Neurobiology of Aging 84 (2019) 238.e25–238.e34

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

The role of C9orf72 in neurodegenerative disorders: a systematic review, an updated meta-analysis, and the creation of an online database

Chrysoula Marogianni [a,1], Dimitrios Rikos [a,1], Antonios Provatas [a], Katerina Dadouli [b], Panagiotis Ntellas [c], Panagiota Tsitsi [g], George Patrinos [d], Efthimios Dardiotis [a], George Hadjigeorgiou [e,f], Georgia Xiromerisiou [a,*]

[a] Department of Neurology, University of Thessaly, University Hospital of Larissa, Larissa, Greece
[b] Department of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, Larissa, Greece
[c] Department of Medical Oncology, University Hospital of Ioannina, Ioannina, Greece
[d] Department of Pharmacy, University of Patras School of Health Sciences, Patras, Greece
[e] Department of Neurology, Laboratory of Neurogenetics, University of Thessaly, University Hospital of Larissa, Larissa, Greece
[f] Department of Neurology, Medical School, University of Cyprus, Nicosia, Cyprus
[g] Department of Clinical Neuroscience, Section of Neurology, Center for Molecular Medicine, Karolinska Institute, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 15 January 2019
Received in revised form 16 April 2019
Accepted 17 April 2019
Available online 24 April 2019

Keywords:
C9orf72
Meta-analysis
Systematic review
Online database
Amyotrophic lateral sclerosis
Neurodegenerative disorders

ABSTRACT

A pathologic expansion of a noncoding GGGGCC hexanucleotide repeat of the C9orf72 gene has been strongly associated with familial amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) cases predominantly in Caucasian populations. In the last decade, scientific interest had been drawn to this gene and many studies conducted have shown a possible correlation with other neurodegenerative diseases as well. We performed an extensive literature search for C9orf72 mutation and its frequency in various neurological and psychiatric diseases. In addition, we performed a meta-analysis of the data related to ALS and familial ALS. An online cloud-based database and an interactive map were developed. The overall mutation frequency of C9orf72 is 20% for familial FTD, 16% for familial ALS and around 6%–8% for sporadic ALS and FTD. The updated meta-analysis that we performed showed that the pooled frequency of C9orf72 repeat expansion in patients with familial ALS was 23% (CI: 18%–28%) and in patients with sporadic ALS 3% (CI: 3%–4%). The subgroup analysis regarding the origin of the population revealed significant differences between Caucasian and Asian patients. Our analysis supports the direct causal relation of the C9orf72 expansion in ALS and FTD. On the contrary, the role of C9orf72 in other neurodegenerative disorders remains controversial. The system that we developed—the online database and the interactive map—is hopefully a stepping stone for an ever-growing platform that will aid scientists from all over the world in contributing to the meta-analysis of C9orf72-related publications.

1. Introduction

The C9orf72 gene is located on the short (p) arm of chromosome 9 open reading frame 72, whereas the protein coded by this gene is found in the cytoplasm of neurons and in presynaptic terminals. Initially, linkage analysis of kindreds with amyotrophic lateral sclerosis (ALS), frontotemporal degeneration (FTD), and ALS-FTD, had suggested a susceptibility locus on the short arm of chromosome 9 (van Es et al., 2009). Later, with the implementation of a genome-wide association approach, the association between the hexanucleotide repeat GGGGCC expansion within the first intron of the C9orf72 gene and ALS, FTD, ALS/FTD was confirmed (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Usually, there are few repeats of this hexanucleotide, typically less than 20–30, but in individuals with the mutation, the repeat can occur in the order of hundreds.

It is known that the mutation interferes with the normal expression of the protein made by C9orf72. Pathogenesis may occur in 2 different ways. One being the loss of function of the C9orf72 gene and the other is because of a toxic gain of function. The latter seems more likely and is associated with the production of

* Corresponding author at: Department of Neurology, University of Thessaly, University Hospital of Larissa, Mezourlo Hill, Larissa, Greece. Tel.: +306973208863; fax: +302413502750.
E-mail address: georgiaxiromerisiou@gmail.com (G. Xiromerisiou).
[1] Equal contribution.

0197-4580/$ – see front matter
https://doi.org/10.1016/j.neurobiolaging.2019.04.012

repetitive sense and antisense RNA and/or repetitive dipeptide repeat proteins (Moens et al., 2017).

The hexanucleotide repeat expansion of the C9orf72 gene has been proven to be a major genetic factor in ALS and FTD patients as it accounts for ~40% of familial ALS (FALS), 30% of familial FTD (FFTD), and ~8% of sporadic ALS (SALS) cases in predominantly Caucasian populations (Majounie et al., 2012b). The identification of C9orf72 mutation in ALS and FTD led the scientific community to the idea that this mutation may be involved in other neurodegenerative disorders as well (Beck et al., 2013). Given the clinical and pathologic overlap between FTD and Alzheimer's disease, several studies were conducted to elucidate any possible association between the C9orf72 hexanucleotide expansion and the susceptibility to Alzheimer's disease (Harms et al., 2013; Majounie et al., 2012a).

Scientists have also investigated the possible pathogenic role of C9orf72 repeat expansion in the spectrum of movement disorders, such as Parkinson's disease, atypical parkinsonian syndromes, and associated clinical phenotypes (Dejesus-Hernandez et al., 2013; Theuns et al., 2014).

Hence, the purpose of our study was to collect the numerous studies published in the recent years regarding C9orf72 and its association with various neurological disorders and to synthesize the research qualitatively by means of a systematic review and quantitatively by means of overall and stratified meta-analyses. Moreover, we created an online database and an interactive geographical map with the different frequencies of C9orf72 mutations in these disorders.

2. Materials and methods

We applied the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Liberati et al., 2009).

2.1. Data sources

We conducted a systematic review of the literature, investigating the frequency of C9orf72 gene mutation in several neurodegenerative diseases. We searched PubMed, Cochrane Library, and Medline (via PubMed) to identify all published studies before 30 May 2018. A combination of the terms "C9orf72", "chromosome 9 open reading frame 72", "ALS", "FTD", "ALS-FTD", "Alzheimer's", "AD", "Parkinson", "PD", "neurodegenerative disease", "psychiatric disorders", "parkinsonism", and "motor neuron disease" were used. All studies presenting original data that reported mutation frequencies of the C9orf72 gene in patients with the above diseases were included for further review. References of selected articles and reviews were also searched for additional records. Three investigators (CM, DR, and AP) independently conducted the database search.

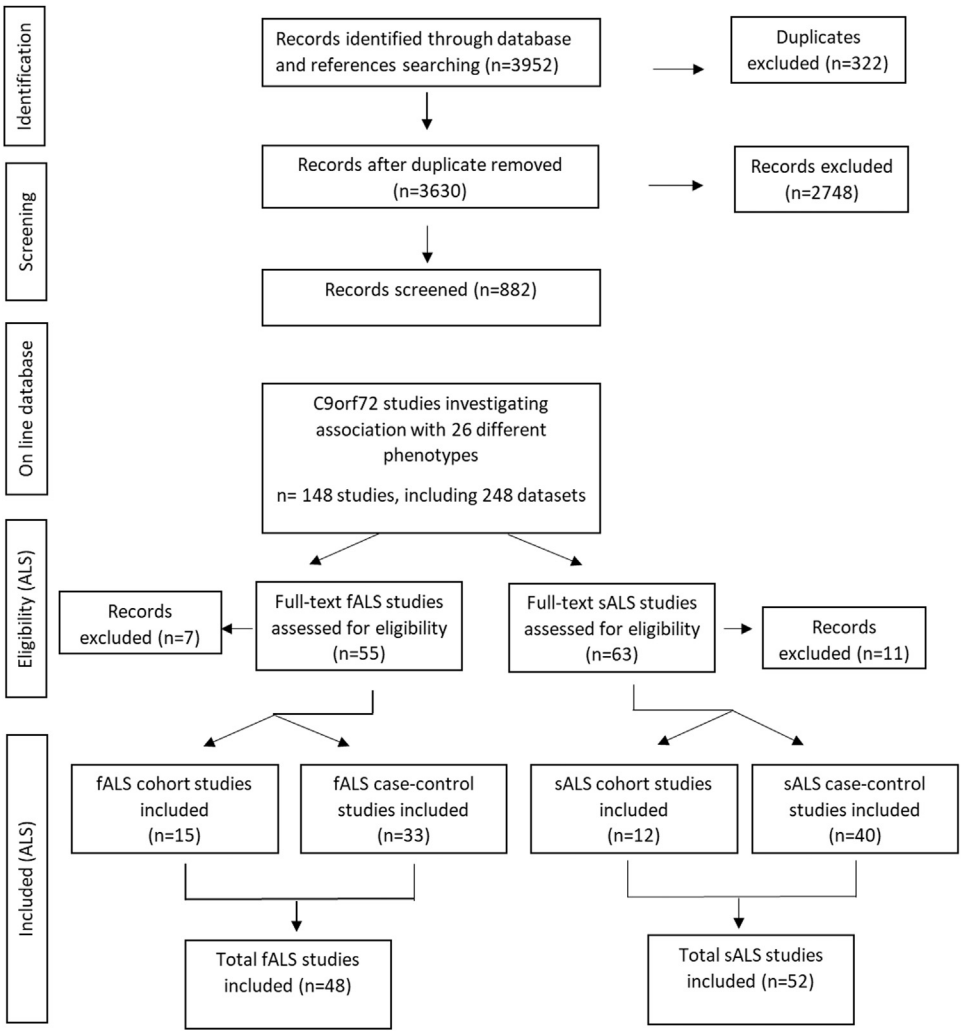

Fig. 1. A flowchart that shows the steps followed for the selection of studies. Abbreviations: ALS, amyotrophic lateral sclerosis; fALS, familial ALS; sALS, sporadic ALS.

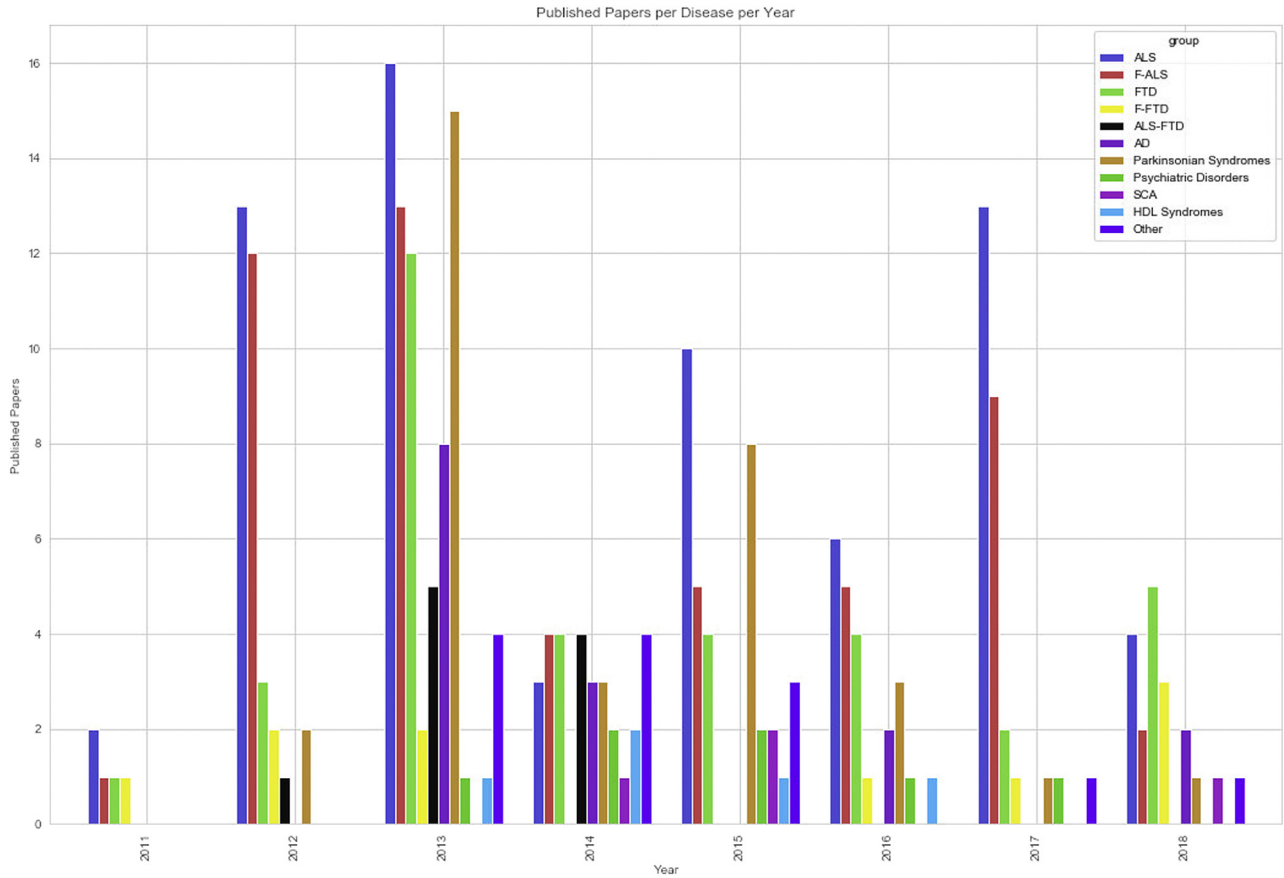

Fig. 2. A graphical presentation of the articles published per year concerning C9orf72 mutations in several neurological and psychiatric disorders. Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; F-ALS, familial ALS; FTD, frontotemporal degeneration; SCA, spinocerebellar ataxia; HDL, Huntington disease–like.

Table 1
Studies and corresponding samples investigating C9orf72 hexanucleotide expansions in various neurological diseases

Disease	Data sets	Cases	C9orf72+ cases	Controls	C9orf72+ controls	Ethnicity[a]
ALS	64	39,634	2609	23,766	21	44.17.3
FALS	55	8191	1349	12,938	6	36.16.3
AD	15	9019	44	13,664	14	11.3.0.1
ALS-FTD	9	419	124	3396	4	7.2.0
FTD	32	8444	634	15,381	18	21.9.2
FFTD	10	930	190	2178	0	7.1.2
CBS	5	407	19	661	0	4.1.0
PD	14	15,294	20	11,608	12	10.4.2
MSA	6	1074	0	1603	0	3.3.2
HDL syndrome	5	1240	20	7579	11	5.0.0
Essential tremor	2	258	0	0	0	1.1.0
Schizophrenia	5	1472	2	337	0	3.2.0
MS	2	1328	6	222	0	2.0.0
PSP	3	347	2	661	0	2.1.0
SCA	4	942	8	637	0	2.2.0
LBD	2	1500	4	0	0	2.0.0
sCJD	1	470	1	7579	11	1.0.0
Spastic paraplegia	2	362	0	308	0	1.1.0
Atypical parkinsonian syndrome	3	265	0	645	0	2.1.0
Dystonia	1	406	0	0	0	0.1.0
SMA	2	442	1	1356	0	2.0.0
RBD	1	344	2	0	0	1.0.0
Psychosis	1	1243	0	1234	0	1.0.0
MND	2	356	5	1536	0	2.0.0
Primary progressive aphasia	1	40	0	0	0	1.0.0
Obsessive-compulsive disorder	1	573	0	0	0	1.0.0
	244	94,606[b]	5008[b]	106,889[b]	97[b]	

Key: ALS, amyotrophic lateral sclerosis; FALS, familial ALS; AD, Alzheimer's disease; FTD, frontotemporal degeneration; FFTD, familial FTD; CBS, corticobasal degeneration; PD Parkinson's disease; MS, multiple sclerosis; MSA, multiple system atrophy; HDL, Huntington disease–like; PSP, progressive supranuclear palsy; SCA, spinocerebellar ataxia; LBD, Lewy body dementia; sCJD, sporadic Creutzfeld-Jacobs disease; SMA, spinal muscular atrophy; RBD, rapid eye movement sleep behavior disorder; MND, motor neuron disease.

[a] Ethnicity: Caucasian, Asian, Latin American, African.

[b] Populations include overlapped samples.

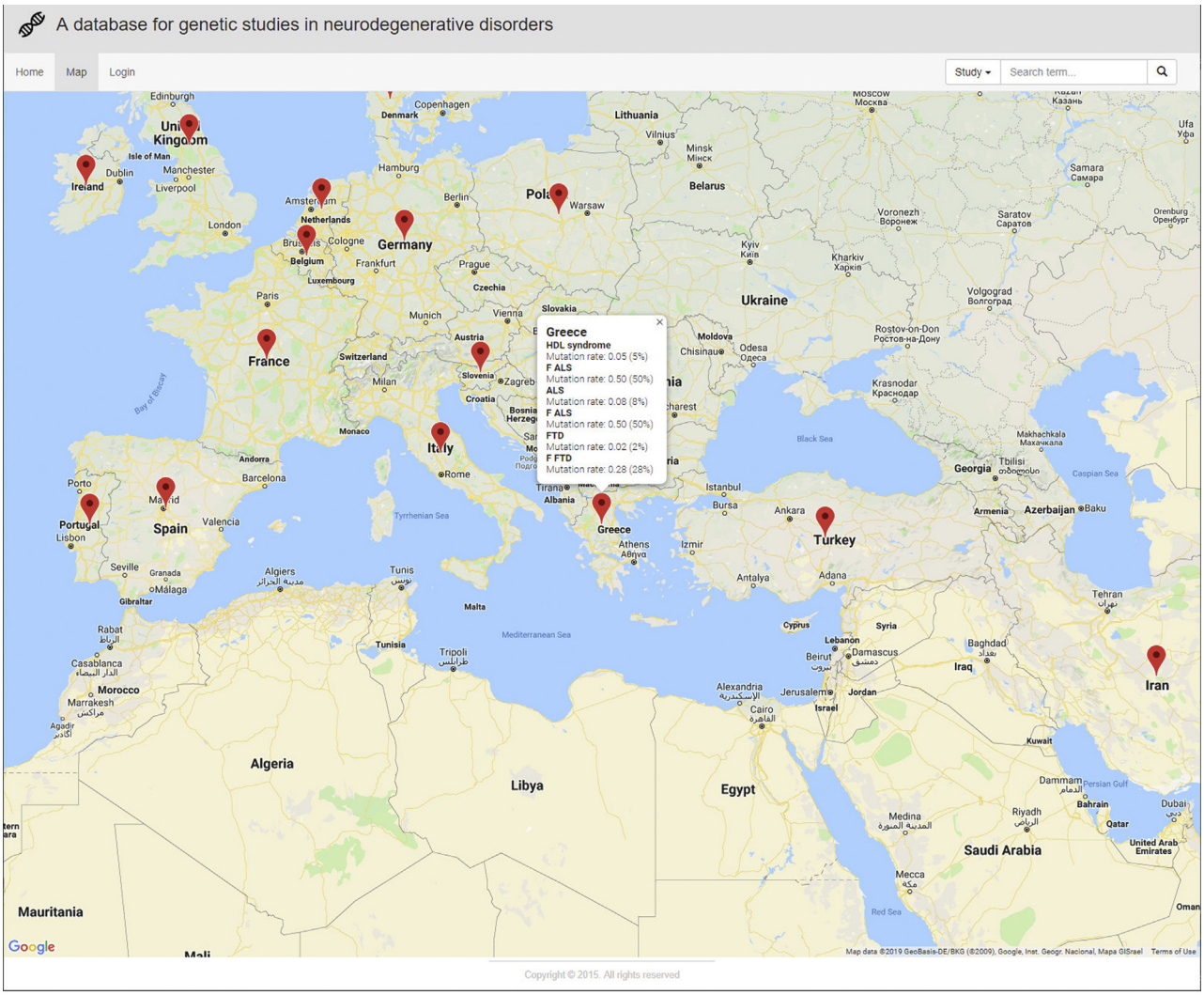

Fig. 3. A live visual representation of the geographic distribution of C9orf72 mutation frequencies. This data changes every time a new publication is added to the data set.

2.2. Data collection and eligibility criteria

Studies reporting C9orf72 mutation frequencies, no matter their design, primary objective, population, or sample size were included in the systematic review and online database. For the FALS and SALS meta-analysis, the additional inclusion criteria were as follows: 1) Both case-control and case series studies. 2) When the same patient population was presented in more than one publication, the one with the largest sample size or most recent publication date was used as the primary study for data extraction. 3) Diagnostic criteria of ALS were clearly stated, as the El Escorial criteria/revised El Escorial or the Awaji criteria and few had only clinical criteria for the diagnosis (Brooks, 1994; Brooks et al., 2000). 4) Familial history of patients with ALS enrolled was clear. 5) Only studies with full text were selected in the systematic review, whereas abstracts presented in scientific meetings, editorials, letters, or commentaries were not included. 6) Languages being limited to English.

2.3. Data extraction

The following information was retrieved from each study: title; author; year of publication; study design; diagnosis criteria category of ALS; criteria of FALS; total cases and controls (if any) screened, total cases, and controls (if any) carrying the mutant allele; the disease group; family history; and the ethnicity of the sample tested. Extracted data were imported on a searchable online database and plotted on a world map according to the country of origin. Data for meta-analysis was extracted from the online database.

2.4. Design of the online database

The platform was created keeping security and speed in mind. Ruby on Rails was used as the basic framework for building the backend of the system. The database is a PostgreSQL hosted on Amazon's RDS servers. The entire system is cloud-based and is currently hosted on Heroku's constant integration cloud platform allowing for swift and seamless updates. The system was designed with scalability as the main concern. It can automatically scale the available bandwidth and serve more users depending on the overall server load. Modularity is another key point. The system has been built with a clear distinction of each component (module) allowing for the seamless addition of new functionality while at the same time reducing any upgrade complexity. We will keep on adding

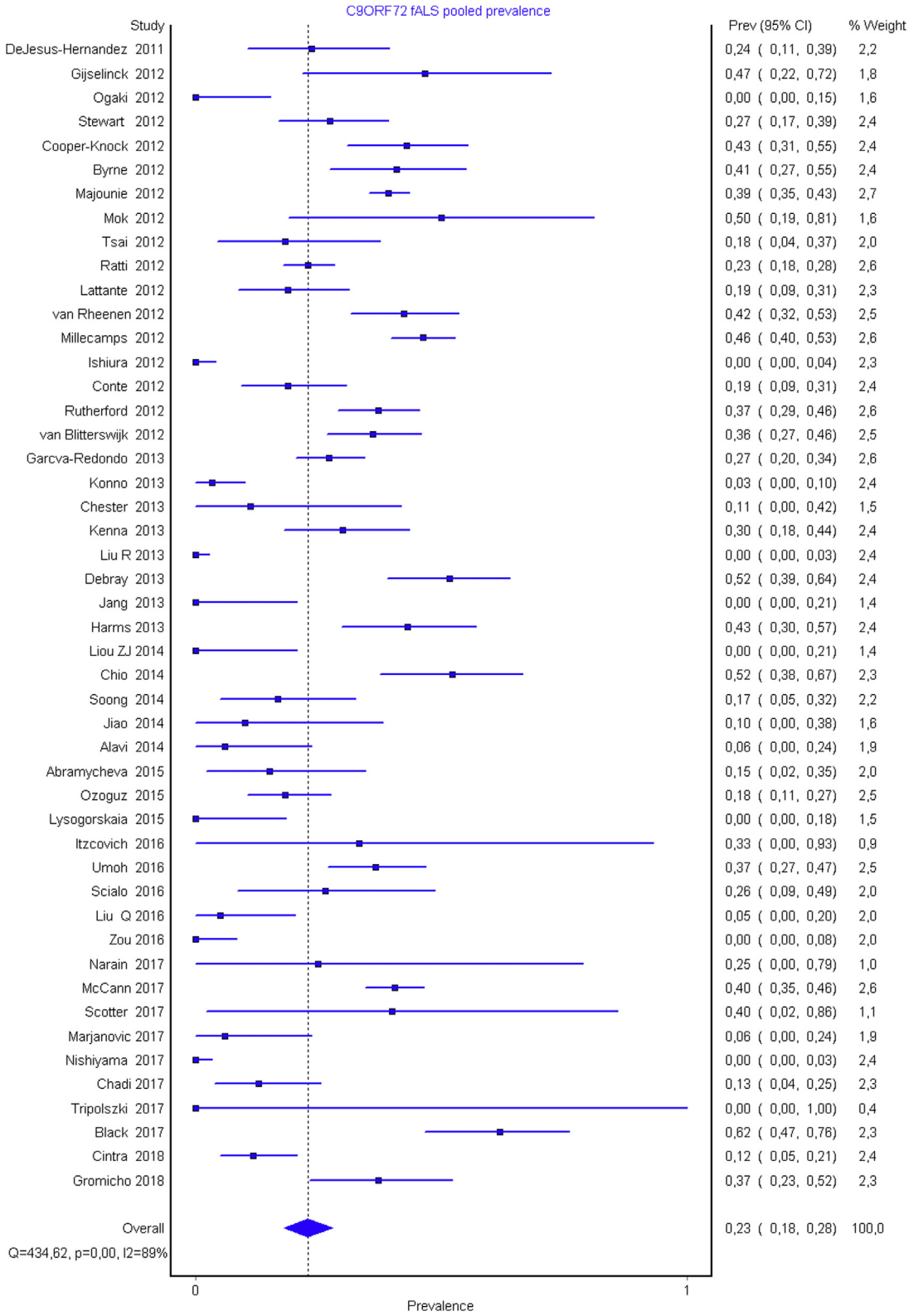

Fig. 4. Forest plot: pooled frequency of C9orf72 repeat expansion in patients with FALS. Abbreviations: FALS, familial ALS.

new functionality to the platform. The new features that will be added will be the result of feedback that we receive from our user base and thorough study of the requirements of the technological scientific scene.

The web database can be accessed via the following link https://neurodegenerative. herokuapp. com/admin/samples. There is a publicly available version with read-only access. Administrative access can be granted to parties that are interested in adding publications.

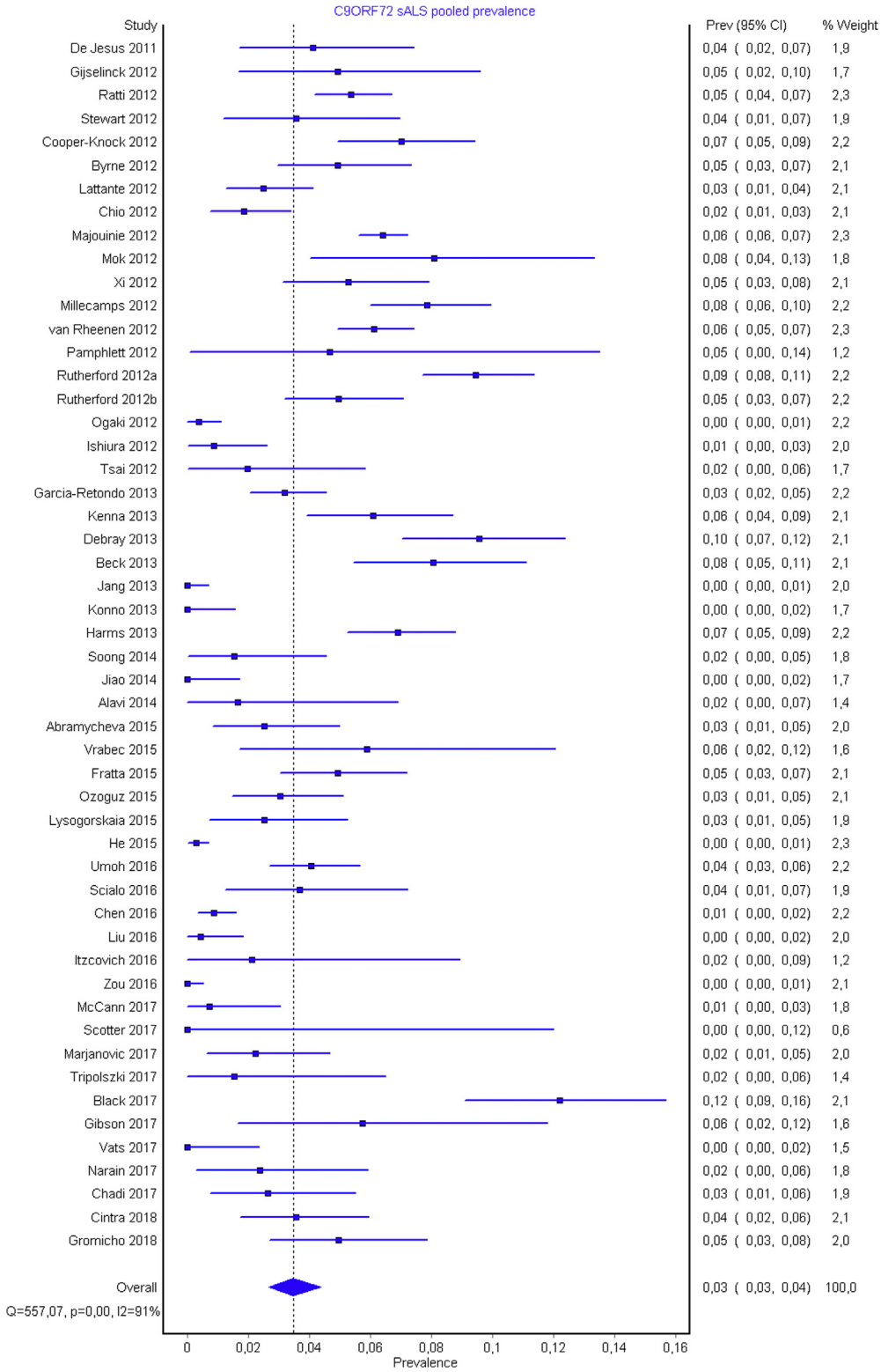

Fig. 5. Forest plot: pooled frequency of C9orf72 repeat expansion in patients with SALS. Abbreviations: SALS, sporadic ALS.

To avoid any unauthorized alteration of the data, any interested party will have to contact our system administrators to request credentials that will be specifically created for them. Usage instructions are shown graphically in Supplementary Figure S1.

2.5. Quality assessment

Quality assessment was performed on each included study by 2 reviewers (DR and AP) independently using the Newcastle-Ottawa Scale (Website: http://www.ohri.ca/programs/clinical_epidemiology/

Table 2
Meta-analysis results of familial and sporadic ALS studies

Disease type	Subgroup	Number of studies	Mutation prevalence	OR	CI	Q	I^2
FALS		48	23%		18%–28%	434.63	89%
	Caucasian	34	32%		28%–37%	157.4	79%
	Asian	14	4%		2%–8%	28.69	55%
	Case-control	33		192.94	113.2–328.8	38.82	18%
SALS		52	3%		3%–4%	557.1	91%
	Caucasian	38	5%		4%–6%	162.38	77%
	Asian	14	1%		0%–1%	19.93	35%
	Case-control	40		28.02	19.6–40.0	45.95	15%

Key: FALS, familial ALS; SALS, sporadic ALS; mutation prevalence, pooled prevalence of C9orf72 repeat expansion in random effects model; OR, pooled odds ratio of C9orf72 repeat expansion in mixed effects model.

oxford.asp. Available from: URL: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp).

2.6. Statistical analysis

Basic descriptive statistics for the systematic review were extracted from the online database. It is worth mentioning that the studies include overlapping populations and the frequencies reported are an indication of magnitude. These features are not suitable for further statistical analysis.

For the FALS and SALS meta-analysis, mutation frequencies of C9orf72 gene were reported as the number of the repeat expansion carriers among all cases and controls of FALS and SALS screened. Meta-analysis was conducted in 2 separate data sets in each phenotype (SALS and FALS). First, we calculated the pooled prevalence of the mutation in all included studies (both cohort and case-control) omitting the control samples. We furthermore calculated the pooled odds ratio in a, case-control studies only, meta-analysis. In case of substantial heterogeneity (I^2>50%), a random effects model was used. Otherwise, we used the Mantel-Haenszel method of fixed effects. All meta-analysis calculations were conducted using the metaXL (www.epigear.com) add-in for Microsoft Excel (Microsoft Office 365 ProPlus).

Statistical heterogeneity among studies was evaluated using Cochrane's Q test and I^2 statistics. A *p*-value lower than 0.10 for the Cochran Q statistic and I^2 exceeding 50% were used as a cutoff for statistically significant heterogeneity. Sensitivity analysis was further performed to access the impact of each study on the pooled result. To explain heterogeneity across studies, we performed subgroup analysis according to population (Caucasian or Asian) and study design (cohort or case-control study). Publication bias was evaluated graphically using funnel plots and Doi plots and numerically using the LFK index (Furuya-Kanamori et al., 2018). The Egger linear regression test method was applied to evaluate the asymmetry of the funnel plot and statistically significant publication bias was considered when the *p*-value was less than 0.10.

3. Results

3.1. Study selection

In total, we gathered 3952 articles of which 322 articles were excluded because of duplication. Subsequently, 2748 articles were excluded because of incorrect type of studies (animal studies, in vitro studies, clinical trials) or because of their type (e.g., conference proceedings, reviews, editorials). We retrieved 882 studies providing original information on C9orf72 mutations in patients with various neurodegenerative diseases. We further excluded 734 studies, that did not meet the inclusion criteria, after deeper review. A total of 148 separate studies were retained for the systematic review. The flowchart in Fig. 1 depicts the selection of studies.

3.2. Systematic review

The hexanucleotide expansion in the C9orf72 was studied in the past few years, in correlation with ALS and FTD, familial and sporadic forms. Based on the encouraging results, researchers investigated any possible correlation that the pathologic mutation might have with other neurodegenerative diseases and psychiatric disorders. Studies were conducted, investigating C9orf72 gene and possible relation to Parkinson's disease, other parkinsonian syndromes, Alzheimer's, Lewy Body dementia, psychosis, etc. This ongoing research is reflected in the published studies per year from 2011 until 2018 in Fig. 2. The included 148 studies in our systematic review comprise a total of 248 separate data sets given that in many studies, several phenotypes are studied simultaneously. A total of 26 different neurodegenerative diseases were studied for the C9orf72 mutation, and the participants (patients and controls) were 202.289. Most of the studies investigated the relation of C9orf72 with ALS (63 studies, 64 data sets) and FALS (53), which, along with FTD (32), FFTD (10), and FTD/ALS (9), were the first, where the pathologic repeat expansion of C9orf72, was identified. Parkinson's (14) and Alzheimer's (15) were also studied while few articles covered the topic of C9orf72 mutation in atypical parkinsonian syndromes and psychiatric diseases such as schizophrenia, psychosis, and obsessive-compulsive disorder.

Several articles examined a population of Asian origin (65 data sets), 10 data sets studied Latin Americans and the remaining 172, studied Caucasian populations. Only one investigated a population of African descent. From the 39,634 patients with ALS, that were included, 2609 were found positive for hexanucleotide repeat GGGGCC expansion, a mutation rate that reaches 6.6%. The corresponding rate for controls was 0.09%. In FALS, the percentage is approximately 16% for the patients tested positive with the mutation, whereas in FTD and FFTD the positive mutation rates are 7.5% and 20.4%, respectively. The highest frequency was found in ALS/FTD cases and was almost 30%. All the above rates match the already published findings, as far as ALS and FTD are concerned. The frequency of C9orf72 repeats in other diseases barely amount to 1%. For Parkinson's disease, the frequency is 0.13% given a population of 15,294, whereas Alzheimer's frequency was estimated at 0.49% in 9019 patients. Furthermore, in the case of schizophrenia, in 1472 patients, the positive cases were 0.13%. Another interesting observation is that of multiple system atrophy, with a population of 1074 patients, of which none were found to have the mutation. The 2 syndromes that stand out were Huntington-like syndrome with a frequency of 1.6% and corticobasal syndrome, with a population of 407 and 19 positive cases. It should be noted that the above frequencies are indicative of a tendency due to the probability of having overlapping populations in the included studies. (Table 1).

A visual representation of the geographic distribution of the various C9orf72 mutation frequencies is depicted on the interactive

map that we created. This data changes every time a new publication is added to the data set (Fig. 3).

3.3. Meta-analysis

The meta-analysis was focused on FALS and SALS. Further application of the inclusion criteria for the meta-analysis resulted in the exclusion of 7 FALS and 11 SALS studies.

Forty-eight and 52 studies were included reporting C9orf72 repeat expansions in 3106 FALS and 22,263 SALS patients respectively (supplementary file S5). Pooled frequency of C9orf72 repeat expansion in FALS patients was 23% (CI: 18%–28%) and in SALS patients 3% (CI: 3%–4%) (Figure 4 and 5). A further meta-analysis of 33 case-control studies including 2110 FALS cases and 12,174 controls revealed a pooled OR of 192.94 (CI: 113.2–328.8). Meta-analysis of 40 case-control studies including 22.263 SALS patients and 24.342 controls yielded a pooled OR of 28.02 (CI: 19.6–40.0) (Table 2 and Supplementary Figures S2 and S3).

3.4. Heterogeneity—subgroup analysis

Substantial heterogeneity was found in the pooled prevalence meta-analysis on both FALS and SALS overall data sets ($I^{2:}$ 89% and 91% respectively). Case-control studies had a much lower heterogeneity in both FALS and SALS groups (I^2: 18% and 15% respectively) indicating that study design (either a cohort or a case-control study) is a major source of heterogeneity.

Subgroup analysis of the (overall) FALS group regarding the origin population (Caucasian–Asian) showed a pooled mutation prevalence in Caucasians of 32% (CI: 28%–376%) and Asians of 4% (CI: 2%–8%). Five percent (CI: 4%–6%) of Caucasian origin and 1% (CI: 0%–1%) of Asian origin SALS patients had C9orf72 repeat expansions. The studies regarding Asian SALS patients were less heterogeneous (I^2: 35%) (Table 2). Subgroup analysis into Caucasians and Asians of the case-control only samples further strengthened the difference between these 2 populations regarding the pooled OR for having the mutant allele (data not shown).

No single study showed a significant drop in heterogeneity after sensitivity analysis.

3.5. Publication bias

Significant funnel plot asymmetry and LFK index suggestive of major asymmetry was found on both (prevalence and OR) groups of SALS meta-analysis but only on prevalence meta-analysis of FALS patients (Table 2 and Figure S4).

4. Discussion

In general, there is a vast amount of evidence to support the direct causal relation of the C9orf72 expansion in ALS and FTD. On the contrary, the role of C9orf72 in other neurodegenerative disorders remains controversial. It is quite impressive that 26 different neurodegenerative and psychiatric disorders have been studied so far. However, the only interesting finding is the association of C9orf72 expansion with Huntington-like syndrome–phenocopies. Specifically, according to several studies (Beck et al., 2013), C9orf72 evaluation may be considered for individuals negative for HD genetic testing. Another more recent association refers to the identification of C9orf72 expansion in patients with corticobasal syndrome (Lindquist et al., 2013). However, the overall number of patients studied is quite small and most patients manifested a possible family history of motor neuron features.

The identification of the pathological expansion in C9orf72 took place in 2011 and there was an explosion in the number of publications related to the estimation of mutation frequency in various populations in 2013. Since then, the annual number of publications referring to C9orf72 remains steady. This indicates that it is still an interesting topic and research is ongoing. Previous meta-analysis was conducted several years ago and they included far fewer studies than us (Chiò et al., 2013; Diekstra et al., 2014). During the last 2 years, the number of C9orf72-related publications rose at an exponential rate. Most of them were case-control studies investigating the prevalence of C9orf72 pathogenic expansion in SALS and FALS (Gibson et al., 2017; Ji et al., 2017). Therefore, a need was created for an updated meta-analysis with all these available studies.

We replicated the results from previous studies showing that C9orf72 repeat expansions were correlated with the risk of FALS and SALS (Shu et al., 2016; Zou et al., 2017). We also managed to demonstrate the differences in the risk of FALS and SALS between Asian and Caucasian populations. A previous study clearly showed that the genetic architecture of FALS and SALS in European and Asian populations varies significantly (Zou et al., 2017). According to this, in Asian populations with ALS, SOD1 mutations should be screened first, whereas C9orf72 expansion is a priority test in European populations. Our study highlighted this priority in European populations. In the subgroup analysis that we conducted, the pooled mutation rate for FALS in Caucasians was 32% while in Asians was 4%. We had similar results regarding SALS and C9orf72 repeat expansions (Caucasians 5%-Asians 1%). However, we must emphasize the fact that studies regarding Asian patients with ALS are far fewer than studies on Caucasian patients.

There were a few limitations in our study. Recent publications have focused on the size of the repeat and found an association between the C9orf72 repeat size and age at onset of the disease (Gijselinck et al., 2016). The pathogenic nature of the repeat depends on its size, but the cutoff between normal and pathogenic alleles is not well established yet. As a result of the current status, some studies consider repeats of >30 units as pathogenic, whereas others use an upper limit of >60 units (Cooper-Knock et al., 2014). Most of the studies in our meta-analysis use a cutoff of 30 units; however, of the eligible studies, not all included the cutoff used. Therefore, it was not feasible to perform a subgroup analysis regarding the number of C9orf72 repeats considered as pathogenic. Hence, this difference could be a source of bias and heterogeneity. Further potential source of bias may be the reliance exclusively on English-language studies. Although relevant studies found no evidence of a systematic bias from the use of language restrictions in systematic review-based meta-analyses this possibility should not be overseen (Morrison et al., 2012).

The presence of statistical heterogeneity is always indicative of variation and could be attributed to several sources. In our study, potential sources of heterogeneity could be the origin of the studied population, the diagnostic criteria that were employed for each study, the study methodology that was used (case-control, cohort study, etc.), and even the cutoff between normal and pathogenic alleles that we thoroughly analyzed previously. A test of heterogeneity was performed for the metanalysis within each subgroup that clearly indicated that the most prevalent incremental factor is the methodology of the study.

Sex differences in clinical prevalence have been identified in ALS previously (Curtis et al., 2017). A recent study revealed a higher prevalence of female patients with C9orf72-related ALS, a finding that contradicts the fact that men are known to have a higher prevalence of ALS overall. We chose not to calculate sex-specific prevalence in our meta-analysis because many studies did not provide sex-segregated data on mutation and nonmutation carriers. The system that we have created, the online database and the interactive map, is hopefully a stepping stone for an ever-growing

platform that will aid scientists from all over the world in contributing to the meta-analysis of C9orf72-related publications. Eventually, this will prove its significance toward the development of an ethnicity-based diagnostic algorithm.

The interactive map is the best way to demonstrate the geographical distribution of the affected population graphically. This map keeps on evolving as more contributions are being made to the systematic review data set.

As a result of this project, we hope, in the near future, we can achieve a milestone where all meta-analysis concerning major publications will be organized and stored in a similar manner. Setting this as a standard will alleviate the required research time.

Disclosure

All authors declare that no conflicts of interest exist.

Acknowledgements

This article is produced as part of the Hellenic ALS Research Network (www.alshellas.org.gr)

The authors would like to thank Ivan Gomarteli and Sergios Venetikidis for their essential contribution to the development and technical support of our platform.

Authors' contributions: GX contributed to the conception and design of the research project. Literature search was conducted separately by CM, DR, GX and AP. Data extraction was performed by CM, DR, and AP. Application of inclusion criteria for the systematic review was carried out by CM, DR, and AP. Disagreements were resolved through discussion and consensus among all authors. Additional inclusion criteria for the meta-analysis was performed by CM, DR, AP, PD, and KD. Statistical analysis was performed by DR, PD, and KD. GH, ED, GP were responsible for its review and critique. All authors participated in the manuscript preparation and final approval.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.04.012.

References

Beck, J., Poulter, M., Hensman, D., Rohrer, J.D., Mahoney, C.J., Adamson, G., Campbell, T., Uphill, J., Borg, A., Fratta, P., Orrell, R.W., Malaspina, A., Rowe, J., Brown, J., Hodges, J., Sidle, K., Polke, J.M., Houlden, H., Schott, J.M., Fox, N.C., Rossor, M.N., Tabrizi, S.J., Isaacs, A.M., Hardy, J., Warren, J.D., Collinge, J., Mead, S., 2013. Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. Am. J. Hum. Genet. 92, 345–353.

Brooks, B.R., 1994. El Escorial world federation of neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on motor neuron diseases/amyotrophic lateral sclerosis of the world federation of neurology research group on neuromuscular diseases and the El Escorial "clinical limits of amyotrophic lateral sclerosis" workshop contributors. J. Neurol. Sci. 124 (Suppl), 96–107.

Brooks, B.R., Miller, R.G., Swash, M., Munsat, T.L., 2000. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Other Mot. Neuron Disord 1, 293–299.

Chiò, A., Logroscino, G., Traynor, B.J., Collins, J., Simeone, J.C., Goldstein, L.A., White, L.A., 2013. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology 41, 118–130.

Cooper-Knock, J., Shaw, P.J., Kirby, J., 2014. The widening spectrum of C9ORF72-related disease; genotype/phenotype correlations and potential modifiers of clinical phenotype. Acta Neuropathol. 127, 333–345.

Curtis, A.F., Masellis, M., Hsiung, G.R., Moineddin, R., Zhang, K., Au, B., Millett, G., Mackenzie, I., Rogaeva, E., Tierney, M.C., 2017. Sex differences in the prevalence of genetic mutations in FTD and ALS: a meta-analysis. Neurology 89, 1633–1642.

DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Gilmer, H.F., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.-Y.R., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D., Petersen, R., Miller, B.L., Dickson, D., Boylan, K., Graff-Radford, N., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in non-coding region of C9ORF72 causes chromosome 9p-linked frontotemporal dementia and amyotrophic lateral sclerosis. Neuron 72, 245–256.

Dejesus-Hernandez, M., Rayaprolu, S., Soto-Ortolaza, A.I., Rutherford, N.J., Heckman, M.G., Traynor, S., Strongosky, A., Graff-Radford, N., Van Gerpen, J., Uitti, R.J., Shih, J.J., Lin, S.C., Wszolek, Z.K., Rademakers, R., Ross, O.A., 2013. Analysis of the C9orf72 repeat in Parkinson's disease, essential tremor and restless legs syndrome. Parkinsonism Relat. Disord. 19, 198–201.

Diekstra, F.P., Van Deerlin, V.M., van Swieten, J.C., Al-Chalabi, A., Ludolph, A.C., Weishaupt, J.H., Hardiman, O., Landers, J.E., Brown Jr., R.H., van Es, M.A., Pasterkamp, R.J., Koppers, M., Andersen, P.M., Estrada, K., Rivadeneira, F., Hofman, A., Uitterlinden, A.G., van Damme, P., Melki, J., Meininger, V., Shatunov, A., Shaw, C.E., Leigh, P.N., Shaw, P.J., Morrison, K.E., Fogh, I., Chio, A., Traynor, B.J., Czell, D., Weber, M., Heutink, P., de Bakker, P.I., Silani, V., Robberecht, W., van den Berg, L.H., Veldink, J.H., 2014. C9orf72 and UNC13A are shared risk loci for amyotrophic lateral sclerosis and frontotemporal dementia: a genome-wide meta-analysis. Ann. Neurol. 76, 120–133.

Furuya-Kanamori, L., Barendregt, J.J., Doi, S.A.R., 2018. A new improved graphical and quantitative method for detecting bias in meta-analysis. Int. J. Evid. Based Healthc. 16, 195–203.

Gibson, S.B., Downie, J.M., Tsetsou, S., Feusier, J.E., Figueroa, K.P., Bromberg, M.B., Jorde, L.B., Pulst, S.M., 2017. The evolving genetic risk for sporadic ALS. Neurology 89, 226–233.

Gijselinck, I., Van Mossevelde, S., van der Zee, J., Sieben, A., Engelborghs, S., De Bleecker, J., Ivanoiu, A., Deryck, O., Edbauer, D., Zhang, M., Heeman, B., Baumer, V., Van den Broeck, M., Mattheijssens, M., Peeters, K., Rogaeva, E., De Jonghe, P., Cras, P., Martin, J.J., de Deyn, P.P., Cruts, M., Van Broeckhoven, C., 2016. The C9orf72 repeat size correlates with onset age of disease, DNA methylation and transcriptional downregulation of the promoter. Mol. Psychiatry 21, 1112–1124.

Harms, M., Benitez, B.A., Cairns, N., Cooper, B., Cooper, P., Mayo, K., Carrell, D., Faber, K., Williamson, J., Bird, T., Diaz-Arrastia, R., Foroud, T.M., Boeve, B.F., Graff-Radford, N.R., Mayeux, R., Chakraverty, S., Goate, A.M., Cruchaga, C., 2013. C9orf72 hexanucleotide repeat expansions in clinical Alzheimer disease. JAMA Neurol. 70, 736–741.

Ji, A.L., Zhang, X., Chen, W.W., Huang, W.J., 2017. Genetics insight into the amyotrophic lateral sclerosis/frontotemporal dementia spectrum. J. Med. Genet. 54, 145–154.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P.C., Ioannidis, J.P., Clarke, M., Devereaux, P.J., Kleijnen, J., Moher, D., 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 6, e1000100.

Lindquist, S.G., Duno, M., Batbayli, M., Puschmann, A., Braendgaard, H., Mardosiene, S., Svenstrup, K., Pinborg, L.H., Vestergaard, K., Hjermind, L.E., Stokholm, J., Andersen, B.B., Johannsen, P., Nielsen, J.E., 2013. Corticobasal and ataxia syndromes widen the spectrum of C9ORF72 hexanucleotide expansion disease. Clin. Genet. 83, 279–283.

Majounie, E., Abramzon, Y., Renton, A.E., Perry, R., Bassett, S.S., Pletnikova, O., Troncoso, J.C., Hardy, J., Singleton, A.B., Traynor, B.J., 2012a. Repeat expansion in C9ORF72 in Alzheimer's disease. N. Engl. J. Med. 366, 283–284.

Majounie, E., Renton, A.E., Mok, K., Dopper, E.G., Waite, A., Rollinson, S., Chio, A., Restagno, G., Nicolaou, N., Simon-Sanchez, J., van Swieten, J.C., Abramzon, Y., Johnson, J.O., Sendtner, M., Pamphlett, R., Orrell, R.W., Mead, S., Sidle, K.C., Houlden, H., Rohrer, J.D., Morrison, K.E., Pall, H., Talbot, K., Ansorge, O., Hernandez, D.G., Arepalli, S., Sabatelli, M., Mora, G., Corbo, M., Giannini, F., Calvo, A., Englund, E., Borghero, G., Floris, G.L., Remes, A.M., Laaksovirta, H., McCluskey, L., Trojanowski, J.Q., Van Deerlin, V.M., Schellenberg, G.D., Nalls, M.A., Drory, V.E., Lu, C.S., Yeh, T.H., Ishiura, H., Takahashi, Y., Tsuji, S., Le Ber, I., Brice, A., Drepper, C., Williams, N., Kirby, J., Shaw, P., Hardy, J., Tienari, P.J., Heutink, P., Morris, H.R., Pickering-Brown, S., Traynor, B.J., 2012b. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol. 11, 323–330.

Moens, T.G., Partridge, L., Isaacs, A.M., 2017. Genetic models of C9orf72: what is toxic? Curr. Opin. Genet. Dev. 44, 92–101.

Morrison, A., Polisena, J., Husereau, D., Moulton, K., Clark, M., Fiander, M., Mierzwinski-Urban, M., Clifford, T., Hutton, B., Rabb, D., 2012. The effect of English-language restriction on systematic review-based meta-analyses: a systematic review of empirical studies. Int. J. Technol. Assess. Health Care 28, 138–144.

Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peuralinna, T., Jansson, L., Isoviita, V.M., Kaivorinne, A.-L., Hölttä-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chiò, A., Restagno, G., Borghero, G., Sabatelli, M., Heckerman, D., Rogaeva, E.,

Zinman, L., Rothstein, J., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257–268.

Shu, L., Sun, Q., Zhang, Y., Xu, Q., Guo, J., Yan, X., Tang, B., 2016. The association between C9orf72 repeats and risk of Alzheimer's disease and amyotrophic lateral sclerosis: a meta-analysis. Parkinsons Dis. 2016, 5731734.

Theuns, J., Verstraeten, A., Sleegers, K., Wauters, E., Gijselinck, I., Smolders, S., Crosiers, D., Corsmit, E., Elinck, E., Sharma, M., Kruger, R., Lesage, S., Brice, A., Chung, S.J., Kim, M.J., Kim, Y.J., Ross, O.A., Wszolek, Z.K., Rogaeva, E., Xi, Z., Lang, A.E., Klein, C., Weissbach, A., Mellick, G.D., Silburn, P.A., Hadjigeorgiou, G.M., Dardiotis, E., Hattori, N., Ogaki, K., Tan, E.K., Zhao, Y., Aasly, J., Valente, E.M., Petrucci, S., Annesi, G., Quattrone, A., Ferrarese, C., Brighina, L., Deutschlander, A., Puschmann, A., Nilsson, C., Garraux, G., LeDoux, M.S., Pfeiffer, R.F., Boczarska-Jedynak, M., Opala, G., Maraganore, D.M., Engelborghs, S., De Deyn, P.P., Cras, P., Cruts, M., Van Broeckhoven, C., 2014. Global investigation and meta-analysis of the C9orf72 (G4C2)n repeat in Parkinson disease. Neurology 83, 1906–1913.

van Es, M.A., Veldink, J.H., Saris, C.G., Blauw, H.M., van Vught, P.W., Birve, A., Lemmens, R., Schelhaas, H.J., Groen, E.J., Huisman, M.H., van der Kooi, A.J., de Visser, M., Dahlberg, C., Estrada, K., Rivadeneira, F., Hofman, A., Zwarts, M.J., van Doormaal, P.T., Rujescu, D., Strengman, E., Giegling, I., Muglia, P., Tomik, B., Slowik, A., Uitterlinden, A.G., Hendrich, C., Waibel, S., Meyer, T., Ludolph, A.C., Glass, J.D., Purcell, S., Cichon, S., Nothen, M.M., Wichmann, H.E., Schreiber, S., Vermeulen, S.H., Kiemeney, L.A., Wokke, J.H., Cronin, S., McLaughlin, R.L., Hardiman, O., Fumoto, K., Pasterkamp, R.J., Meininger, V., Melki, J., Leigh, P.N., Shaw, C.E., Landers, J.E., Al-Chalabi, A., Brown Jr., R.H., Robberecht, W., Andersen, P.M., Ophoff, R.A., van den Berg, L.H., 2009. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. Nat. Genet. 41, 1083–1087.

Zou, Z.Y., Zhou, Z.R., Che, C.H., Liu, C.Y., He, R.L., Huang, H.P., 2017. Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. J. Neurol. Neurosurg. Psychiatry 88, 540–549.