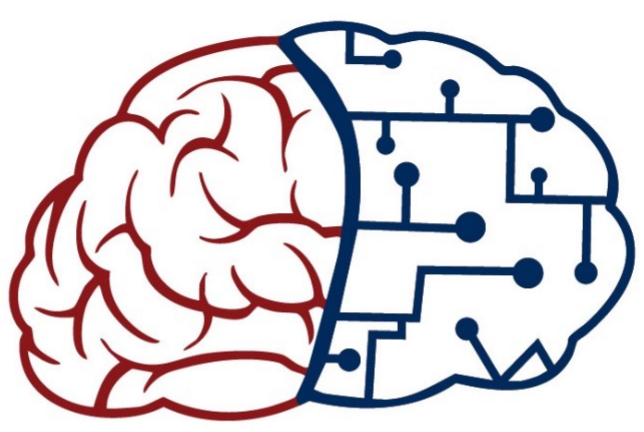




BACKGROUND

Pathogenic hexanucleotide *C9orf72* repeat expansions (C9+), the most common genetic cause of frontotemporal degeneration (FTD) and amyotrophic lateral sclerosis (ALS), are not completely penetrant [1].

A previously-reported *C9orf72* penetrance score (*K*-score) aims to estimate familial disease risk [2]. However, *K*-scores have not been investigated in independent cohorts. The *K*-score ranges from 0-1, with 1 expected to reflect complete penetrance.

$$K = \frac{A}{A + \sum (C * p)_n}$$

Number of family members affected by FTD/ALS
Estimated relatedness between family member <i>n</i> and C9+ proband 0.5 for first-degree relatives; 0.25 for second-degree relatives
Age- and sex-related proportion of risk survived for family member <i>n</i> [3] 0 for affected individuals, 1 for unaffected individuals >80 years old

Moreover, prior work suggests that epigenetic C9 promoter methylation (C9pm) ≥10% is a neuroprotective modifier of pathological burden, age at death, disease duration, and neurodegeneration in C9+ individuals [4,5,6]. C9pm values tend to be concordant within families and not tissue-specific, suggesting that additional genetic variation may contribute to this disease modifier.

Hypotheses:

- C9+ FTD/ALS families with concordantly high C9pm have lower *K*-scores (expected to reflect incomplete penetrance).
- Rare genetic variants related to high C9pm can be identified in C9+ FTD/ALS families with discordant C9pm.

Importance: Further understanding the genetic modifiers of *C9orf72* penetrance and clinico-pathological heterogeneity.

Higher *K*-scores relate to: the number of affected and unaffected family members

K-scores are higher with more affected contributors ($r=0.694, p<0.001$, Figure 3A).
K-scores are lower with more unaffected contributors ($r=-0.585, p<0.001$, Figure 3B).
This relationship holds as a ratio (Figure 3C).

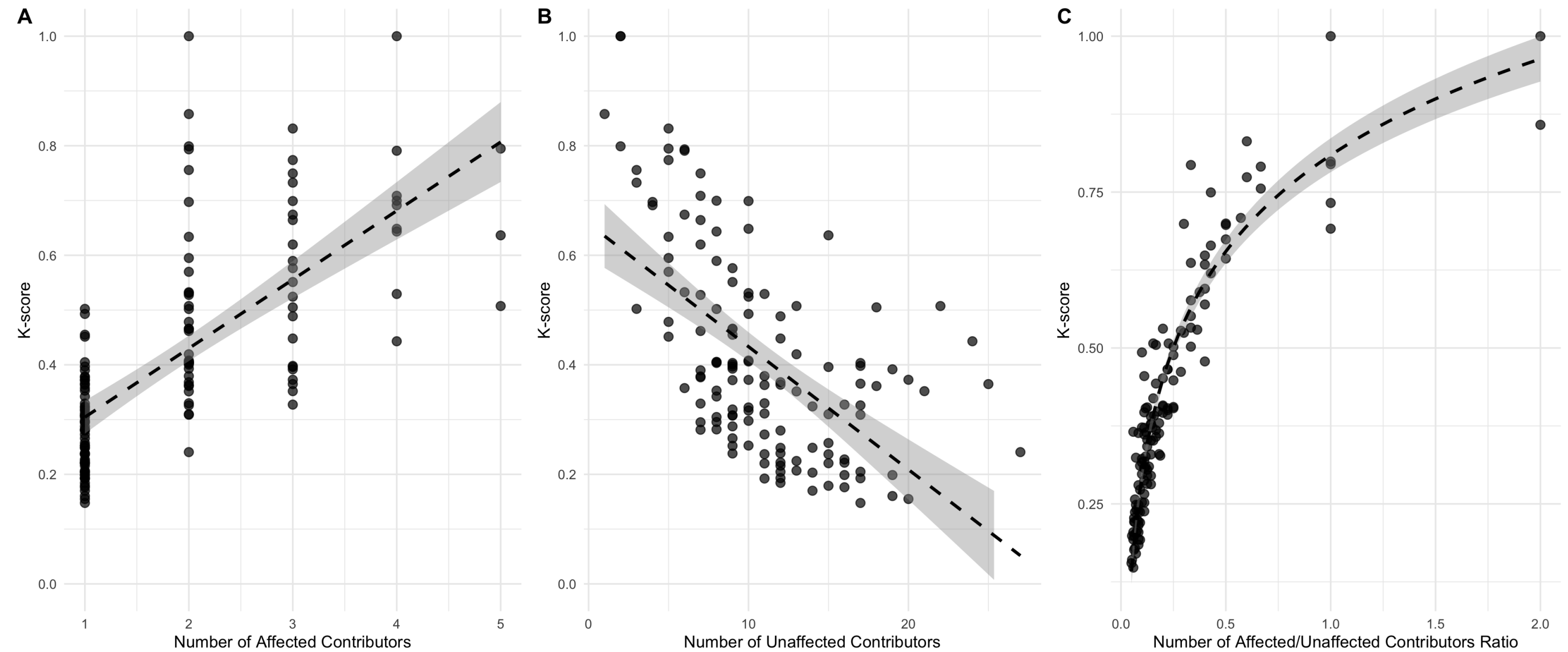


Figure 3. Comparison of factors influencing *K*-scores in UNICORN.

Higher *K*-scores for: families in “high” category of Wood Pedigree Classification

Families with “High” Wood Pedigree Classification have higher *K*-scores (Figure 4), however, there is still variability: some families with low *K*-scores, and some families with high *K*-scores. 12 families with concordantly high C9pm have *K*-scores ranging from 0.31-0.83.

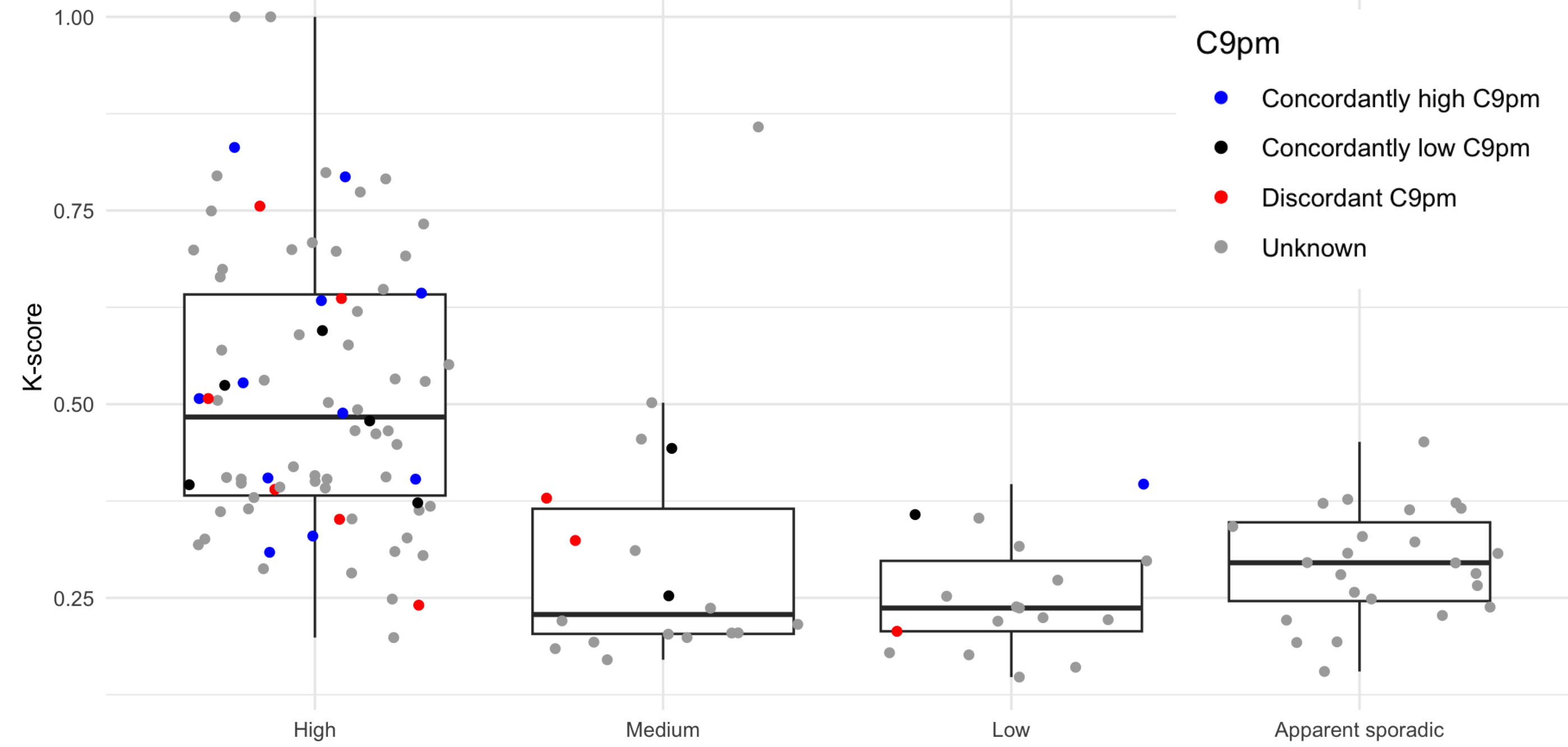


Figure 4. Comparison of familial *K*-scores by Wood Pedigree Classification [8].

METHODS

C9orf72 FTD/ALS Families

Calculated *K*-scores for 137 C9+ families in the University of Pennsylvania Centralized Observational Research Repository on Neurodegenerative Disease (UNICORN) study

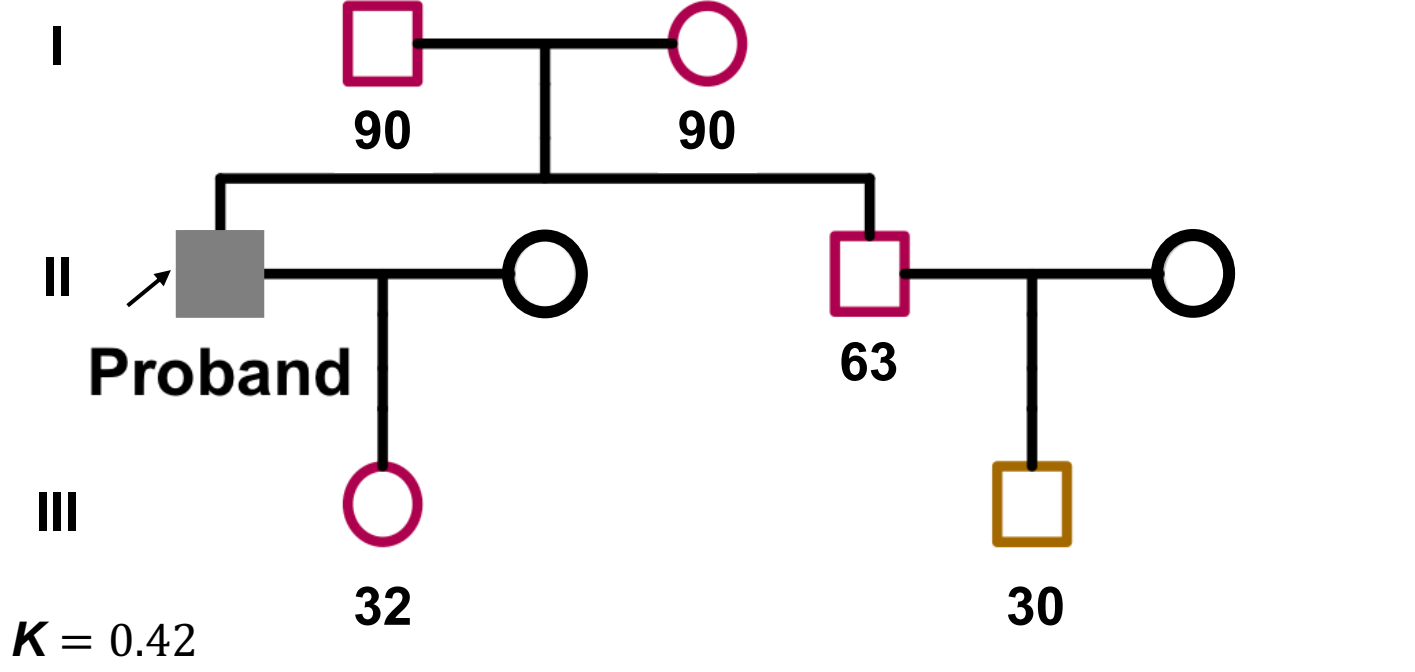


Figure 1. Example family and *K*-score. Squares=males, circles=females, with age (years) underneath. Proband is C9+. Maroon outline=first-degree relative; gold outline=second-degree relative. Grey fill=affected by FTD/ALS.

C9orf72 Promoter Methylation

Obtained Illumina Methylation Screening Array data for C9+ individuals from 29 families (N=2-5 individuals per family)

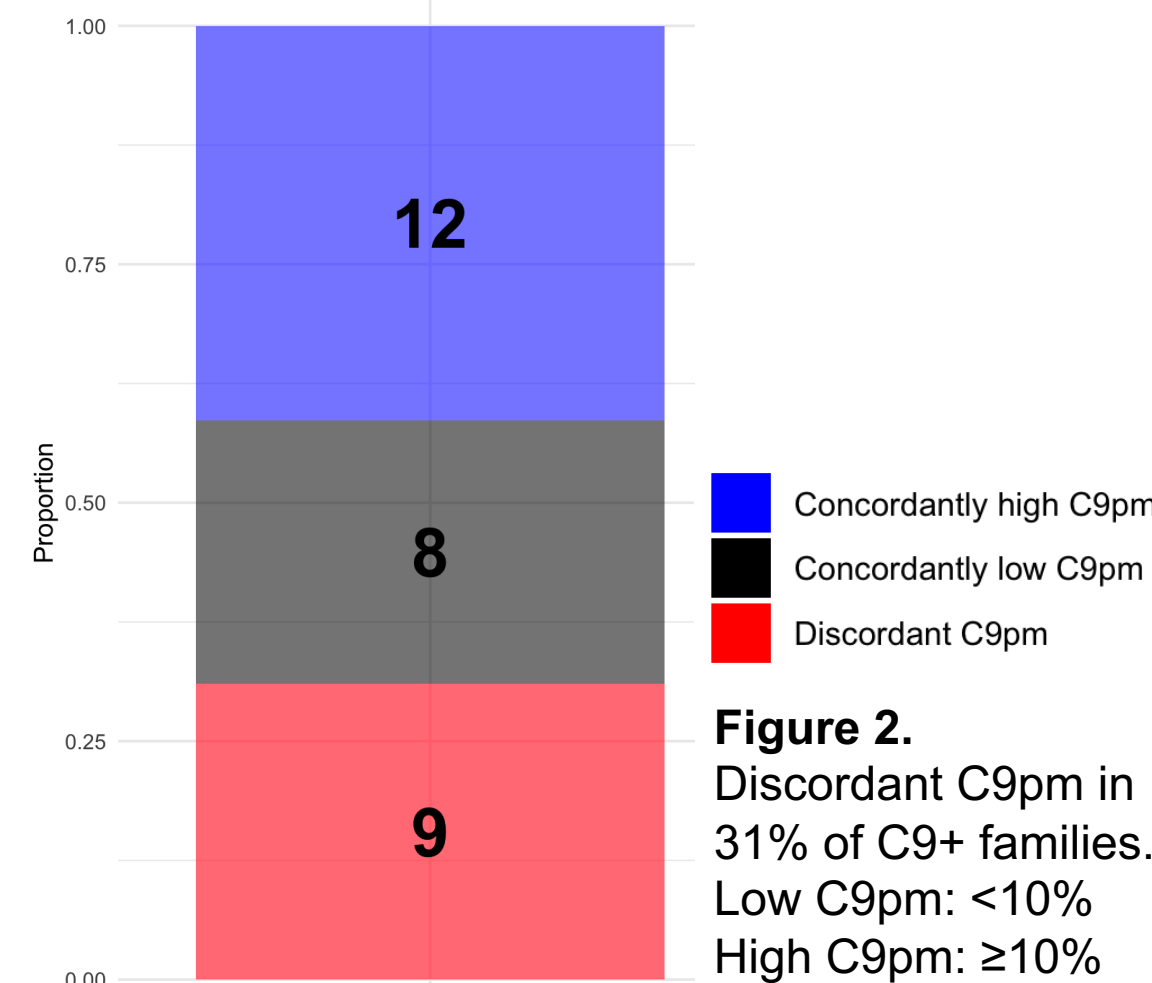


Figure 2. Discordant C9pm in 31% of C9+ families. Low C9pm: <10% High C9pm: ≥10%

Rare Genetic Variants

Obtained genome sequencing data for 11 families (3 with discordant C9pm)

In families with discordant C9pm, screened for rare genetic variants present in C9+ family members with high C9pm:

- Ran Exomiser [7] with human phenotype ontology terms for FTD (HP:0002145) and ALS (HP:0007354)
- Filtered to keep variants with:
 - Frequency ≤0.01% in population databases
 - Genotype quality ≥99

Rare genetic variants identified in C9+ family members with discordant C9pm

Across 3 C9+ families with discordant C9pm, we identified a total of 149 genetic variants related to high C9pm. We highlight variants from each family (Figure 5).

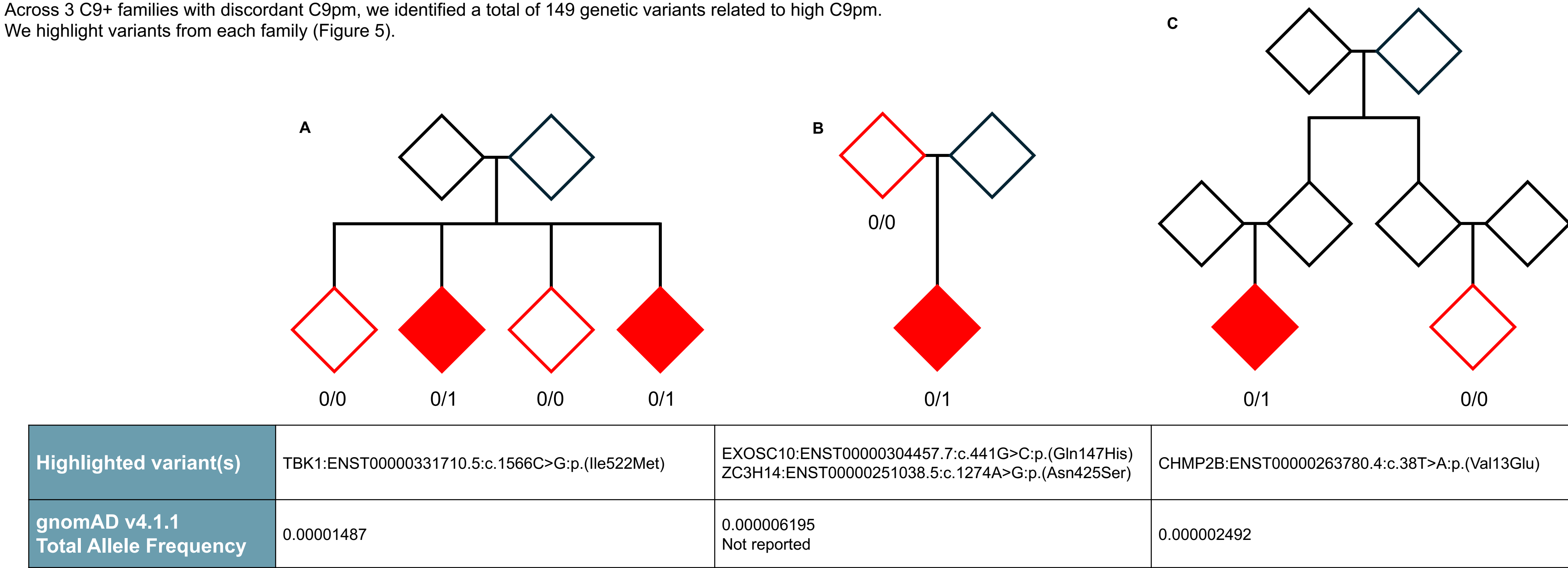


Figure 5. (A) 13 variants met our criteria including a heterozygous *TBK1* variant in individuals with high C9pm; this variant was absent in the C9+ siblings with low C9pm and was confirmed by Sanger sequencing. DNA was not available for the parents. (B) 42 variants met our criteria including heterozygous variants in *EXOSC10*, and *ZC3H14/MSUT2* in the child with high C9pm; these variants were absent in the C9+ parent with low C9pm. DNA was not available for the presumed C9- parent. (C) 94 variants met our criteria including a heterozygous *CHMP2B* variant in the individual with high C9pm; this variant was absent in the C9+ cousin with low C9pm. DNA was not available for older generations.

Red outline = C9+ individual with low C9pm; Red fill = C9+ individuals with high C9pm; Black outline = no DNA available to test for C9 status or C9pm.

CONCLUSIONS

- We observed that, as expected, higher *K*-scores relate to a higher ratio of affected/unaffected contributors. Not all families with concordantly high C9pm had lower *K*-scores, perhaps highlighting the difference between genomic modifiers of C9+ clinico-pathological heterogeneity (C9pm) and *C9orf72* penetrance (affected/unaffected status).
 - Future work will evaluate if these familial scores provide a risk/susceptibility biomarker on an individual basis and to identify the genetic and environmental modifiers of *C9orf72* penetrance.
- We studied C9+ families with discordant C9pm and identified rare genetic variants related to high C9pm, highlighting genes involved in autophagy, rRNA processing in the nucleolus and cytosol and the endolysosomal system. These cellular functions are maintained by multiple parallel and tightly regulated pathways; it is important to determine how altered activity in one component of these networks may modify C9pm. This analysis provides a template for understanding genetic modifiers of heterogeneity in C9+ disease.

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