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# *C9orf72* Repeat Expansion Discordance in 6 Multigenerational Kindreds

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## Abstract

### Background and Objectives

A hexanucleotide repeat expansion in the noncoding region of the *C9orf72* gene is the most common genetically identifiable cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia in populations of European ancestry. Pedigrees associated with this expansion exhibit phenotypic heterogeneity and incomplete disease penetrance, the basis of which is poorly understood. Relatives of those carrying the *C9orf72* repeat expansion exhibit a characteristic cognitive endophenotype independent of carrier status. To examine whether additional shared genetic or environmental risks within kindreds could compel this observation, we have conducted a detailed cross-sectional study of the inheritance within multigenerational Irish kindreds carrying the *C9orf72* repeat expansion.

### Methods

One hundred thirty-one familial ALS pedigrees, 59 of which carried the *C9orf72* repeat expansion (45.0% [95% CI 36.7–53.5]), were identified through the Irish population-based ALS register. *C9orf72* genotyping was performed using repeat-primed PCR with amplicon fragment length analysis. Pedigrees were further investigated using SNP, targeted sequencing data, whole-exome sequencing, and whole-genome sequencing.

### Results

We identified 21 kindreds where at least 1 family member with ALS carried the *C9orf72* repeat expansion and from whom DNA was available from multiple affected family members. Of these, 6 kindreds (28.6% [95% CI 11.8–48.3]) exhibited discordant segregation. The *C9orf72* haplotype was studied in 2 families and was found to segregate with the *C9orf72*-positive affected relative but not the *C9orf72*-negative affected relative. No other ALS pathogenic variants were identified within these discordant kindreds.

### Discussion

Family members of kindreds associated with the *C9orf72* repeat expansion may carry an increased risk of developing ALS independent of their observed carrier status. This has implications for assessment and counseling of asymptomatic individuals regarding their genetic risk.

## Introduction

Amyotrophic lateral sclerosis (ALS) is a complex genetic disorder in which a familial pattern of inheritance is present in up to 15% of cases.[1] Four major genes are associated with ALS, namely *SOD1*, *TARDBP*, *FUS*, and *C9orf72* in populations of European extraction. Of these, the G4C2

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## Glossary

**ALS** = amyotrophic lateral sclerosis; **FTD** = frontotemporal dementia; **IBD** = identity by descent; **WES** = whole-exome sequencing; **WGS** = whole-genome sequencing.

hexanucleotide repeat expansion in the noncoding region of the *C9orf72* gene is the most common genetically identifiable cause of ALS and frontotemporal dementia (FTD) in populations of European ancestry.[2]

Effective genomic therapies have been developed for *SOD1*-associated disease[3] and are in clinical trial for other variants including those associated with *C9orf72*. Accordingly, and to better characterize disease onset, presymptomatic cohorts have been established for gene carriers of *SOD1* and *C9orf72* variants. Kindred studies have established that the phenotypic expression of *C9orf72* repeat expansion is more complex than that of *SOD1* and includes a wide range of manifestations including ALS, FTD, chorea, and neuropsychiatric symptoms.[4] Moreover, population studies using the multistep model of ALS show that while the presence of the *C9orf72* repeat expansion reduces the number of steps required for disease development,[5] other factors also contribute to the clinical presentation of disease.

We have previously shown that asymptomatic first-degree relatives of probands carrying a *C9orf72* repeat expansion exhibit a characteristic cognitive endophenotype that segregates from controls.[6] This endophenotype is independent of carrier status and suggests that family members of those carrying the *C9orf72* repeat expansion may also carry additional genetic or shared environmental risks. To further explore this hypothesis, we have conducted a detailed cross-sectional study of the inheritance within multigenerational Irish kindreds carrying the *C9orf72* repeat expansion.

## Methods

### Data Collection

Data from the population-based Irish ALS register were examined to identify all patients with familial ALS diagnosed between January 1, 1994, and December 31, 2021.[1] Enrollment on the register is possible for only those confirmed to have possible, probable, or definite ALS according to El Escorial criteria.[7] Where multiple kindred members were identified, extensive pedigrees comprising first-degree, second-degree, and greater-degree relatives were generated. Data were cross-referenced with the Irish ALS DNA biobank, and those individuals and kindreds who carried the *C9orf72* repeat expansion were identified. Where DNA samples were available for more than 1 affected family member, inheritance patterns were examined. Data on kindred size, phenotype, age at symptom onset, and survival from onset were obtained from the Irish ALS register. Where available, cognitive assessment data for patients with ALS and asymptomatic relatives were also examined.

### Genomic Analysis

#### *C9orf72* Genotyping

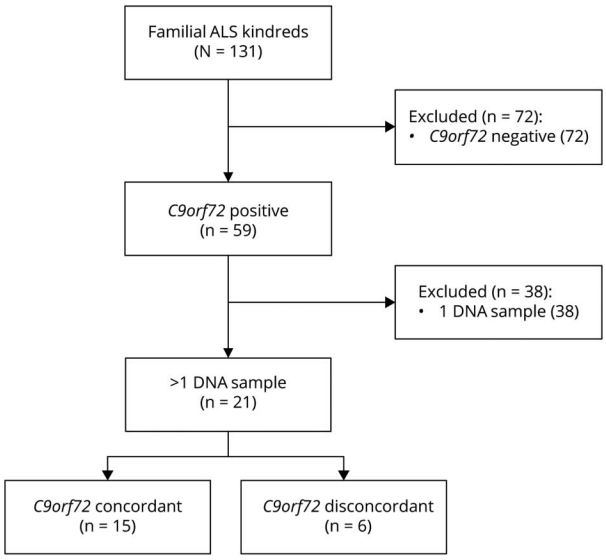
*C9orf72* genotyping of Irish patients with ALS has been performed for Irish ALS patients since 2011. All DNA samples are tested for the repeat expansion using repeat-primed PCR (rpPCR) with amplicon fragment length analysis[8,9] and results confirmed with repeat testing in house. This methodology has previously been validated with positive and negative controls using Southern blotting.[8] Amplified fragments are measured by capillary electrophoresis on an Applied Biosystems 3500 Series Genetic Analyzer and visualized using Gene Mapper v4.0. Patients with 30 hexanucleotide repeats or above were deemed positive for the expansion. Most Irish ALS DNA samples deemed negative for the *C9orf72* repeat expansion carried less than 10 repeats, with the most frequently observed repeat length of 1–2 units.[10] Where paired-end, PCR-free, whole-genome sequencing (WGS) data were available, the presence or absence of the repeat expansion was further confirmed using ExpansionHunter v2 and ExpansionHunter v3.[11] Finally, genotypes from *C9orf72* discordant pedigrees were confirmed by blinded external analysis with a different assay in King's College London.[12]

#### Pedigree Analysis

Pedigrees were investigated using all available genotyping data (SNP, targeted sequencing data, whole-exome sequencing [WES], and WGS). Where SNP genotyping data were available, familial relatedness was confirmed using identity by descent (IBD). IBD matrices were calculated by first filtering to common SNPs (MAF >0.35), removing SNPs that were absent in more than 5% of samples, and removing SNPs that were significantly out of Hardy-Weinberg equilibrium (HWE) in controls. SNPs sharing linkage were pruned by removing SNPs within a 50-bp range of a lead SNP with an $R^2$ exceeding 0.2. SNP genotyping data from 5 Irish cohorts (eTable 1, links.lww.com/NXG/A659) were analyzed to study the haplotype surrounding the *C9orf72* repeat expansion in families with discordant repeat expansion genotyping. Each data set was phased with Beagle v4.1[13] using 1,000 Genomes Project Phase 3 reference data.[14] Plink v1.9[15] was used to perform data quality control separately for each data set.

Paired-end, PCR-free WGS data were generated and processed as previously described.[16] WES was performed to a target depth of 90X on an Illumina NovaSeq following Agilent SureSelect enrichment. Targeted sequencing data were available for a previously described cohort.[17] WES and targeted sequencing data were processed from FASTQ to variant calling following the GATK best practices pipeline.[18] Where data were available, participants were screened for exonic and

**Figure 1** Flowchart of Irish ALS Kindreds Carrying the *C9orf72* Repeat Expansion




splice-site variants in the exons of 37 genes linked to ALS (*ALS2, ANG, ATXN2, C21orf2, CHCHD10, CHMP2B, DAO, DCTN1, ELP3, ERBB4, ERLIN1, ERLIN2, FIG4, FUS, hnRNPA1, KIF5A, LMNB1, MATR3, NEFH, NEK1, OPTN, PARK7, PFN1, PRPH, SETX, SIGMAR1, SOD1, SPAST, SPG11, SQSTM1, TAF15, TARDBP, TBK1, UBQLN2, UNC13A, VAPB,* and *VCP*).[19] The pathogenicity of putative variants was assessed in accordance with the American College of Medical Genetics (ACMG) guidelines.[20]

## Statistical Analysis

We calculated the rate of *C9orf72*-negative ALS among first-degree relatives of *C9orf72* kindreds where DNA samples were available for more than 1 affected family member. Kindred size was determined based on number of first-degree relatives for most recently diagnosed proband. In 1 *C9orf72* pedigree, where kindred size was not available, data were imputed (mean kindred size of 11). The probabilities of a given number of relatives developing ALS within a specified kindred size (inclusive of first-degree relatives only) were calculated using a binomial distribution model[21] and assuming a lifetime risk of ALS in the general population of 1 in 385 people.[22] Pedigrees were plotted using CeGAT pedigree chart designer.[23] Where data ascertainment was incomplete for individual cases, missing data were excluded from individual analyses, but the cases were retained in the data set. Unless otherwise stated, confidence intervals are calculated using Bayesian posterior priors. Unless otherwise stated, statistical analysis was conducted in R v3.6 1.

## Standard Protocol Approvals, Registrations, and Patient Consents

Informed written consent for this study was obtained from all study participants. Ethical approval for this project was granted by Beaumont Hospital Ethics Medical Research Committee (REC reference 15/40). A data protection impact assessment was completed and approved by the Data Protection Officer in Beaumont Hospital.

## Data Availability

Data are not publicly available to preserve participant anonymity.

# Results

Of 131 families with ALS in which DNA samples were available for analysis, 59 (45.0% [95% CI 36.7–53.5]) had at least 1 relative with ALS who carried a pathogenic *C9orf72* repeat expansion. In 21 families in which at least 1 patient carried the *C9orf72* repeat expansion, DNA samples were available for more than 1 affected family member. Of these 21 kindreds, 15 demonstrated cosegregation of the *C9orf72* repeat expansion with disease (Figure 1). However, 6 kindreds (28.6% [95% CI: 11.8–48.3]), comprising 28 individuals with ALS exhibited discordant segregation (i.e., some relatives with ALS carried the pathogenic expansion while other relatives with ALS did not). Of these discordant kindreds, 2 were discordant parent-offspring ALS pairs, 2 included discordant ALS siblings, and 2 families comprised discordant cousins (Table). There were no statistically significant differences between gene carriers and noncarriers in terms of age at onset or clinical phenotype.

The rate of *C9orf72*-negative ALS among first-degree relatives from the 21 *C9orf72* kindreds where DNA samples were available for more than 1 affected family member was calculated. Because only first-degree relatives were included in kindred size determination, 2 *C9orf72*-negative individuals from discordant kindreds were not included in the nominator. The denominator included a total of 222 relatives (*C9orf72* discordant [78], *C9orf72* concordant [144]) giving a rate of *C9orf72*-negative ALS among relatives from *C9orf72* kindreds of 1.8% (95% CI: 0.5–4.6).

## Additional Genomic Analysis

Sufficient data were available to perform further genetic analysis for 3 large families only, as detailed further. Data regarding the 3 remaining *C9orf72* discordant kindreds are available in eFigure 1 (links.lww.com/NXG/A658).

### Pedigree A

Pedigree A includes 6 siblings who developed ALS or FTD or both, of a sibship of 12 (Figure 2A). Two siblings with ALS were confirmed carriers of the *C9orf72* repeat expansion, while their sibling who developed ALSFTD did not carry the expansion. During writing, 4 other siblings are still alive, aged older than 65 years, and asymptomatic. These include 1 *C9orf72* carrier and 1 *C9orf72* noncarrier. The *C9orf72*-positive and *C9orf72*-negative relatives scored in the abnormal and normal ranges on the total Edinburgh Cognitive and Behavioral ALS Screen (ECAS) score, respectively.

**Table** Composition of *C9orf72* Discordant ALS Kindreds

| Kindred | No. of FDR with ALS | Kindred size[a] | p[+] | Reg ID | *C9orf72* status | Phenotype | Age at onset (y) | Survival from onset (mo) | Relationship |
|---|---|---|---|---|---|---|---|---|---|
| **A** | 5 | 19 | 0.00000000132553 | II.1 | Positive | ALS | 47 | 51 | Sibling |
| | | | | II.4 | Positive | ALS-FTD | 65 | 16 | Sibling |
| | | | | II.11 | Negative | ALS-FTD | 59 | 79 | Sibling |
| **B** | 4 | 14 | 0.00000004439112 | III.1 | Positive | ALS | 57 | 41 | Sibling |
| | | | | III.4 | Positive | ALS-ci | 60 | 32 | Sibling. Parent of IV.1 |
| | | | | IV.1 | Negative | ALS | 31 | 53 | Child of III.4 |
| **C** | 2 | 10 | 0.00029734114785 | IV.1 | Positive | ALS-ci | 62 | 18 | Sibling |
| | | | | IV.2 | Positive | ALS-ci | 74 | 20 | Sibling |
| | | | | V.3 | Negative | ALS | 54 | 16 | Cousin |
| **D** | 4 | 15 | 0.00000006037612 | II.1 | Positive | ALS-FTD | 65 | 25 | Sibling |
| | | | | II.6 | Positive | ALS | 60 | 42 | Sibling |
| | | | | II.8 | Negative | ALS-FTD | 67 | 23 | Sibling |
| **E** | 2 | 7 | 0.00013984608411 | IV.6 | Positive | ALS | 43 | 36 | Sibling |
| | | | | IV.7 | Positive | ALS | 60 | 18 | Sibling |
| | | | | IV.2 | Negative | ALS | 68 | 18 | Cousin |
| **F** | 6 | 13 | 0.00000000000052 | II.6 | Positive | ALS-FTD | 64 | 66 | Sibling. Child of I.1 |
| | | | | I.1 | Negative | ALS-FTD | n/a | n/a | Parent of II.6 |

Abbreviations: ALS = amyotrophic lateral sclerosis; ci = cognitive impairment (Strong criteria); FDR = first-degree relative; FTD = frontotemporal dementia; n/a = not available.
[a] Inclusive of first-degree relatives only. + Probability of the exact specified number of first-degree relatives developing ALS within the specified kindred size (inclusive of first-degree relatives only).

WGS was available for the *C9orf72*-negative ALSFTD sibling (II.11). ExpansionHunter v2 and v3 provided further confirmation that the negative patient is heterozygous for 2 and 5 GGGGCC repeat motifs.

SNP genotyping was available for 1 *C9orf72*-positive ALS sibling (II.1) and the negative ALSFTD sibling (II.11). Sibling relatedness was confirmed (pi-hat = 0.5383), verifying both that the negative sibling is truly related to the family and that the result is not attributable to a laboratory or clerical error. SNP genotyping confirms that the positive ALS sibling carries the elongated *C9orf72* haplotype (Figure 3). The negative ALSFTD sample is homozygous for the nonrisk allele at 2 critical SNPs (rs3849942 and rs10812605).

Targeted NGS was available for the 2 *C9orf72*-positive ALS siblings, and WGS was available for the *C9orf72*-negative ALSFTD sibling. The only putative variant observed in the negative patient (II.11) was *ATXN2:c.224A>G(p.[D75G])*. This variant is predicted to be benign by in silico tools, and to date, only expanded and intermediate CAG repeat expansion in *ATXN2* have been linked to ALS pathogenesis.[24,25] *ATXN2* was not included in the ALS-targeted NGS panel, so could not be confirmed in the 2 positive siblings.

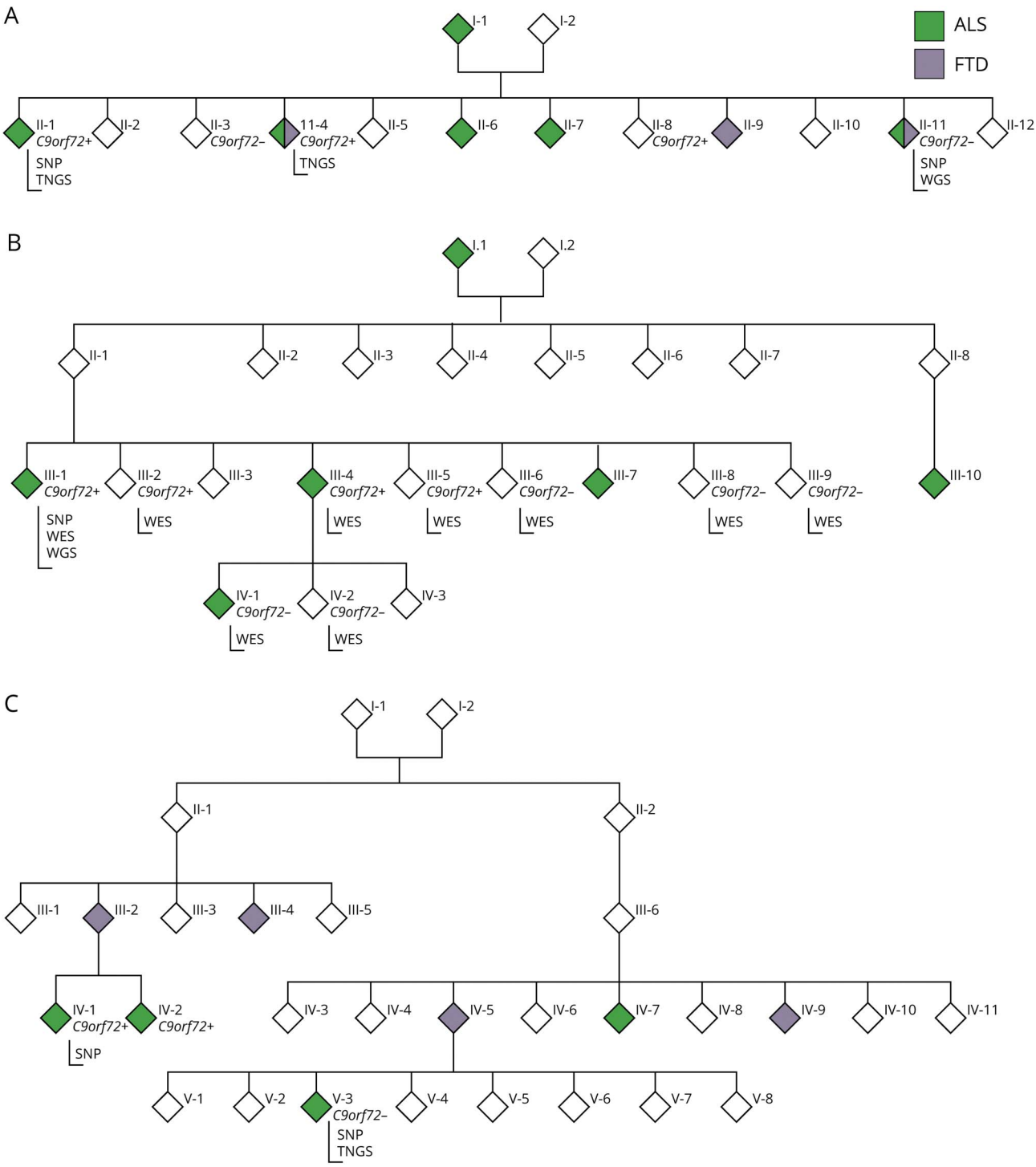
### Pedigree B

Pedigree B is a 4-generation kindred with 6 recorded cases of ALS (Figure 2B). Two siblings with ALS were confirmed carriers of the *C9orf72* repeat expansion. Notably, the offspring of one of these siblings developed ALS despite not carrying the pathogenic expansion. Five siblings from generation 3 remain alive and asymptomatic at an age older than 60 years. Two of these carry the pathogenic repeat expansion, while 3 others do not. One *C9orf72*-positive relative scored in the abnormal range on total ECAS score, while 2 *C9orf72*-negative relatives had normal total ECAS scores. No cognitive assessment data were available on the remaining relatives.

WES was performed for multiple members of this extended pedigree. The expected relatedness percentages were confirmed with the *C9orf72*-negative ALS patient (IV.1) having 50% relatedness to their affected *C9orf72*-positive parent (III.4).

In patient III.1, the presence of the *C9orf72* repeat expansion was confirmed with ExpansionHunter v2 and v3 with allele predictions of 2/238 and 2/99, respectively. In addition, individual III.1 also carries *KIF5A:c.2953G>A(p.[G985S]*). This variant is predicted to be benign by in silico tools and crucially is also absent in the *C9orf72*-negative patient (IV.1) and their

**Figure 2** *C9orf72* Discordant Families




(A) refers to Pedigree A, (B) to Pedigree B and (C) to Pedigree C. *C9orf72+* indicates a carrier of the repeat expansion as confirmed with rpPCR in 2 independent laboratories. *C9orf72-* indicates the individual does not carry the pathogenic expansion. SNP indicates that there is SNP genotyping available. TNGS indicates that there is targeted sequencing data available. WES indicates that there is whole-exome sequencing data available. WGS indicates that there is whole-genome sequencing data available.

affected parent (III.4), so is unlikely to be contributing to the observed discordance.

### Pedigree C

Pedigree C is a 4-generation kindred that includes 4 individuals with ALS and 4 individuals with FTD (Figure 2C). Two siblings with ALS are confirmed to carry the *C9orf72* repeat expansion, while their cousin with ALS (fifth-degree relative) does not carry the repeat expansion. SNP genotyping for an affected patient (IV.1) and the distant cousin (V.3) confirms that the *C9orf72*-negative patient either did not inherit the haplotype or that recombination occurred in the inherited

**Figure 3** *C9orf72* Haplotype Analysis for Pedigrees A and C

| | | | | | | Pedigree: | A | | | | | C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP_ID | Allele | Finnish_haplotype | European_haplotype | UK_haplotype | Swedish_haplotype | II.I_haplotype_a | II.I_haplotype_b | II.XI_haplotype_a | II.XI_haplotype_b | II.XI_WGS_alleles | II.XI_WGS_depth | IV.I_haplotype_a | IV.I_haplotype_b | V.III_haplotype_a | V.III_haplotype_b |
| rs10511816 | G | A | A | | A | | | C | C | G/G | 44 | C | A | C | C |
| rs10967952 | T | | | | T | T | T | C | T | T/C | 18/21 | T | T | T | T |
| rs1444533 | T | A | | A | A | T | T | | | T/T | 40 | | | | |
| rs1822723 | C | C | | C | C | C | C | | | C/T | 25/17 | C | C | C | C |
| rs10967958 | C | | | | C | C | C | C | C | C/C | 45 | | | | |
| rs4879515 | T | T | T | T | T | T | T | T | C | C/T | 21/15 | | | | |
| rs10967959 | C | | | | C | C | C | | | C/T | 14/17 | | | | |
| rs12350089 | T | | | | T | G | T | T | T | T/T | 45 | | | | |
| rs895023 | A | T | | T | T | A | A | A | A | A/A | 25 | A | A | A | A |
| rs2440622 | T | T | | T | T | T | T | | | T/T | 39 | | | | |
| rs1977661 | C | C | | C | C | C | C | A | C | C/A | 27/22 | C | C | C | C |
| rs2166128 | C | | | | C | C | C | C | C | C/C | 40 | C | C | C | C |
| rs10812605 | C | | C | | C | T | C | T | T | T/T | 37 | T | C | T | T |
| rs11792285 | C | | | | C | C | C | C | T | C/T | 16/21 | T | C | T | T |
| rs13290599 | G | | | | G | | | G | G | G.G | 43 | G | G | G | G |
| rs3849942 | T | T | T | T | T | C | T | C | C | C/C | 50 | C | T | C | C |
| rs10967976 | G | | | | G | G | G | | | G/A | 14/21 | | | | |
| rs10122902 | G | G | | G | G | A | G | G | G | G/G | 36 | G | G | G | G |
| rs10757665 | T | T | | T | T | T | T | | | T/C | 23/27 | | | | |
| rs774359 | C | C | C | C | C | T | C | | | T/C | 17/16 | T | C | T | T |
| rs2282241 | C | C | | C | C | A | C | C | C | C/C | 53 | C | C | C | A |
| *C9orf72* RE | | | | | | | | | | | | | | | |
| rs1948522 | C | C | | C | C | T | C | C | C | C/C | 41 | C | C | C | C |
| rs1982915 | G | G | | G | G | G | G | G | A | A/G | 27/16 | A | G | A | A |
| rs12002175 | G | | | | G | G | G | G | G | G/G | 42 | G | G | G | G |
| rs7868845 | T | | | T/C | T/C | T | T | T | T | T/T | 41 | C | C | C | C |
| rs10757670 | T | | | | T | T | T | T | T | T/T | 39 | | | | |
| rs2453556 | G | G | | G | G | | | G | G | G/G | 32 | A | G | A | A |
| rs702231 | A | A | | A | A | | | | | C/A | 13/17 | | | | |
| rs696826 | G | G | | G | G | A | G | | | A/G | 19/22 | | | | |
| rs2477518 | T | | | T/C | T/C | C | T | T | C | T/C | 14/12 | C | C | T | T |

The yellow highlight indicates that the 2 positive samples carry the established elongated *C9orf72* haplotype. The red highlight indicates 2 loci where the *C9orf72*-negative patients in pedigrees A and C are homozygous for the nonrisk allele indicating that they either did not inherit the haplotype or recombination occurred in the inherited haplotype. Both whole-genome sequencing (WGS) and SNP genotyping were available for individual II.XI in pedigree A. The presence of heterozygous genotypes eliminates the possibility that the observed haplotype is attributable to a large deletion in the region.

haplotype (Figure 3). Relatedness is observed to be 3.5%, which is at the background level of the population but is not unexpected for distant cousins. Targeted sequencing was available for the *C9orf72*-negative cousin (V.3), and no putative variants were observed.

## Discussion

We have detected nonsegregation of disease with the *C9orf72* expansion in 28.6% of Irish kindreds from which DNA of more than 2 affected relatives was available. The 6 kindreds in which *C9orf72* discordance was observed had an average of 4 or more relatives with ALS, rendering it unlikely that such familial clustering occurred by chance alone. In all pedigrees with sufficient genetic data, familial relationships were verified. *C9orf72* repeat expansion carrier status was verified in all patients from the discordant families through blinded reanalysis, both in-house and in a second laboratory, using different methodologic approaches. The convergence of these results suggests that the finding of frequent discordant inheritance in *C9orf72* kindreds is a true observation.

We have estimated that 1.8% of individuals from *C9orf72* kindreds will develop ALS without exhibiting the repeat expansion. While this estimate is limited by the small numbers of patients and relatives studied and lack of genetic data on all relatives from these *C9orf72* kindreds, it is, nonetheless, noteworthy that this estimate mirrors our previously published data that the lifetime risk of developing ALS in first-degree relatives of individuals with ALS whose genetic status is unknown is 1.4%.[22] Together, these

findings suggest that being a first-degree relative from a *C9orf72*-positive kindred is in itself a risk factor of ALS, even if the individual under consideration does not carry the *C9orf72* repeat expansion and exceeds the expected background population risk of ALS (lifetime risk, 0.3%).[22]

Multiple variants in ALS-associated genes have been previously been found among *C9orf72* kindreds, consistent with an oligogenic model of ALS.[26] However, analysis of targeted NGS, WES, and WGS did not reveal any other pathogenic variants among our *C9orf72* discordant kindreds. The observation of isolated probably benign variants most likely reflects the background variant carrier rate.[27]

Haplotype analysis was performed in *C9orf72*-positive and *C9orf72*-negative patients where SNP data were available. The *C9orf72* repeat expansion is widely linked to a "Finnish" haplotype,[28,29] which has been identified in association with the repeat expansion among all Irish patients with ALS to date.[8] Cosegregation of the previously described Finnish risk haplotype with the repeat expansion among carriers and its absence in apparent noncarriers support true absence in all tissues rather than failure of detection of the variant. However, where the Finnish haplotype is not observed, we cannot fully exclude the possibility that both *C9orf72*-negative samples with available SNP genotyping may have inherited a recombined version of the *C9orf72* haplotype because previous analysis has shown that approximately 2% of cases carry the nonrisk alleles at these loci. We consider this unlikely because the discordant inheritance was also observed in those carrying the more common Finnish haplotype.

A possible explanation for failure of cosegregation is somatic instability of the *C9orf72* repeat expansion. *C9orf72* expansion length may vary considerably between relatives and between tissues within individuals,[30] at times demonstrating markedly increased expansion lengths in neural compared with non-neural tissues.[31,32] It is possible that a patient carries a repeat expansion within the neuroaxis, which is derived from the ectoderm during embryogenesis, but not in their blood, which is derived from the mesoderm. However, the absence of the Finnish haplotype suggests that this is not a plausible explanation, although as noted, we cannot entirely eliminate the possibility that these individuals have inherited a shortened, recombined version of the haplotype.

At present, no nonblood-derived DNA samples from individuals in our discordant kindreds are available for analysis. A future study measuring repeat expansion length in both patient's blood and in tissue deriving from the same germ layer as motor neurons (e.g., epithelial sample) could definitively determine whether somatic mosaicism results underestimate the true frequency of the *C9orf72* repeat expansion.

However, if these patients are indeed exhibiting somatic mosaicism, the finding implies that measuring repeat expansion in DNA extracted from blood is inaccurate, which has immediate implications both for symptomatic and asymptomatic genetic testing in ALS, as well as important inferences for research (e.g., recruitment for clinical trials).

An alternative explanation is that kindreds carrying the *C9orf72* repeat expansion may carry an additional genetic burden that increases the risk of developing the disease, independent of the presence of the expansion. This hypothesis is consistent with our observation that cognitive endophenotypes within these kindreds do not segregate with the presence of the expansion and our previous observation of increased disease penetrance with maternal transmission of disease.[22]

Familial clustering of ALS may be due to genetic or environmental factors or a combination of both. While in kindreds with a known pathogenic variant, clustering of disease will often be attributed to the variant, an understanding is evolving that *C9orf72* expansions may not be pathogenic in isolation. Additional genetic or environmental insults may be required to initiate disease or modify its phenotypic presentation. In this study, we have endeavored to comprehensively assess potential genetic modifiers within *C9orf72* kindreds. However, the presence of environmental variables associated with risk of ALS were not directly assessed in *C9orf72* kindreds during this study, and further studies will be required to address this question.

Approximately one-third of Irish *C9orf72* kindreds demonstrate incomplete cosegregation of the repeat expansion with ALS. These observations have implications for assessment and advice regarding genetic risk in asymptomatic individuals from kindreds carrying the *C9orf72* repeat expansion. Our findings emphasize the importance of testing all affected family members for pathogenic variants. The findings also support our conjecture that family members from kindreds carrying the *C9orf72* repeat expansion are not suitable as controls in clinical studies and that longitudinal cohort studies should enroll both gene-positive and gene-negative family members as study participants.

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## Disclosure

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