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RESEARCH

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C9orf72 intermediate expansions of 24–30 repeats are associated with ALS



Alfredo Iacoangeli^{1,2*} , Ahmad Al Khleifat², Ashley R. Jones², William Sproviero², Aleksey Shatunov², Sarah Opie-Martin², Alzheimer's Disease Neuroimaging Initiative³, Karen E. Morrison⁴, Pamela J. Shaw⁵, Christopher E. Shaw^{2,6}, Isabella Fogh^{2,7}, Richard J. Dobson^{1,8,9}, Stephen J. Newhouse^{1,8,9} and Ammar Al-Chalabi^{2,10}

Abstract

The expansion of a hexanucleotide repeat GGGGCC in C9orf72 is the most common known cause of ALS accounting for ~40% familial cases and ~7% sporadic cases in the European population. In most people, the repeat length is 2, but in people with ALS, hundreds to thousands of repeats may be observed. A small proportion of people have an intermediate expansion, of the order of 20 to 30 repeats in size, and it remains unknown whether intermediate expansions confer risk of ALS in the same way that massive expansions do. We investigated the association of this intermediate repeat with ALS by performing a meta-analysis of four previously published studies and a new British/Alzheimer's Disease Neuroimaging Initiative dataset of 1295 cases and 613 controls. The final dataset comprised 5071 cases and 3747 controls. Our meta-analysis showed association between ALS and intermediate C9orf72 repeats of 24 to 30 repeats in size (random-effects model OR = 4.2, 95% CI = 1.23–14.35, p -value = 0.02). Furthermore, we showed a different frequency of the repeat between the northern and southern European populations (Fisher's exact test p -value = 5×10^{-3}). Our findings provide evidence for the association between intermediate repeats and ALS (p -value = 2×10^{-4}) with direct relevance for research and clinical practice by showing that an expansion of 24 or more repeats should be considered pathogenic.

Keywords: C9orf72, Repeat expansion, ALS, Genetics, Whole-genome sequencing, Next-generation sequencing

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, primarily affecting upper and lower motor neurons, resulting in progressive weakness and culminating in death from neuromuscular respiratory failure, typically 2–5 years after diagnosis [1]. The incidence is 1–2 per 100,000 person-years, point prevalence is 3 to 5 per 100,000 people in Europe and the United States, and the lifetime risk is 1 in 300 [2–4].

Following initial linkage and association studies, the massive expansion of a hexanucleotide repeat in the C9orf72 gene was found to be the most frequent cause of ALS [5–10]. In most people, the repeat length is 2, but in people with ALS, hundreds to thousands of repeats may be observed [11]. A small proportion of people have an intermediate expansion, of the order of 20 to 30 repeats in size and several studies suggest that a threshold of 20 or 23 repeats could be used to discriminate between pathogenic and neutral expansions [7, 11–16]. However, the rarity of intermediate repeats and the consequential lack of statistical power, has limited the validity of these results and it remains unknown whether intermediate expansions confer risk of ALS in the same way that massive expansions do. At present, a repeat length of > 30 is typically used as the threshold to distinguish between neutral and pathogenic expansions [5].

One meta-analysis based on four studies, suggested an association of intermediate expansions between 24 and 30 repeats in size with sporadic ALS (chi-squared

* Correspondence: alfredo.iacoangeli@kcl.ac.uk

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

¹Department of Biostatistics and Health Informatics, King's College London, London, UK

²Department of Basic and Clinical Neuroscience, King's College London, Maurice Wohl Clinical Neuroscience Institute, London, UK

Full list of author information is available at the end of the article



p -value = 0.03, fixed-effect model) [17]. However, while this meta-analysis was rigorous, it had a few limitations which prevented the results translating into research and medical practice. First, several studies were excluded because data on repeats of length < 30 were not available [17]. Second, the fixed-effect model used in the meta-analysis assumes that the genetic effects are the same across the combined investigations, and that all differences are due to chance [18, 19]. While this assumption appears to be supported by the lack of heterogeneity across the involved studies (Q -test p -value = 0.61), heterogeneity may be masked. Genetic effects may vary across different populations for various reasons, including both genuine differences and differential biases and errors across studies [20, 21], and the *C9orf72* repeat expansion exemplifies this variation [13, 15, 16, 22, 23]. Moreover, the Q -test and I -squared do not describe heterogeneity well when the number of studies is small [24, 25] and the datasets did not provide sufficient power to properly test the meta-analysis studies for heterogeneity. Third, other studies of intermediate repeat sizes have been inconclusive [5, 7, 14].

Taking these considerations into account, we therefore analysed a new group of 1295 people with ALS and 613 controls, all sized for *C9orf72* repeats, and included the findings in a meta-analysis of studies for which there are data on intermediate repeats of size 24 or greater. We did not investigate intermediate repeats of size 20 to 23 since such repeats have been observed both in ALS patients and controls with no differences in allele distribution in several studies [5, 16, 23], including in our new cohort (Fig. 1 and Additional file 1: **Table S1**).

Methods

Whole-genome sequencing samples

Whole-genome sequencing (WGS) data were obtained from blood samples of 1908 individuals, comprising 1295 people with ALS (all apparently sporadic cases) and 613 unaffected controls from two groups: a UK set of 1295 ALS cases, 340 matched controls [26], and 273 controls from the Alzheimer's Disease Neuroimaging Initiative (ADNI) WGS dataset (<http://adni.loni.usc.edu>). The UK dataset was generated as part of Project MinE [26, 27], on the Illumina HiSeq X platform (150 bp paired-end reads) and the ADNI dataset on the Illumina 2000 platform (100 bp paired-end reads).

Genotyping

We used ExpansionHunter [28] to size the *C9orf72* GGGGCC repeat in the WGS data. ExpansionHunter has been previously validated for *C9orf72* repeat sizing in 5787 WGS samples from Project MinE that were also genotyped using repeat-primed polymerase chain reaction (PCR) and fluorescence PCR. Using the repeat-primed PCR calls as the gold standard, ExpansionHunter showed an expansion detection accuracy $> 99\%$ [28]. ExpansionHunter also showed high concordance with PCR and Sanger sequencing in repeats whose total length did not exceed the read-length [28, 29], while providing a confidence interval for longer repeats. We used the ExpansionHunter repeat length called when repeat sizes had a read length < 25 repeats for the UK dataset and < 17 repeats for the ADNI dataset to account for the difference in sequencing platform, and we used the

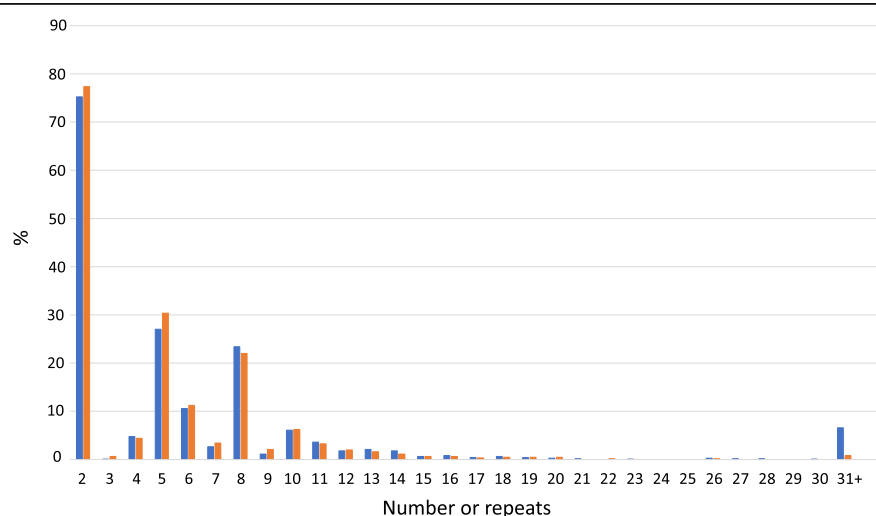


Fig. 1 Distribution of the samples carrying at least one allele of N repeats (x axis) in our dataset (British/ADNI) for cases (blue) and controls (orange)

middle value of the confidence interval for longer repeats.

Meta-analysis and statistics

For meta-analysis, we selected studies for which data were available for repeats of size 24 or more [17]. Association between the number of repeats and disease status was analysed by chi-square and Fisher's exact tests, with the samples split into two categories: samples with an intermediate expansion of 24–30 repeats and the remainder including those with repeat length > 30. Heterogeneity across studies was examined using the *I*-squared statistic and chi-square-based *Q*-tests. The *Q*-test power was calculated using the Jackson method [25]. Both fixed-effect and random-effects models were used. Sensitivity analyses were conducted to evaluate the influence of individual results on the pooled estimate after sequential removal of each study. Potential publication bias was assessed by both Begg and Egger tests. The Kolmogorov-Smirnov test was used to assess the difference of the repeat length distributions between cases and controls. To test the phenotype differences between our intermediate repeat carriers (> 23 and <= 30), the expansion carriers (> 30) and the non-expanded patients (< 24), we used the log-rank test to compare the survival distributions, Pearson's chi-squared test for gender and site of onset and the Kolmogorov-Smirnov test for the age at onset. The *Q*-test power calculation was made using Stata 15 (Stata Corp, College Station, TX, USA). All other analyses were done in R 3.4.4 using the *Metabin* function of the *Meta* library [30]. The *p*-value threshold used in this study to indicate statistical significance was 0.05.

Alzheimer's Disease Neuroimaging Initiative

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD).

Results

C9orf72 repeat expansion distribution in the new cohort

For the British dataset, the median age of ALS onset was 62.4 years and for controls, 60.1 years, with a male-female ratio of 62:38. In the ADNI dataset the median age was 73.2 and the male-female ratio 49:51.

We identified no difference in the distributions of repeat size between ALS and controls in the 1–23

repeat interval (Kolmogorov-Smirnov test, *p*-value = 0.96). Nine cases and one control were found to carry 24–30 repeats. Five controls were found to carry expansions whose length is rarely seen in unaffected controls and among which a few were hundreds of repeats long (Fig. 1 and Additional file 1: **Table S1**). Specifically, five British controls carried large expansions up to hundreds of repeats (sizes 60, 357, 406, 413 and 484) and one British control carried an intermediate repeat expansion of 26 repeats. 6.6% of cases had a repeat of size > 30.

Meta-analysis of the 24–30 repeat expansion

A literature review [17] identified four studies which provided original data on the number of patients carrying 24–30 repeats, including one study from North America [23], one study from Italy [16], one study from France [15], and one study from Spain [13]. We extended the review to all articles published up to January 2019 and identified one further study [28] providing data suitable for our meta-analysis. However, in this additional study, most of the ALS cases overlapped with our British dataset, and we therefore excluded it from our analysis. Therefore, after reproducing the previously published meta-analysis, we performed two new meta-analyses. In the first we added our British samples (1295 cases and 340 controls) and in the second we added our British/ADNI dataset (1295 cases and 613 controls). The final dataset used in this study comprised 5071 cases and 3747 controls. We were able to reproduce the association between the *C9orf72* 24–30 repeat expansion and ALS using the previously used four datasets (fixed-effect model odds ratio = 5.13, 95% confidence interval = 1.28–22.24, *p*-value = 0.03) (Table 1A). The association was replicated after including our cohorts, both when the British cohort was added (fixed-effect model odds ratio = 4.01, 95% confidence interval = 1.21–13.26 *p*-value = 0.02) (Table 1B) and when the British/ADNI dataset was added (fixed-effect model odds ratio = 4.82, 95% confidence interval = 1.45–15.96, *p*-value = 0.01) (Table 1C). The use of the fixed-effect model was suggested by the chi-square *Q*-test which did not show heterogeneity (*p*-value = 0.61), but this was underpowered to detect heterogeneity (power = 12.07%), suggesting that a random-effects model is a more appropriate model to use. Under a random-effects model, we did not show association using the four studies from the previous meta-analysis only (*p*-value = 0.07). Using the UK/ADNI dataset we were able to show this association under the random-effects model hypothesis also (odds ratio = 4.2, 95% confidence interval = 1.23–14.35, *p*-value = 0.02) (Table 1C).

No evidence of publication bias from either the Egger (*p*-value = 0.69) or Begg (*p*-value = 0.14) test

Table 1 Meta-analyses on the influence of *C9orf72* 24–30 repeat-long expansions on the risk of ALS. (A) Replication of the previously published meta-analysis (Y. Chen et al.). (B) Adding our British dataset to the 4 used in Y. Chen et al. (C) Adding our British/ADNI dataset to the 4 used in Y. Chen et al

	N. of samples 24–30 repeats Cases/Controls	Total N. of samples Cases/Controls	OR	95% Conf. Interval	% Weight Fixed/Random	
A - Y. Chen et al. meta-analysis datasets						
Garcia-Redondo et al.	1/0	781/248	0.95	0.04	23.52	32.8/22.7
Ratti et al.	1/0	1275/862	2.03	0.08	49.89	25.8/22.7
Rutherford et al.	3/0	995/1444	10.19	0.53	197.45	17.6/26.5
Millecamps et al.	6/0	725/580	10.19	0.59	186.57	23.8/28.1
M-H pooled (fixed-effect)	11/0	3776/3134	5.13	1.18	22.24	100.0
M-H pooled (random-effects)	11/0	3776/3134	4.16	0.90	19.14	100.0
Heterogeneity chi-squared $Q = 1.82$ (d.f. = 3) $p = 0.61$, power = 12.07%						
I-squared (variation in OR attributable to heterogeneity) = 0.0% (0.0–74.8%)						
Fixed-effect Test of OR = 5.13: $z = 2.18$ $p = 0.03$						
Random-effects Test of OR = 4.16: $z = 1.83$ $p = 0.07$						
B - Y. Chen et al. meta-analysis datasets + our British dataset						
Garcia-Redondo et al.	1/0	781/248	0.95	0.04	23.52	19.5/14.7
Ratti et al.	1/0	1275/862	2.03	0.08	49.89	15.3/14.7
Rutherford et al.	3/0	995/1444	10.19	0.53	197.45	10.5/17.2
Millecamps et al.	6/0	725/580	10.49	0.59	186.57	14.2/18.2
British dataset	9/1	1295/340	2.37	0.30	18.79	40.5/35.2
M-H pooled (fixed-effect)	20/1	5071/3474	4.01	1.21	13.26	100.0
M-H pooled (random-effects)	20/1	5071/3474	3.41	1.00	11.66	100.0
Heterogeneity chi-squared $Q = 2.00$ (d.f. = 4), $p = 0.74$, power = 12.83%						
I-squared (variation in OR attributable to heterogeneity) = 0.0% (0.0–58.4%)						
Fixed-effect Test of OR = 4.01: $z = 2.28$ $p = 0.02$						
Random-effects Test of OR = 3.41: $z = 1.96$ $p = 0.05$						
C - Y. Chen et al. meta-analysis datasets + our British/ADNI dataset						
Garcia-Redondo et al.	1/0	781/248	0.95	0.04	23.52	20.7/14.7
Ratti et al.	1/0	1275/862	2.03	0.08	49.89	16.3/14.7
Rutherford et al.	3/0	995/1444	10.19	0.53	197.45	11.1/17.2
Millecamps et al.	6/0	725/580	10.49	0.59	186.57	15.0/18.2
British/ADNI dataset	9/1	1295/613	4.28	0.54	33.88	36.9/35.2
M-H pooled (fixed-effect)	20/1	5071/3747	4.82	1.45	15.96	100.0
M-H pooled (random-effects)	20/1	5071/3747	4.20	1.23	14.35	100.0
Heterogeneity chi-squared $Q = 1.80$ (d.f. = 4), $p = 0.77$, power = 13.01%						
I-squared (variation in OR attributable to heterogeneity) = 0.0% (0.0–53.7%)						
Fixed-effect Test of OR = 4.82: $z = 2.57$ $p = 0.01$						
Random-effects Test of OR = 4.20: $z = 2.29$ $p = 0.02$						

was observed (Additional file 1: **Figure S1**). Sensitivity testing, recalculating the pooled odds ratios by omitting one study per iteration (Additional file 1: **Table S2**) showed that results were consistent, without major fluctuations.

Association between *C9orf72* intermediate expansion on the aggregated dataset

We also investigated the association between the intermediate repeats and ALS in a final dataset generated from the aggregation of the 5 datasets used for the

meta-analysis (5071 cases and 3747 controls). The association was demonstrated both with the chi-squared test (p -value = 5×10^{-4} , odds ratio = 14.83, 95% confidence interval 1.99–110.57) and the Fisher's exact test (p -value = 2×10^{-4}). Considering that the frequency of the *C9orf72* expanded repeat greatly varies across European populations [5, 7, 13–15, 31], we attempted to investigate whether this is also true for the intermediate repeats. To do so we split the aggregated dataset cases in two groups, 3015 northern European patients (Americans, French and British) and 2056 southern European patients (Italians and Spanish). Both the chi-squared test (p -value = 5×10^{-3} , odds ratio = 6.17, 95% confidence interval 1.43–26.61) and the Fisher's exact test (p -value = 5×10^{-3}) showed a significant difference between the two groups. On the basis of intermediate repeat heterogeneity between the two groups, we tested the association between the intermediate repeats and ALS in the two groups separately. For the northern European dataset (3015 cases and 2637 controls) the resulting odds ratio was 15.83 (95% confidence interval 2.11–118.67, chi-squared p -value = 3×10^{-4} and Fisher's exact test p -value = 10^{-4}). In the southern European dataset (2056 cases and 1110 controls) the association was not significant (Fisher's exact test p -value = 0.54). However, the low frequency of intermediate expansions (<0.1% in cases and not present in controls) limits the statistical power to conclude if there is an association in this group (power < 10%).

Clinical phenotype analysis

For our 1295 British patients, we compared the age at onset, time from first symptom to death (or last visit for censored cases) and site of onset (bulbar or spinal), between the patients who carried a non-expanded number of repeats (< 24 in 1201 patients), an intermediate number of repeats (> 23 and \leq 30 in 9 patients) and a full expansion (> 30 in 85 patients). We found no significant differences between the intermediate repeat carriers and the other patients. Consistent with previous findings [11, 32–34], patients carrying a full expansion had a shorter survival time (p -value < 0.0001, median = 2.49 years and 25–75% quantiles = 1.88–3.71 years) and a younger age at onset (p -value = 0.009, mean = 58.39 ± 8.46 years) compared to patients carrying a non-expanded number of repeats (survival median = 3.01 years and 25–75% quantiles = 2.10–4.55 years, mean age at onset = 60.90 ± 11.13 years). No difference in terms of gender prevalence and site of onset were observed between the three groups. A demographic table for the three groups is available in the Supplementary material (Additional file 1: **Table S2**).

Discussion

We have shown that intermediate repeat expansions in the *C9orf72* gene are associated with ALS. An expanded hexanucleotide repeat in *C9orf72* is the most common cause of ALS. While expansions of hundreds of repeats in size are clearly associated with the disease, a lower limit has not yet been identified. A few studies have suggested that 24–30 repeats might be sufficient to cause the disease [12, 17]. However, considering that the statistical power of the datasets used was limited, further investigation was essential. We have added 1295 cases and 613 controls to the meta-analysis and demonstrated the association of such intermediate expansions with ALS both under a fixed-effect (p -value = 0.01) and a random-effects model (p -value = 0.02). The latter tells us that the association between 24 and 30 repeats and the disease exists without assuming that the effect of this mutation is homogenous across the patient groups. This up-to-date figure obtained from a meta-analysis on 5071 ALS patients and 3747 controls from 5 populations (UK, USA, Spain, Italy and France) provides definitive evidence for the association between intermediate repeats and ALS (p -value = 2×10^{-4}) and expands the role of *C9orf72* in the development of ALS by enlarging the number of cases that can be explained, with direct implications for research and clinical practice. Indeed, we suggest the threshold to discriminate between neutral and pathogenic *C9orf72* expansions should be lowered to 23, considering as pathogenic, expansions of 24 or more repeats, in agreement with previously reported observations [5, 17].

Expansions of 20–23 repeats have contradictory evidence of association with ALS. This suggests that if such an association exists, their contribution to disease risk is weaker than for longer expansions, and we need larger datasets to investigate it. Due to the very low frequency of intermediate repeats, further investigation and larger sample sizes are needed to consolidate our results and determine whether to lower the threshold further. International initiatives such as Project MinE, whose aim is to collect genetic data (including the length of the *C9orf72* repeat) of over 10,000 thousand people with ALS, will be crucial to this end. Interestingly, we also observed a few controls with very large expansions that are only rarely observed in non-affected individuals. We have openly released the repeat frequency in our cohort for a wide range of lengths, to allow further analysis when new cohorts become available (Additional file 1: **Table S1**). Finally, we looked at age at onset, survival time, gender prevalence and site of onset of the intermediate repeat carriers, and we found no significant differences from the other patients.

However, this analysis was limited by their small number (9), which does not allow us to make any conclusion about the clinical characterization of this sub-group of patients.

Additional file

Additional file 1: Figure S1. Bias detection in the meta-analysis: A) Egger test; B) Begg test. C) Funnel plot of the 5 studies used in our meta analysis.

Table S1. *C9orf72* expansion analysis results obtained with ExpansionHunter on the British/ADNI dataset. **Table S2.** Pooled odds ratios by omitting one study per iteration. Demographic table. (DOCX 3047 kb)

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Al: analysis of the data and writing the manuscript. Al and AAC: design and interpretation of data. Al, IF, ARJ, AAC, WS: conception of the study. Al, AAK, ARJ, WS, AS: acquisition of data. All authors: revising the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

patients were analysed under ethical approval reference 08/H0405/60 from the Trent Research ethics committee.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author details

¹Department of Biostatistics and Health Informatics, King's College London, London, UK. ²Department of Basic and Clinical Neuroscience, King's College London, Maurice Wohl Clinical Neuroscience Institute, London, UK. ³The Alzheimer's Disease Neuroimaging Initiative, Center for Imaging of Neurodegenerative Disease, San Francisco VA Medical Center, University of California, San Francisco, USA. ⁴Faculty of Medicine, University of Southampton, University Hospital Southampton, Southampton, UK. ⁵Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK. ⁶UK Dementia Research Institute, King's College London, London, UK. ⁷Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy. ⁸Farr Institute of Health Informatics Research, UCL Institute of Health Informatics, University College London, London, UK. ⁹National Institute for Health Research (NIHR) Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London, London, UK. ¹⁰King's College Hospital, Bessemer Road, London SE5 9RS, UK.

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References

- Al-Chalabi A, Hardiman O, Kiernan MC, Chiò A, Rix-Brooks B, van den Berg LH (2016) Amyotrophic lateral sclerosis: moving towards a new classification system. *The Lancet Neurology* 15:1182–1194
- Chiò A, Logroscino G, Traynor B, Collins J, Simeone J, Goldstein L, White L (2013) Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. *Neuroepidemiology* 41:118–130
- Johnston CA, Stanton BR, Turner MR, Gray R, Blunt AH-M, Butt D, Among M-A, Shaw CE, Leigh PN, Al-Chalabi A (2006) Amyotrophic lateral sclerosis in an urban setting. *J Neurol* 253:1642–1643
- Robberecht W, Philips T (2013) The changing scene of amyotrophic lateral sclerosis. *Nat Rev Neurosci* 14:248
- DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of *C9ORF72* causes chromosome 9p-linked FTD and ALS. *Neuron* 72:245–256
- Morita M, Al-Chalabi A, Andersen P, Hosler B, Sapp P, Englund E, Mitchell J, Habgood J, De Belleruche J, Xi J (2006) A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. *Neurology* 66:839–844
- Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, Van Swieten JC, Myllykangas L (2011) A hexanucleotide repeat expansion in *C9ORF72* is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72:257–268
- Shatunov A, Mok K, Newhouse S, Weale ME, Smith B, Vance C, Johnson L, Veldink JH, van Es MA, van den Berg LH (2010) Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: a genome-wide association study. *The Lancet Neurology* 9:986–994
- van Es MA, Veldink JH, Saris CG, Blauw HM, van Vught PW, Birve A, Lemmens R, Schelhaas HJ, Groen EJ, Huisman MH (2009) Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat Genet* 41:1083
- Vance C, Al-Chalabi A, Ruddy D, Smith BN, Hu X, Sreedharan J, Siddique T, Schelhaas HJ, Kusters B, Troost D (2006) Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2–21.3. *Brain* 129:868–876
- Van Mossevelde S, van der Zee J, Cruts M, Van Broeckhoven C (2017) Relationship between *C9orf72* repeat size and clinical phenotype. *Curr Opin Genet Dev* 44:117–124
- Byrne S, Heverin M, Elamin M, Walsh C, Hardiman O (2014) Intermediate repeat expansion length in *C9orf72* may be pathological in amyotrophic

- lateral sclerosis. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* 15:148–150
13. García-Redondo A, Dols-Icardo O, Rojas-García R, Esteban-Pérez J, Cordero-Vázquez P, Muñoz-Blanco JL, Catalina I, González-Muñoz M, Varona L, Sarasola E (2013) Analysis of the C9orf72 gene in patients with amyotrophic lateral sclerosis in Spain and different populations worldwide. *Hum Mutat* 34:79–82
 14. Gijssels I, Van Langenhove T, van der Zee J, Slegers K, Philtjens S, Kleinberger G, Janssens J, Bettens K, Van Cauwenberghe C, Pereson S (2012) A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. *The Lancet Neurology* 11:54–65
 15. Millicamps S, Boillée S, Le Ber I, Seilhean D, Teyssou E, Giraudeau M, Moigneu C, Vandenberghe N, Danel-Brunaud V, Corcia P (2012) Phenotype difference between ALS patients with expanded repeats in C9ORF72 and patients with mutations in other ALS-related genes. *J Med Genet* 49:258–263
 16. Ratti A, Corrado L, Castellotti B, Del Bo R, Fogh I, Cereda C, Tiloca C, D'Ascenzo C, Bagarotti A, Pensato V (2012) C9ORF72 repeat expansion in a large Italian ALS cohort: evidence of a founder effect. *Neurobiol Aging* 33:2528. e2527–2528. e2514
 17. Chen Y, Lin Z, Chen X, Cao B, Wei Q, Ou R, Zhao B, Song W, Wu Y, Shang H-F (2016) Large C9orf72 repeat expansions are seen in Chinese patients with sporadic amyotrophic lateral sclerosis. *Neurobiol Aging* 38:217. e215–217. e222
 18. Lau J, Ioannidis JP, Schmid CH (1997) Quantitative synthesis in systematic reviews. *Ann Intern Med* 127:820–826
 19. Sutton AJ, Abrams KR, Jones DR, Jones DR, Sheldon TA, Song F (2000) Methods for meta-analysis in medical research
 20. Ioannidis JP (2007) Non-replication and inconsistency in the genome-wide association setting. *Hum Hered* 64:203–213
 21. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG (2001) Replication validity of genetic association studies. *Nat Genet* 29:306
 22. Majounie E, Renton AE, Mok K, Doppler EG, Waite A, Rollinson S, Chiò A, Restagno G, Nicolaou N, Simon-Sanchez J (2012) Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *The Lancet Neurology* 11:323–330
 23. Rutherford NJ, Heckman MG, DeJesus-Hernandez M, Baker MC, Soto-Ortolaza AI, Rayaprolu S, Stewart H, Finger E, Volkening K, Seeley WW (2012) Length of normal alleles of C9ORF72 GGGGCC repeat do not influence disease phenotype. *Neurobiol Aging* 33:2950. e2955–2950. e2957
 24. Gavaghan DJ, Moore RA, McQuay HJ (2000) An evaluation of homogeneity tests in meta-analyses in pain using simulations of individual patient data. *Pain* 85:415–424
 25. Jackson D (2006) The power of the standard test for the presence of heterogeneity in meta-analysis. *Stat Med* 25:2688–2699
 26. Consortium PMAS (2018) Project MinE: study design and pilot analyses of a large-scale whole-genome sequencing study in amyotrophic lateral sclerosis. *Eur J Hum Genet* 26:1537
 27. van der Spek RA, van Rheenen W, Pulit SL, Kenna KP, McLaughlin RL, Moisse M, Dekker AM, Tazelaar GH, Kenna B, van Eijk KR (2018) The project MinE databrowser: bringing large-scale whole-genome sequencing in ALS to researchers and the public. *bioRxiv*:377911
 28. Dolzhenko E, van Vugt JJ, Shaw RJ, Bekrisky MA, van Blitterswijk M, Narzisi G, Ajay SS, Rajan V, Lajoie B, Johnson NH (2017) Detection of long repeat expansions from PCR-free whole-genome sequence data. *Genome research*:gr.225672.225117
 29. Tazelaar GH, Dekker AM, van Vugt JJ, van der Spek RA, Westeneng H-J, Kool LJ, Kenna KP, van Rheenen W, Pulit SL, McLaughlin RL (2018) Association of NIPA1 repeat expansions with amyotrophic lateral sclerosis in a large international cohort. *Neurobiology of Aging*
 30. Schwarzer G (2007) Meta: an R package for meta-analysis. *R news* 7:40–45
 31. Cooper-Knock J, Hewitt C, Highley JR, Brockington A, Milano A, Man S, Martindale J, Hartley J, Walsh T, Gelsthorpe C (2012) Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. *Brain* 135:751–764
 32. Irwin DJ, McMillan CT, Brettschneider J, Libon DJ, Powers J, Rascovsky K, Toledo JB, Boller A, Bekisz J, Chandrasekaran K (2013) Cognitive decline and reduced survival in C9orf72 expansion frontotemporal degeneration and amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 84:163–169
 33. Mehta PR, Jones AR, Opie-Martin S, Shatunov A, Iacoangeli A, Al Khleifat A, Smith BN, Topp S, Morrison KE, Shaw PJ (2019) Younger age of onset in familial amyotrophic lateral sclerosis is a result of pathogenic gene variants, rather than ascertainment bias. *J Neurol Neurosurg Psychiatry* 90:268–271
 34. Stewart H, Rutherford NJ, Briemberg H, Krieger C, Cashman N, Fabros M, Baker M, Fok A, DeJesus-Hernandez M, Eisen A et al (2012) Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. *Acta Neuropathol* 123:409–417. <https://doi.org/10.1007/s00401-011-0937-5>

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