HHS Public Access

Author manuscript

Neurobiol Aging. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Neurobiol Aging. 2018 April ; 64: 160.e1–160.e7. doi:10.1016/j.neurobiolaging.2017.12.015.

High frequency of C9orf72 hexanucleotide repeat expansion in amyotrophic lateral sclerosis patients from two founder populations sharing the same risk haplotype

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Abstract

We characterized the C9orf72 hexanucleotide repeat expansion (RE) mutation in amyotrophic lateral sclerosis (ALS) patients of 2 distinct origins, Ashkenazi and North Africa Jews (AJ, NAJ), its frequency, and genotype-phenotype correlations. In AJ, 80% of familial ALS (fALS) and 11% of sporadic ALS carried the RE, a total of 12.9% of all AJ-ALS compared to 0.3% in AJ controls (odds ratio [OR] = 44.3, $p < 0.0001$). In NAJ, 10% of fALS and 9% of sporadic ALS carried the RE, a total of 9.1% of all NAJ-ALS compared to 1% in controls (OR = 9.9, $p = 0.0006$). We identified a risk haplotype shared among all ALS patients, although an association with age at disease onset, fALS, and dementia were observed only in AJ. Variations were identified downstream the repeats. The risk haplotype and these polymorphisms were at high frequencies in alleles with 8 repeats or more, suggesting sequence instability. The different genotype-phenotype correlations and OR, together with the large range in age at onset, suggest that other modifiers and risk factors may affect penetrance and phenotype in ALS.

1. Introduction

The G4C2 hexanucleotide repeat expansion in C9orf72 gene is the most common genetic mutation that causes amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Since first identified (DeJesus-Hernandez et al., 2011; Renton et al., 2011), the mutation was reported in different cohorts around the world with various frequencies among familial ALS (fALS), sporadic ALS (sALS), FTD, and ethnic groups (Abramycheva et al., 2015; Beck et al., 2013; Borghero et al., 2014; Byrne et al., 2012; Chen et al., 2016; Gijselinck et al., 2012; Hubers et al., 2014; Itzcovich et al., 2016; Majounie et al., 2012; Scotter et al., 2017; Simon-Sanchez et al., 2012; Snowden et al., 2012; Stewart et al., 2012;

Disclosure statement

Bryan J Traynor has a patent pending on the clinical testing and therapeutic intervention for the hexanucleotide repeat expansion of C9orf72; Avi Orr-Urtreger received research support from ALS Association and from Kahn and Adelis Foundations; and Vivian E Drory received research support from ALS Association and from Adelis Foundation. Other authors report no conflicts of interest.

Umoh et al., 2016; Vrabec et al., 2015). Common to all reports is the observation that the expansion of the repeat is more frequent in fALS. Moreover, the expansion is more frequent in ALS patients with dementia, as well as patients affected with FTD. In most studies, a risk haplotype was identified, although some rare cases of expansions on a nonrisk haplotype were reported.

The normal function of C9orf72 protein is still unclear but evidence supports roles in membrane trafficking and autophagy (Ciura et al., 2016; Farg et al., 2014; Sellier et al., 2016; Sullivan et al., 2016; Webster et al., 2016; Yang et al., 2016). Recently, it was shown that the normal C9orf72 protein localizes to processing bodies and is recruited to stress granules upon stress-related stimuli (Maharjan et al., 2017). Moreover, knockdown of C9orf72 completely abolishes stress granule formation and accelerates cell death.

We report here for the first time a high frequency of the G4C2 nucleotide repeat expansion in C9orf72 in a large cohort of ALS patients of Ashkenazi (AJ) and North Africa Jewish (NAJ) origins, which are genetically homogeneous populations and already proved valuable to study the genetics of neurodegenerative disorders, such as Parkinson's disease (Gan-Or et al., 2008; Orr-Urtreger et al., 2007). We further identified the mutation to be on a common, unstable risk haplotype and evaluated genotype-phenotype correlations.

2. Material and methods

2.1. Samples

DNA was extracted from the peripheral blood using standard protocols. Samples included 459 unrelated Jewish ALS patients followed at the ALS Clinic at Tel Aviv Sourasky Medical Center, Tel Aviv, Israel (Table 1). All patients had a diagnosis of clinically definite or probable ALS according to the revised El Escorial criteria (Brooks et al., 2000). The recruitment interval spanned from 2004 to 2016. Included in this cohort are 22 patients with known mutations in OPTN (Goldstein et al., 2016), PFN1 (Wu et al., 2012), and SOD1. Of the 459 patients, 349 were AJ (76%), 76 were Jews of Moroccan descent (16.6%, MJ), and 34 were of other North Africa origins (7.4%, NAJ-nm).

A total of 900 control samples were analyzed, that included 671 anonymous DNA samples from young healthy individuals, aged 20–45 years, mostly women (300 MJ and 371 AJ), who underwent routine genetic screening tests and were randomly selected and 229 healthy elderly AJ (age range: 44–87 years; average age 68.3 ± 8.9 years; 92 males and 137 females) (Goldstein et al., 2016).

All patients underwent an interview to disclose ancestry, family history of ALS, dementia or other neurodegenerative diseases, age at onset (AAO) defined as the age when the first symptoms appeared, and affected site at disease onset (limb or bulbar). Disease duration, defined as time from first symptoms to death or tracheotomy, was recorded for all patients, as well as the existence of cognitive or behavioral disturbances.

2.2. Standard protocol approvals, registrations, and patient consents

All participants provided informed consent before entering the study. All DNA samples were coded and tested in an anonymous manner. The Institutional and National Supreme Helsinki Institutional Review Board Committees for Genetics Studies approved the study protocol and the informed consent forms.

2.3. C9orf72 expansion genotyping

Determining the number of the G4C2 repeats was done as previously described (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Briefly, allele determination was based on 2 polymerase chain reactions (PCRs), 1 using primers flanking the repeat and 1 using repeat-primed PCR methodology. Some primers were modified (Table A.1, Fig. A.1). An expansion allele was recorded when the number of repeats was 30 or more (C9Positive [C9Pos]). The rest of the alleles of less than 30 repeats were considered negative for the expansion (C9Negative [C9Neg]). Fragment analysis was conducted on an ABI 3130xl Genetic Analyzer, and peaks were visualized using GeneMapper 4.0 (Applied Biosciences).

2.4. Deletion/insertion characterization

Primers flanking the repeats were designed to sequence alleles suspicious of having deletions or insertions (Table A.1-B, primer pairs 1–3). Sequence was performed using BigDye V1.1 chemistry and an ABI 3130xl Genetic Analyzer.

2.5. SNP genotyping and haplotype analysis

One hundred thirty-nine AJ-ALS and 127 AJ controls were genotyped on Affymetrix Genome-Wide Human single nucleotide polymorphism (SNP) 6.0 array. Of the 139 AJ-ALS, 14 were C9orf72 carriers. The analysis was done using SNP & Variation Suite v8.2 (Golden Helix, Inc.). Genotypes on chromosome 9 were aligned to identify the minimal linkage disequilibrium (LD) interval and to determine the risk haplotype. Six nonredundant SNPs were sequenced in 6 MJ-ALS and 4 NAJ-nm-ALS patients, carriers of the C9orf72 expansion (Table A.1–C).

2.6. Statistical analysis

Unless otherwise mentioned, all calculations were carried out using SPSS software V.15 (SPSS Inc., Chicago, IL) with 2-sided significant tests. Independence of categorical variables was tested using the $\chi 2$ distribution. For small cell counts, a Fisher's exact test (2-tailed) was used. AAO and disease duration were tested for association with expansion using independent t-test. Mann-Whitney U-test was used to determine if the distribution of wild-type alleles is the same across categories of ethnicity and risk or nonrisk haplotype. Survival curve was generated using Kaplan-Meier test.

3. Results

3.1. Wild-type alleles in the Jewish population

A total of 459 unrelated ALS patients and 900 ethnically matched controls were tested. Among the nonexpanded alleles, the most frequent number of repeats was 2, 5, and 8 in all

groups (Fig. 1A, Table A.2, 60.0%, 11.1%, 12.7% of all alleles, respectively). The rest of the nonexpanded alleles were each observed at 0%–4.6% (Table A.2). Intermediate repeats (20–29 repeats) were rare and observed in 7 individuals (Fig. 1B, Table A.2). No difference in the nonexpanded allele distribution was observed between ALS and controls of Ashkenazi origin ($p = 0.618$) or of Moroccan origin ($p = 0.759$).

3.2. C9orf72 expansion is strongly associated with ALS in the AJ population and is correlated with fALS, dementia, and earlier AAO

Of the 349 AJ-ALS, 45 carried the expansion (12.9%), compared to 2 AJ controls (Table 2, $p = 2.04 \times 10-18$, odds ratio [OR] = 44.26, 95% confidence interval [CI]: 10.66–183.68, $p < 0.0001$; Fig. 1C). One patient was suggestive of having 2 expanded alleles because he showed an expansion pattern (Fig. 1D) with no PCR product when using the flanking primers, and primers incompatibility was excluded by using 9 different primer pairs (data not shown).

Of the 10 AJ-ALS with evident familial history (fALS), 8 were C9orf72 mutation carriers (Fisher's exact test, $p = 1.57 \times 10-6$, Table 3). Three of them were previously reported (Majounie et al., 2012). Additional 3 patients with a family history of dementia were C9Pos.

In patients, a significant association with dementia was detected (Pearson $\chi 2 = 6.29$, df = 1, $p = 0.012$, Table 3), as well as with earlier AAO (58.7 ± 9.6 years in C9Pos compared to 63.6 ± 11.8 years in C9Neg [<30 repeats, t-test, $p = 0.008$]). C9orf72 expansion mutation did not correlate with gender, site of disease onset, or disease duration (Table 3). C9Pos did not show a significant shorter survival from the disease onset (median survival 37 months) than the C9Neg (median survival 33 months; $p = 0.987$, $p = 0.458$, $p = 0.642$ for log-rank, Breslow, and Tarone-Ware tests, respectively; with 13.3% and 14.8% censored of C9Pos and C9Neg, respectively).

3.3. C9orf72 expansion is strongly associated with ALS in the NAJ population

Of the MJ-ALS, 6 carried the expansion (7.9%), compared to 3 in MJ controls (OR = 8.49, 95% CI: 2.07–34.76, $p = 0.003$, Table 2). Four C9Pos ALS patients were observed in North Africa patients who are not of Moroccan origin (11.8%, OR = 13.2, 95% CI: 2.82–61.78, $p = 0.001$, Table 2). When looking at all these 10 North Africa ALS patients, the OR is 9.9 with $p = 0.0006$ (Table 2). In MJ-ALS, a tendency toward a correlation between the G4C2 expansion and dementia was suspected, as 28.6% of ALS with dementia were C9Pos ($p = 0.094$, Fisher's exact test), and only a weak significant correlation with site of onset was found ($p = 0.046$, Fisher's exact test, Table 3). These 2 observations do not exist when looking at the entire cohort of all North Africa patients (Table 3).

3.4. C9orf72 expansion mutations in the Ashkenazi Jews and North Africa Jews share a risk haplotype

We analyzed whole-genome SNP genotypes of 139 AJ-ALS and 127 matched controls. Of the AJ-ALS, 14 carried the C9orf72 expansion mutation. We tested to see if they carry the original Finnish risk haplotype (Laaksovirta et al., 2010; Majounie et al., 2012). A 107-Kb minimal LD interval was identified (chr9:27484575–27591569) that included 44 informative

SNPs, encompassed the complete C9orf72 gene (Fig. 2A), and was the same as the reported Finnish haplotype. A minimal number of 6 nonredundant SNPs determining the risk haplotype were genotyped in all 6 MJ-ALS and 4 NAJ-nm-ALS C9Pos patients (Fig. 2A). All these 10 patients carried the risk haplotype, with the exception of 1 patient from Egypt who was homozygous to the nonrisk allele at the proximal end of the LD interval (rs10967965, Fig. 2B) reducing the LD to 92.8 Kb. This suggests a common ancestor to all Jewish C9Pos ALS patients from Ashkenazi and North Africa origins.

3.5. The C9orf72 background risk haplotype of Ashkenazi

Of the AJ-ALS patients who were C9Neg (n = 125), 28 carried the risk haplotype (22.4%). Of the 127 controls which were C9Neg, 25 carried the risk haplotype (19.7%). For all 252 individuals, we scored the allele with the highest number of repeats and evaluated the correlation between these alleles and the risk haplotype. In the ALS group, all 28 that carried the risk haplotype had 8 repeats or more (100%) compared to only 11.3% of patients without the risk haplotype ($p < 0.0001$, Mann-Whitney U test, Fig. 2C). In AJ controls, 23 of the 25 that carried the risk haplotype had 8 repeats or more (92%) compared to 20 of 102 that did not carry the risk haplotype (19.6%) ($p < 0.0001$, Mann-Whitney U test, Fig. 2C). The same significant result was observed when looking at all AJ (n = 252, data not shown).

3.6. The number of repeats in the unexpanded alleles is higher in MJ compared to AJ

Interestingly, among the control individuals, the distribution of the wild-type alleles was significantly different in MJ compared to AJ, with higher number of repeats in the MJ group ($p < 0.0001$, Mann-Whitney U test). This might explain why expansion is more frequent in the MJ controls (1.0%) than in the AJ controls (0.3%). This was also observed in the ALS groups ($p = 0.001$, Mann-Whitney U test).

We further explored the possibility that higher number of repeats in wild-type alleles also affects the overall lower AAO observed in MJ-ALS patients compared to AJ-ALS ($p = 2.99 \times 10{-}8$, t-test), but we did not find a significant correlation to AAO, regardless of the patient's ethnicity (data not shown), suggesting that the difference in AAO between MJ-ALS and AJ-ALS is not related to higher number of repeats in nonexpanded C9orf72 alleles.

3.7. Sequence instability is significantly more frequent in chromosomes bearing at least 8 repeats

We observed 5 types of deletions/insertions downstream the repeat: (1) 12-bp deletion in a wild-type allele (n = 4, Fig. A.2-A and H) or in an expanded allele (n = 19, Fig. A.2-B and H); (2) 12-bp duplication (n = 2, Fig. A.2-C and H); (3) 11-bp duplication (n = 5, Fig. A.2-D and H); (4) indel of 11-bp deletion and 1-bp insertion (n = 24, Fig. A.2-E and H); and (5) 24-bp insertion (n = 1, Fig. A.2-F and H). Two samples did not show a pattern of repeats when using protocol 2 and did show an expanded allele when using protocol 3; therefore, their variation at the 3′ end was not determined (Fig. A.2-G1 and G2). Altogether, these insertions and deletions, localized to highly GC-rich region, were relatively rare (n = 55, 2.0% of all alleles) and mostly present on chromosomes bearing 8 repeats or more (45/55, 81.8%), suggesting a tendency toward sequence instability when number of repeats is at least 8. The 12-bp deletion and the indel (11-bp deletion and 1-bp insertion) were mostly

observed in AJ (20/23 and 23/24, respectively), while the 11-bp duplication was unique to MJ and the 12-bp duplication unique to AJ. Furthermore, 31.1% (19/61) of the C9orf72 expansion alleles were adjacent to the 12-bp deletion, compared to only 36 alleles bearing variation of 2655 C9Neg alleles ($\chi 2 = 251.95$, df = 1, $p < 0.0001$). Finally, we did not find a significant difference in AAO or disease duration between AJ-ALS patients carrying the C9orf72 expansion mutation with the deletion at the 3′ end compared to those C9Pos that did not have the deletion (t-test, p = 0.72 and p = 0.29, respectively).

4. Discussion

We hereby describe for the first time the frequency of C9orf72 expansion mutation in a large cohort of ALS patients from different Jewish origins, demonstrating high carrier rates. Our study indicates that the C9orf72 expansion is the most common genetic cause of ALS in AJ (13%) but only second to the founder OPTN691_692insAG mutation in MJ-ALS patients (8% compared to 14.5%) (Goldstein et al., 2016). Mutations in C9orf72 and OPTN together explain 16% and 20% of all AJ and MJ-ALS, respectively. Because both genes are involved in autophagy, our data further emphasize the important contribution of this pathway to ALS. Our results help understand the genetic background of ALS in patients of Jewish origin and will further improve decision making during the genetic workup and genetic consultation of patients and families, strongly supporting the need to screen all ALS patients for the C9orf72 mutation.

Phenotype-genotype correlation showed different results depending on the ethnicity of the patients. In ALS patients of North Africa origin, we did not detect any significant correlation between the expansion and different clinical characteristics of the disease. This might be due to the significantly earlier AAO in NAJ ALS patients compared to the AJ and the worldwide average, and the factors causing this difference might mask the correlation. In AJ, the correlation to fALS was very high compared to other populations reported [80% compared to 0% and 5% in Middle Eastern and Asian patients, respectively, the smallest extreme (Majounie et al., 2012), and 46.7% and 57.9% in Belgian and Sardinia, respectively, the highest extreme (Gijselinck et al., 2012; Majounie et al., 2012)]. Even when including the sALS cases with familial dementia history with fALS, this percentage stays high (61.1%). Moreover, C9orf72 mutation is also high in sALS compared to other populations (11% in AJ and 9% in NAJ), compared to a 6.3% found in a cross-sectional study that included 11 different European countries and populations in the United States (Majounie et al., 2012).

Our results also point to the need of studying a well characterized clinical cohort of FTD Jewish patients to determine the frequency of C9orf72 expansion mutation in this group. A genome-wide study on these 2 closely related neurodegenerative diseases, in a genetically homogeneous group, might decipher the genetic factors that lead to the development of one phenotype over the other.

The AJ are considered a relatively homogeneous population due to historic bottle necks and religious constraints. However, even within such a homogeneous group, we found a large variation in AAO among carriers, aged from 32 to 73 years, as well as a very large difference in the length of disease duration, from 5 to 138 months. Another example for the

phenotypic diversity is the coexistence of the motor neuron phenotype with dementia in only about 16% of the C9Pos patients. These facts suggest the presence of other modifiers, and the need for whole-genome analyses, in addition to further accurate determination of the expansion length, using other methods, as well as the determination of the methylation status of the region.

The C9orf72 expansion mutation among Jews shares a risk haplotype. Comparing the risk haplotype to previously reported risk haplotypes in other populations suggests common ancestor with the European haplotype (Laaksovirta et al., 2010; Majounie et al., 2012; Smith et al., 2013). This risk haplotype was found in 22.4% of AJ controls and in high frequencies in the European and African populations (Garcia-Redondo et al., 2013), suggesting an ancient haplotype. It is still debatable if the expansion occurred only once and was inherited as an unstable allele or if chromosomes bearing 8 repeats or more are more prone to expansion. We identified 1 AJ-ALS C9Pos patient with 32 repeats. Another case of ALS patient was previously described with 28 repeats on the risk haplotype chromosome (Garcia-Redondo et al., 2013). Although possible, it is less likely that such a large shrinking from hundreds of repeats took place. Therefore, different mutation events within this risk chromosome could have occurred independently, with yet unexplained mechanism. This model can also be supported by the finding that in the control MJ population, the number of repeats is significantly higher than in AJ and the frequency of expansion carriers is higher as well (1.0% compared to 0.3%), although we did not show anticipation or a higher percentage of C9-ALS in MJ compared to AJ.

We found several variations downstream the repeat, all within the first 23 bp after the repeat. This region is GC rich (the first 100 bp after the repeat has 87% GC content), and the instability is observed mostly on chromosomes bearing 8 repeats or more. Interestingly, the indel variation (11-bp deletion and 1-bp insertion), which we observed in AJ and 1 MJ, was previously reported as unique to samples of Portuguese origin in a large-scale multinational study (Nordin et al., 2017). Also of interest is that some of the variations were observed on chromosomes that had less than 8 repeats. It might be that these rare cases represent rare recombinations that occurred between chromosomes with and without the expansion. The effect of this variation on gene function, if at all, is yet to be determined.

One of our ALS patients carried both the C9orf72 expansion mutation and OPTN 691-692AG insertion mutation in heterozygous state. This is yet another example of double mutations (Lattante et al., 2015). The surprisingly high number of ALS cases carrying the OPTN 691-692AG insertion mutation or the C9orf72 expansion mutation, and more specifically, the high frequency (1%) of each of these mutations in the controls, suggest this cohort as a good model to shed a light on the penetrance of these mutations and to find additional genetic modifiers of ALS, or protective alleles, and by that hinting to potential therapeutic interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Tova Naiman for her technical assistance. The authors are grateful to the patients and their families who participated in this study.

This work was supported by Adelis Foundation, by ALS Association (grant number 47717), and by Kahn Foundation.

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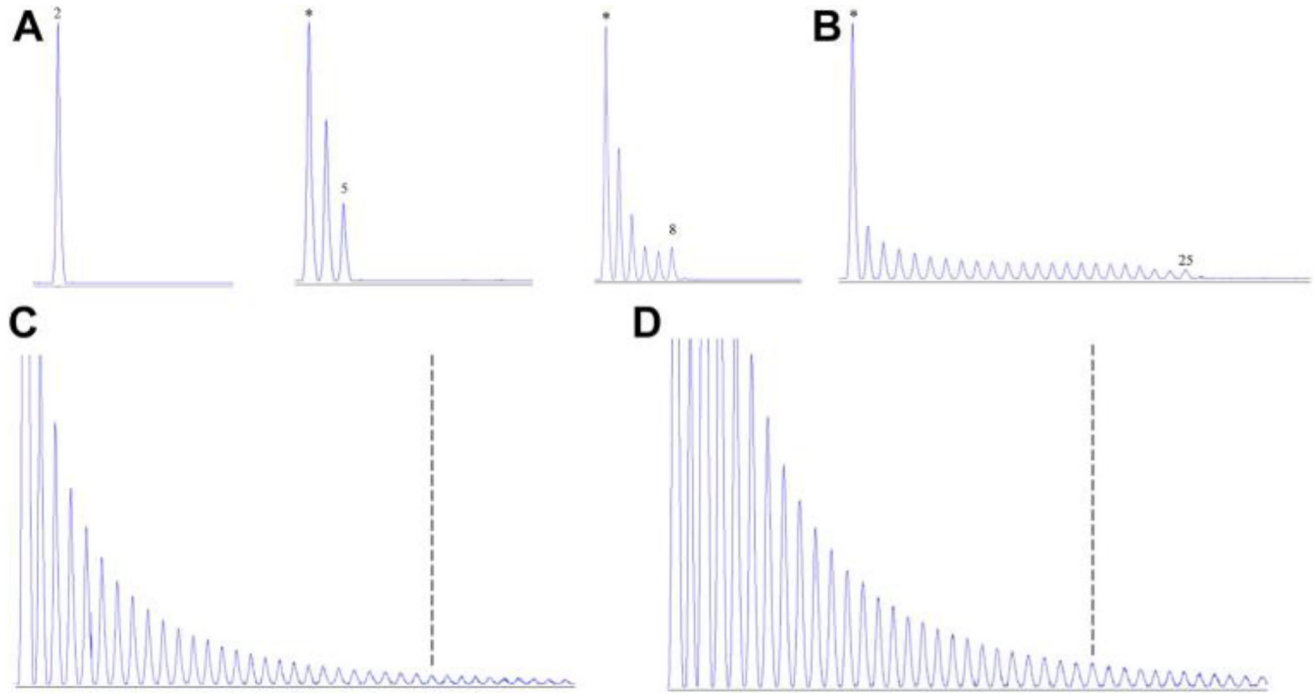
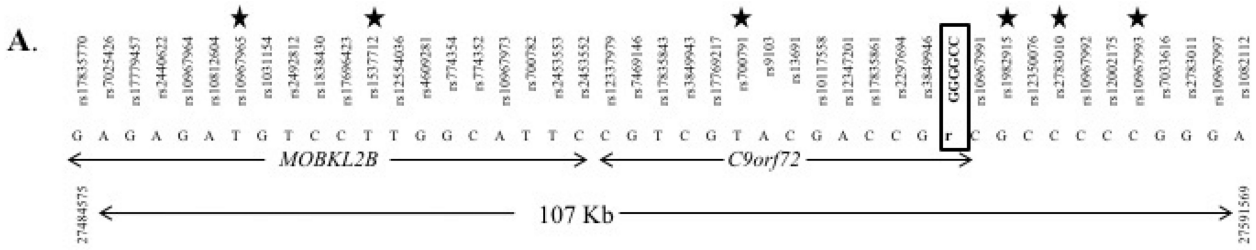


Fig. 1. C9orf72 G4C2 hexanucleotide repeat capillary-based sequence traces of the repeat-primed PCR (protocol 2)

(A) The 3 most common alleles in the Jewish population are 2, 5, and 8 repeats. * 1/4 The first peak represent 3 repeats when number of repeats is higher than 2. (B) Intermediate repeat size of 25 is observed. (C) Expanded allele is observed with a saw-tooth tail pattern that extends beyond 30 repeats (dotted line) with a 6-bp periodicity. (D) Expanded allele is observed in a sample that failed to give any PCR results using flanking primers, suggesting this individual carries 2 expanded alleles. Abbreviation: PCR, polymerase chain reaction.



B.

	rs10967965	rs1537712	rs700791	rs1982915	rs2783010	rs10967993
	27,497,988	27,508,489	27,545,960	27,579,560	27,581,239	27,582,311
MJ-1	TT	TT	TT	GG	CC	CC
MJ-2	TT	TT	TT	GG	CC	CC
MJ-3	AT	CT	GT	GG	CC	CC
MJ-4	AT	CT	GT	GG	CC	CC
MJ-5	AT	CT	GT	AG	CT	CT
MJ-6	TT	TT	TT	GG	CC	CT
NAJ-1	AT	TT	GT	GG	CC	CC
NAJ-2	AT	CT	GT	AG	CC	CT
NAJ-3	AT	CT	GT	GG	CC	CC
NAJ-4	AA	CT	GT	GG	CT	CC
	T	T	T	G	C	C

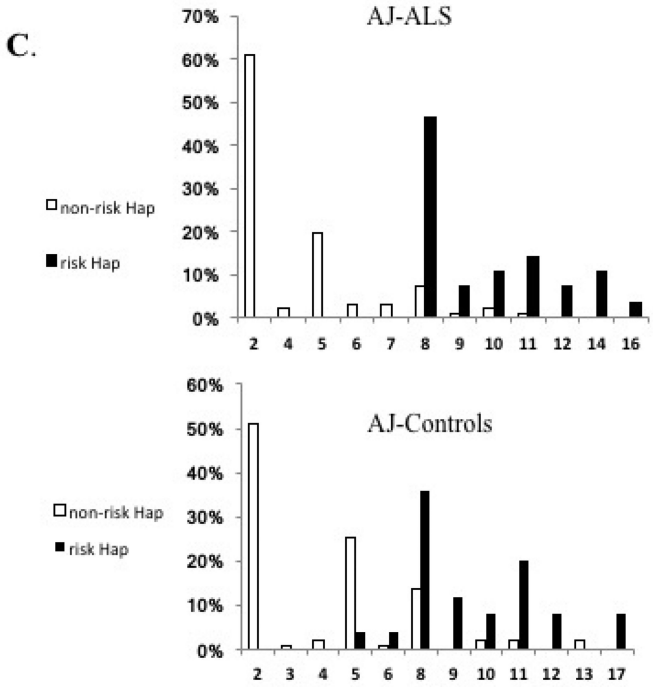


Fig. 2. C9orf72 gene region haplotype analysis

(A) 107-Kb risk haplotype is shared among AJ-ALS patients carrying the C9orf72 expansion, between rs17835770 and rs1082112 (107 Kb), that includes the complete C9orf72 gene. Marked with starts are the 6 nonredundant SNPs that define the risk haplotype. (B) Haplotype analysis of the 10 ALS patients of North Africa origin carrying the C9orf72 expansion. All patients carry the same risk haplotype as observed in the AJ-ALS, marked with bold letters and highlighted in the last row, with the exception of 1 patient (NAJ-nm-4) that is homozygous to the nonrisk allele at the proximal end of the LD interval (rs10967965, AA, Chr9:27,497,988) reducing the LD to 92.8 Kb. (C) Repeat allele frequencies for risk and nonrisk haplotypes in the AJ-ALS patients and AJ controls. The repeat sizes are higher for the risk haplotype in both groups and are highly significant ($p < 0.0001$). Abbreviation: AJ-ALS, ALS patients screened for the C9orf72 expansion mutation in Jews of Ashkenazi origin; ALS, amyotrophic lateral sclerosis; Hap, haplotype; LD, linkage disequilibrium; NAJ-nm, North Africa Jews that are not of Morocco descent.

Table 1

The ALS patients screened for the *C9orf72* expansion mutation included Jews of Ashkenazi origin (AJ-ALS), Morocco (MJ-ALS) and other North Africa origins (NAJ-ALS).

	AJ-ALS	MJ-ALS	NAJ-ALS	Total
Number of unrelated patients (% of total)	349 (76.0)	76 (16.6)	34 (7.4)	459
Age at onset, yrs (±SD)	63.0 (±11.6)	54.5 (±13.1)	56.4 (±13.1)	61.1 (±12.4)
Males (%)	208 (59.6)	44 (57.9)	16 (47)	268 (58.4)
Familial ALS (%)	10[a] (2.9)	7[b] (9.2)	3[c] (8.8)	20 (4.3)
Disease Duration, months[d] (±SD), number of patients	36.8 (±27.9), n=293	38.2 (±28.9) n=57	34.4 (±22.0) n=26	36.8 (±27.6) n=376
Site of onset (%)				
Bulbar	95 (27.2)	21 (27.6)	12 (35.3)	128 (27.9)
Limb	254 (72.8)	55 (72.4)	22 (64.7)	331 (72.1)

[a] Seventeen patients had a questionable positive family history and were not included in the percentage calculation and analysis for association with familial ALS.

[b] Six patients had a questionable positive family history and were not included in the percentage calculation and analysis for association with familial ALS.

[c] One patient had a questionable positive family history and was not included in the percentage calculation and analysis for association with familial ALS.

[d] Disease duration was calculated only for patients deceased or with tracheotomy

Table 2

The association between *C9orf72* expansion and ALS.

Study group / Genotype		No. Tested	Carriers of the C9orf72 expansion (30 repeats or more)	Both alleles are less than 30 repeats	*p*-value (Fisher's Exact test, *df*=1, two tailed)	OR for heterozygous (95% CI, p-value of OR)
ALS patients:	AJ	349	45[a] (12.9)	304 (87.1)	$p = 2.04\times10^{-18}$	44.26 (10.66–183.68, <0.0001)
	MJ	76	6 (7.9)	70 (92.1)	$p = 0.0029$	8.49 (2.07–34.76, 0.003)
	NAJ	34	4[b] (11.8)	30 (88.2)	$p = 0.0025$	13.20 (2.82–61.78, 0.001)
Total North Africa: MJ+NAJ		110	10 (9.1)	100 (90.9)	$p = 1.87\times10^{-4}$	9.9 (2.67–36.69, 0.0006)
Total (AJ, MJ, NAJ)		459	55 (12.0)	404 (88.0)	$p = 1.091\times10^{-21}$	24.37 (9.68–61.33, <0.0001)
Controls[c]:	AJ	600	2 (0.3)	598 (99.7)		
	MJ	300	3 (1.0)	297 (99)		
Total (AJ, MJ)		900	5 (0.5)	895 (99.5)		

[a] One ALS patient had both alleles with more than 30 repeats.

[b] One from Algier, one from Egypt, one from Tunis and one from Egypt/Tunis

[c] Control groups are in Hardy-Weinberg equilibrium

Abbreviations: ALS= amyotrophic lateral sclerosis; CI= confidence interval; OR= odds ratio; AJ= Ashkenazi Jews; MJ= Moroccan Jews; NAJ= North Africa Jews that are not of Morocco descent.

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Table 3

Genotype-phenotype correlations of the *C9orf72* expansion in Jewish ALS patients.

		AJ-ALS			MJ-ALS			All North-Africa Jewish ALS (MJ+NAJ)		
		C9 expansion (≥30) (n=45)	**No expansion (<30) (n=304)**	***p*-value[a] (χ^2)**	**C9 expansion (≥30)**	**No expansion (<30)**	***p*-value[a]**	**C9 expansion (≥30)**	**No expansion (<30)**	***p*-value[a]**
ALS class[b]	fALS (%)	8 (80)	2 (20)	$p=1.57\times10^{-6}$	1 (14.3)	6 (85.7)	$p=0.452$	1 (10)	9 (90)	$p=1.00$
	sALS (%)	37 (10.9)	302 (89.1)		5 (7.2)	64 (92.8)		9 (9)	91 (91)	
Dementia[c]	Dementia (%)	7 (29.2)	17 (70.8)	$p=0.012$ (6.29)	2 (28.6)	5 (71.4)	$p=0.094$	2 (18.2)	9 (81.8)	$p=0.265$
	No dementia (%)	37 (11.5)	285 (88.5)		4 (5.9)	64 (94.1)		8 (8.2)	90 (91.8)	
Gender	Male	24 (11.5)	184 (88.5)	$p=0.359$ (0.84)	2 (4.5)	42(95.6)	$p=0.233$	3 (5)	57 (95)	$p=0.181$
	Female	21 (14.9)	120 (85.1)		4 (12.5)	28 (87.5)		7 (14)	43 (71.6)	
Site of onset	Limb	29 (11.4)	225 (88.6)	$p=0.178$ (1.81)	2 (3.6)	53 (96.4)	$p=0.046$	5 (6.5)	72 (93.5)	$p=0.163$
	Bulbar	16 (16.8)	79 (83.2)		4 (19.0)	17 (81.0)		5 (15.2)	28 (84.8)	
Age at onset (± SD)		58.7 (±9.6)	63.6 (±11.8)	$p=0.008$ (assuming equal variance)	53.0 (±8.0)	54.6 (±13.5)	$p=0.77$ (assuming equal variance)	54.1 (±6.2)	55.2 (±13.6)	$p=0.647$ (assuming unequal variance)
Disease duration in months, (± SD), for deceased or with tracheotomy only		36.9 (±22.7)	36.8 (±28.6)	$p=0.973$ (assuming equal variance)	32.8 (±7.5)	38.8 (±30.4)	$p=0.265$ (assuming unequal variance)	32.6 (±6.9)	37.4 (±28.0)	$p=0.253$ (assuming unequal variance)

[a] χ^2 test (or Fisher's Exact test when numbers 5 or lower) was done on categorical variants. Independent t-test was done on AAO and Disease duration

[b] See comments a–c in Table 1.

[c] Excluding cases with unclear status of dementia.