**Supplementary Material**

***C9orf72* hexanucleotide repeat allele tagging SNPs:**

**Associations with ALS risk and longevity**

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**SUPPLEMENTARY METHODS**

**Genotyping quality Control**

Genotyping data quality control was performed separately on samples processed with different arrays. First, we removed duplicated samples and related samples (proportion IBD > 0.125), samples with discordant sex information, outlying heterozygosity rate (>4SD) and > 5% missing genotype rate. In per-variant quality-control, we included variants that had a genotyping rate of > 95%, variants in HWE (p>0.000001) and minor allele count >= 3. To harmonize genotyping array data, we included only biallelic non-palindromic SNPs and set allele coding to match the reference genome so that A1 allele was always the alternative allele regardless of minor allele frequency.

**Data availability**

C9orf72 repeat lengths and genotypes used in the case-control analysis can be accessed at <https://github.com/kkaivola/C9orf72_repeat_length_genotype_data/> .

Accessing Finngen data requires permission from Finngen <https://www.finngen.fi/en> .

**FinnGen Ethics statement and materials & methods**

**Release 9**

Patients and control subjects in FinnGen provided informed consent for biobank research, based on the Finnish Biobank Act. Alternatively, separate research cohorts, collected prior the Finnish Biobank Act came into effect (in September 2013) and start of FinnGen (August 2017), were collected based on study-specific consents and later transferred to the Finnish biobanks after approval by Fimea (Finnish Medicines Agency), the National Supervisory Authority for Welfare and Health. Recruitment protocols followed the biobank protocols approved by Fimea. The Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS) statement number for the FinnGen study is Nr HUS/990/2017.

The FinnGen study is approved by Finnish Institute for Health and Welfare (permit numbers: THL/2031/6.02.00/2017, THL/1101/5.05.00/2017, THL/341/6.02.00/2018, THL/2222/6.02.00/2018, THL/283/6.02.00/2019, THL/1721/5.05.00/2019 and THL/1524/5.05.00/2020), Digital and population data service agency (permit numbers: VRK43431/2017-3, VRK/6909/2018-3, VRK/4415/2019-3), the Social Insurance Institution (permit numbers: KELA 58/522/2017, KELA 131/522/2018, KELA 70/522/2019, KELA 98/522/2019, KELA 134/522/2019, KELA 138/522/2019, KELA 2/522/2020, KELA 16/522/2020), Findata permit numbers THL/2364/14.02/2020, THL/4055/14.06.00/2020,,THL/3433/14.06.00/2020, THL/4432/14.06/2020, THL/5189/14.06/2020, THL/5894/14.06.00/2020, THL/6619/14.06.00/2020, THL/209/14.06.00/2021, THL/688/14.06.00/2021, THL/1284/14.06.00/2021, THL/1965/14.06.00/2021, THL/5546/14.02.00/2020, THL/2658/14.06.00/2021, THL/4235/14.06.00/202, Statistics Finland (permit numbers: TK-53-1041-17 and TK/143/07.03.00/2020 (earlier TK-53-90-20) TK/1735/07.03.00/2021, TK/3112/07.03.00/2021) and Finnish Registry for Kidney Diseases permission/extract from the meeting minutes on 4th July 2019.

The Biobank Access Decisions for FinnGen samples and data utilized in FinnGen Data Freeze 9 include: THL Biobank BB2017\_55, BB2017\_111, BB2018\_19, BB\_2018\_34, BB\_2018\_67, BB2018\_71, BB2019\_7, BB2019\_8, BB2019\_26, BB2020\_1, Finnish Red Cross Blood Service Biobank 7.12.2017, Helsinki Biobank HUS/359/2017, HUS/248/2020, Auria Biobank AB17-5154 and amendment #1 (August 17 2020), AB20-5926 and amendment #1 (April 23 2020) and it´s modification (Sep 22 2021), Biobank Borealis of Northern Finland\_2017\_1013, Biobank of Eastern Finland 1186/2018 and amendment 22 § /2020, Finnish Clinical Biobank Tampere MH0004 and amendments (21.02.2020 & 06.10.2020), Central Finland Biobank 1-2017, and Terveystalo Biobank STB 2018001 and amendment 25th Aug 2020.

**Release 10**

Patients and control subjects in FinnGen provided informed consent for biobank research, based on the Finnish Biobank Act. Alternatively, separate research cohorts, collected prior the Finnish Biobank Act came into effect (in September 2013) and start of FinnGen (August 2017), were collected based on study-specific consents and later transferred to the Finnish biobanks after approval by Fimea (Finnish Medicines Agency), the National Supervisory Authority for Welfare and Health. Recruitment protocols followed the biobank protocols approved by Fimea. The Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS) statement number for the FinnGen study is Nr HUS/990/2017.

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The Biobank Access Decisions for FinnGen samples and data utilized in FinnGen Data Freeze 10 include: THL Biobank BB2017\_55, BB2017\_111, BB2018\_19, BB\_2018\_34, BB\_2018\_67, BB2018\_71, BB2019\_7, BB2019\_8, BB2019\_26, BB2020\_1, BB2021\_65, Finnish Red Cross Blood Service Biobank 7.12.2017, Helsinki Biobank HUS/359/2017, HUS/248/2020, HUS/150/2022 § 12, §13, §14, §15, §16, §17, §18, and §23, Auria Biobank AB17-5154 and amendment #1 (August 17 2020) and amendments BB\_2021-0140, BB\_2021-0156 (August 26 2021, Feb 2 2022), BB\_2021-0169, BB\_2021-0179, BB\_2021-0161, AB20-5926 and amendment #1 (April 23 2020)and it´s modification (Sep 22 2021), Biobank Borealis of Northern Finland\_2017\_1013, 2021\_5010, 2021\_5018, 2021\_5015, 2021\_5023, 2021\_5017, 2022\_6001, Biobank of Eastern Finland 1186/2018 and amendment 22 § /2020, 53§/2021, 13§/2022, 14§/2022, 15§/2022, Finnish Clinical Biobank Tampere MH0004 and amendments (21.02.2020 & 06.10.2020), §8/2021, §9/2022, §10/2022, §12/2022, §20/2022, §21/2022, §22/2022, §23/2022, Central Finland Biobank 1-2017, and Terveystalo Biobank STB 2018001 and amendment 25th Aug 2020, Finnish Hematological Registry and Clinical Biobank decision 18th June 2021, Arctic biobank P0844: ARC\_2021\_1001.

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**SUPPLEMENTARY TABLES AND FIGURES**

**Supplementary Table 1. Summary of ALS case-control analysis results (HRE carriers excluded), comparison of tagging SNP and intermediate-length allele analyses**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SNP (present study) | 504 cases n (%) | 3190 controls n (%) | p | OR | 95 % CI |
| rs2814707\*T carrier | 180 (35.7%) | 1002 (31.4%) | 0.057 | 1.21 | 0.99-1.48 |
| rs139185008\*C carrier | 30 (6.0 %) | 91 (2.9%) | 0.0010 | 2.15 | 1.36-3.33 |
| rs2814707\*T homozygous | 25 (5.0%) | 88 (2.8%) | 0.012 | 1.84 | 1.17-2.90 |
| rs2814707\*T homozygous rs139185008\*C carrier | 11 (2.2%) | 14 (0.4%) | 0.00020 | 5.06 | 2.06-12.07 |
| rs2814707\*T homozygous no rs139185008\*C | 14 (2.8%) | 74 (2.3%) | 0.53 | 1.20 | 0.62-2.17 |
| Intermediate-length alleles(from ref. 16) | **525 cases****n (%)** | **3950 controls****n (%)** |  |  |  |
| ≥7 carrier | 185 (35.2%) | 1293 (32.7%) | 0.26 | 1.12 | 0.92–1.36 |
| 17–45 carrier | 19 (3.6%) | 101 (2.6%) | 0.15 | 1.43 | 0.82–2.74 |
| 21–45 carrier | 12 (2.3%) | 66 (1.7%) | 0.29 | 1.38 | 0.67–2.59 |
| ≥7/≥7 | 26 (5.0%) | 104 (2.6%) | 0.005 | 1.93 | 1.19–3.02 |
| ≥7/17–45 | 7 (1.3%) | 10 (0.25%) | 0.002 | 5.32 | 1.71–15.56 |
| ≥7/21–45 | 6 (1.1%) | 3 (0.076%) | 0.00016 | 15.19 | 3.23–94.21 |
| 7–16/7–16 | 19 (3.6%) | 94 (2.4%) | 0.098 | 1.57 | 0.90– 2.62 |

HRE=Hexanucleotide repeat expansion. rs139185008\*C carriers among ALS cases (n=30) include 15 cases without any intermediate-length alleles, these samples may have hidden non-genotyped HREs or SNP imputation errors.

**Supplementary Table 2. Demographic details of final study cohorts**

|  |  |  |
| --- | --- | --- |
|   | **ALS** | **Controls** |
| **N** | 683\* | 3196\* |
| **Male (%)** | 327 (49,38%) | 1568 (49.1%) |
| **Mean age** | 63,4 | 75.2 |
| **Median age** | 63 | 75 |
| **Age of onset - mean** | 58,3 | - |
| **Age of onset - median** | 59 | - |
| **Site of onset** |   | - |
| **Bulbar (%)** | 225 (33,99 %) | - |
| **Lower (%)** | 240 (36,25 %) | - |
| **Upper (%)** | 162 (24,47 %) | - |
| **Limb (%)** | 10 (1,51 %) | - |
| **Resp (%)** | 9 (1,36 %) | - |
| **NA (%)** | 14 (2,27 %) | - |

\*Exact demographic data missing in 23 ALS cases and 6 controls and were excluded in demographic detail calculations

**Supplementary Table 3. Genotype concordance between AmplideX C9orf72 kit and RP-PCR.**

|  |  |  |  |
| --- | --- | --- | --- |
| Sample |  | Amplidex genotype (manually checked genotypes when genotype missing or mismatching) | RP-PCR genotype |
| A14LIALS101 |  | 2, >145 | 2/exp |
| A14LIALS15 |  | 8, 8 (8/10) | 8/10 |
| A14LIALS169 |  | 2, 2 (2/exp) | 2/exp |
| A14LIALS187 |  | 8, 35 | 8/36 |
| A14LIALS236 |  | 2, 19 | 2/19 |
| A14LIALS26 |  | 8, 22 | 8/22 |
| A14LIALS272 |  | 2, 36 | 2/37 |
| A14LIALS279 |  | 2, 11 | 2/11 |
| A14LIALS283 |  | 2, 19 | 2/19 |
| A14LIALS285 |  | 2, 12 (2/12) | 12/12\* |
| A14LIALS300 |  | 2, >145 | 2/exp |
| A14LIALS301 |  | 2, >145 | 2/exp |
| A14LIALS303 |  | 2, >145 | 2/exp |
| A14LIALS312 |  | 2, 2 (2/exp) | 2/exp |
| A14LIALS391 |  | 5, 15 | 5/15 |
| A14LIALS405 |  | 2, 14 | 2/14 |
| A14LIALS45 |  | (2/36) | 2/37 |
| A14LIALS452 |  | 8, 22, 41  | 8/22\* |
| A14LIALS58 |  | 5, >145 | 5/exp |
| A14LIALS66 |  | (2/exp) | 2/exp |
| N19ALS172 |  | 2, >145 | 2/exp |
| N19ALS176 |  | 5, >145 | 5/exp |
| N19ALS187 |  | 2, >145 | 2/exp |
| N19ALS20 |  | 7, 16, 34 (7/16) | 7/16 |
| N19ALS225 |  | 2, >145 | 2/exp |
| N19ALS254 |  | (2/exp) | 2/exp |
| N19ALS326 |  | 2, >145 | 2/exp |
| N19ALS337 |  | 10, 18 | 10/18 |
| N19ALS35 |  | 2, 22 | 2/22 |
| N19ALS43 |  | 5, 23 | 5/23 |
| N19ALS53 |  | 8, 10 | 8/10 |
| S14F13ALS19 |  | 7, 24 | 7/24 |
| S14F13ALS37 |  | 2, 2, >145 (2/145) | 2/exp |
| S14F13ALS45 |  | 2, >145 | 2/exp |
| S14F13ALS54 |  | 8, >145 | 8/exp |
| S14F13ALS62 |  | 8, 8 (8/13) | 8/13 |
| S14F13ALS77 |  | 2, 45 (2/42-48) | 2/exp |
| S14F13ALS79 |  | 5, >145 | 5/exp |
| S14F13ALS8 |  | 8, >145 | 8/exp |
| S14F13ALS80 |  | 2, >145 | 2/exp |

\*Genotype confirmed with over-the-repeat PCR

**Supplementary Figure 1. Statistical power of SNP regression analyses in FinnGen.**

(A) Total sample number needed for 80 % power with different effect sizes (odds ratios) for rs139185008 heterozygosity analysis (MAF 1.3 %, dominant model, binary outcome, case rate 3%). (B) Total sample number needed for 80 % power with different effect sizes (odds ratios) for rs2814707 homozygosity analysis (MAF 17 %, recessive model, binary outcome). Sample size was calculated with genpwr R package.

**A**



**B**

