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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No** | **Study** | **Country** | **Ethnicity** | **Disease group** | **No of cases** | **Repeat size in cases** | **No of controls** | **Repeat size in controls** | **Predominant haplotype** | **Repeat sizing method** | **Cut-off for expansion** | **Remarks** |
| 1 | Abramycheva et al, 2015 | Russia | Russian | Sporadic ALS | 249 | ≤16 | 223  | ≤15 | Finnish haplotype in C9 carriers | Fragment-length analysis and rp-PCR; Southern blot in carriers | stutter amplification pattern detected | Repeat size distribution similar between 249 sporadic ALS subjects and controls |
| C9ORF72-ALS | 9 (3.5%) | >40 |
| 2 | Byrne et al, 2012 | Ireland | Irish | Sporadic ALS | 386 | ≤23  | 188 | ≤23 | Founder haplotype | rp-PCR; Southern blot in carriers | >30 | 16/19 C9 carriers had 20-SNP founder haplotype |
| Familial ALS | 49 |
| 3 | Byrne et al, 2014  | Ireland | Irish | Sporadic ALS | 396 | <30 Only 4/396, (0.6%) had ≥20 repeats  | None | - | Not mentioned | rp-PCR | >30 | Higher number of repeats associated with younger age of diagnosis in cases with <20 repeats: Mean (sd) 47.6 (15.9) years vs 62.8 (sd 11.2), p=0.007. Cases with 20-30 repeats phenotypically similar to those with >30. |
| 4 | Debray et al, 2013 | Belgium | Belgian | Sporadic ALS | 471  | ≤21 | 384 | ≤20 | Not mentioned | rp-PCR and triplet-repeat PCR  | Stutter amplification pattern detected used | Sporadic ALS cohort: no correlation between length of unexpanded allele in carriers with age of onset, survival, or region of disease onset. In expansion carriers (n=45), no correlationbetween size of non-expanded allele and age atonset or survival. |
| Familial ALS | 119  |
| 5 | He et al, 2015 | China | Han Chinese | Sporadic ALS | 1092 | Mean (sd)3.79 ± 2.59 (2-25) | 1062 | Mean (sd) 3.84 ± 2.60(2-23) |  | rp-PCR, fluorescent fragment-length analysis | >30 | Expansion detected in only 3 sALS cases, 2 with genotypes inconsistent with European founder haplotype. |
| 6 | Itcovitch et al, 2016 | Argentina | Latin American | ALS | 50 |  | 73  | <18 | Not studied | Fluorescent fragment lengthAnalysis; rp-PCR | Presumed >30 | No significant difference in allele frequencies between normal controls and FTD or ALS.  |
| 7 | Jang et al, 2013 | Korea | Korean | Familial ALS  | 8 | Median (sd) 3.5 ± 0.3 (2-11) | None | - | Not mentioned | 2-step PCR protocol | Not specified |  |
| Sporadic ALS | 246 |
| 8 | Jiao et al, 2014 | China | Han Chinese | ALS | 110 (including 10 familial ALS) | Combined: Mean (sd) 6.2 ± 4.8 (2-20)  | 150 | Mean (sd) 6.0 ± 3.2(2-11)  | ALS-FTD proband from shared same risk haplotype | FAM fluorescent-labelled PCR then rp-PCR | Not mentioned | No significant difference in distributions of repeat numbers between patients and controls (p=0.23). |
| 9 | Konno et al, 2013 | Japan | Japanese | Sporadic ALS | 110 | Mean (sd) 3.33 ±2.27 | 180 | Mean (sd) 3.62 ±2.44(5.67 ± 2.95 with A-allele; 3.13 ± 2.02 without) | Both carriers had risk-haplotype | rp-PCR and genotyping PCR |  | No significant differences in mean repeat length between groups; but individuals with at least one rs3849942 allele A had significantly longer repeat than those without. Sporadic ALS: 5.68 ± 3.21 with A-allele; 3.07 ± 1.97 without. Familial ALS: 5.90 ± 3.91 with A-allele; 3.36 ± 2.13 without |
| Familial ALS | 58 | Mean (sd) 3.96 ±2.85 |
| 10 | Mok et al, 2012 | Greece | Greek  | Sporadic ALS | 136 | 11 expansion carriers | 228 | Only 4 controls (1.8%) had 20-24 repeats  | Not mentioned | rp-PCR and fragment-length analysis | >30 | Frequency of intermediate alleles in control Greek population similar to 2 UK control series: unpublished 1/85 (1.2%) and 6/361 (1.7%). |
| Familial ALS | 10 | 5 expansion carriers |
| 11 | Ogaki et al, 2012 | Japan | Japanese | Sporadic ALS | 552 | Mean (sd) 3.65 ± 2.43 (2–13) | 197 | Mean (sd) 3.69 ± 2.46 (2–14) | Expansion carriers carried 20-SNP risk haplotype | rp-PCR with fragment length analysis | Not mentioned |  |
| Familial ALS | 11 |
| 12 | Rutherford et al, 2012 | North American  | Not specified; presumed predominantly Caucasian | ALS | 995 | 1-25 | 1444 | ≤23 | Not studied | 2-step PCR protocol | ‘Normal’ range defined as <30 repeats | Maximum repeat length within normal range was 25 in a patient and 23 in a control. No association between repeat length of normal alleles in 211 expansion carriers and disease phenotype or age at onset in C9ORF72 mutation carriers or non-mutation carriers. |
| FTD-ALS | 160 |  |
| 13 | Sabatelli et al, 2012 | Italy | Italian, Sicilian and Sardinian | Sporadic ALS | 1757 | Not defined | 402 | ≤23; lower in Sardinian controls (≤12)  | Not mentioned | rp-PCR | >30 | Mean repeat length in Italian + Sicilian controls was 3 (range, 0–23; IQR, 0–5); in Sardinian controls was 4 (range, 0–12; IQR, 0–5) |
| 14 | Smith et al, 2013 | 5 European cohorts | Northern European  | ALS with or without FTD | 1347 | No range given | 856 | No range given | Common founder in all 137 carriers | rp-PCR | >30 | Average number of pathogenic repeats on the disease haplotype was 8, with a spread of expanded alleles up to 26.  |
| 15 | Xi et al, 2012 | UK, Italy, Spain, North America | European and North American | ALS | 389 |  | 602 | 2-30 | Not mentioned | 2-step PCR – fragment length analysis followed by rp-PCR | >30 | No intermediate or pathological number of repeats for the second allele (2-11 repeats) detected in expansion carriers. A trend toward association between the 10-repeat allele and risk for all 4 disorders (OR 1.72-2.14) seen. |
| 16 | Yeh et al, 2013 | Taiwan | Han Chinese | ALS | 16 | Mean (sd)3.63 ± 2.92 (median 2.5; 1-9) | 100 | Mean (sd)4.23 ± 3.08 (median 4; 1-17) | Not studied | Fluorescent rp-PCR | Not specified |  |

Supplementary Table 2. ALS studies included. ALS = amyotrophic lateral sclerosis; rp-PCR = repeat-primed polymerase chain reaction; SNP = single nucleotide polymorphism; FTD = frontotemporal dementia.