Nazionale, PRIN 20228N7573). This study was performed under the Department of Excellence grant of the Italian Ministry of University and Research to the "Rita Levi Montalcini" Department of Neuroscience, University of Torino, Italy.

This study represents independent research part funded by the National Institute for Health Research (NIHR) Biomedical Research Center at South London and Maudsley NHS Foundation Trust and King's College London. Ammar Al-Chalabi is an NIHR Senior Investigator (NIHR202421).

The Joint Programme on Neurodegenerative Disease (JPND) have funded data collection for patient registries over several decades along with the Charity Research Motor Neuron (RMN), the

Irish Health Research Board (HRB) in Ireland, the Ulla-Carin Lindqvist Foundation in Sweden, and Fundación Miquel Valls in Spain, which played a crucial role in collating the dataset used in this paper. The MND Register of England, Wales and Northern Ireland is funded by the MND Association, with additional support through an EU Joint Programme - Neurodegenerative Disease Research (JPND) project under the egis of JPND - www.jpnd.eu (United Kingdom, Medical Research Council (MR/L501529/1; MR/R024804/1) and Economic and Social Research Council (ES/L008238/1)) and through the My Name'5 Doddie Foundation, and Alan Davidson Foundation.

This paper was made possible through the collaboration of the TRICALS Consortium through the work of the PRECISION ALS Programme. Science Foundation Ireland (SFI) and Biogen provided financial contributions, which supported the data processing and analysis phases of this study. Biogen were given a chance to review and provide comments to this paper prior to submission.

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