C9orf72 Repeat Expansion: A Primer for Those Impacted

Genetics Basics

We all have billions of cells that make up our body. In each of those cells, we carry an instruction manual—called DNA— that guides our cells on how to function properly. Imagine that our DNA contains the instructions for building a house, where each chapter of the manual is a chromosome, and each step in a chapter is a gene. When someone has a pathogenic variant (also known as a mutation), that gene might cause the house to eventually fail. This phenomenon explains why, in genetic adult-onset conditions, we might be born with a well-functioning house, but as we get older and use the house more, it might eventually fail us, because it was not built right from the start.

C9orf72

C9orf72 is an acronym for chromosome 9 open reading frame seventy-two. It is named for a region on the ninth chromosome. This region of the ninth chromosome is present in every individual. It becomes abnormal/pathogenic when there is an increased amount of genetic material in that region, known in this case as a hexanucleotide repeat. You can think of these repeats as a "control + paste" function on a computer that copies and pastes a word over and over again. When it runs off the page, it is considered to be pathogenic, causing instability in the region. The *C9orf72* repeats can range from 2 to over 4,000[1-10]. Normal repeat sizes range between 2 and 24 units[11,12]. There is debate in the research community and between genetic testing labs on what the risk of disease is for those with 25-29 repeats, but generally speaking, the presence of 30 or more repeats is considered an increased risk for developing frontotemporal dementia (FTD) or amyotrophic lateral sclerosis (ALS). The presence of the *C9orf72* repeat expansions was first discovered in 2011.

Research has shown that the presence of the C9orf72 repeat expansion causes:

1. Less of the normal protein coded by *C9orf72*; this is known in genetic diseases as a loss of function.
2. Additional messenger RNA (mRNA) created by the mutation that are not present in those without the mutation.
3. These additional mRNA create proteins that are not present in people without the mutation. Numbers 2 and 3s are known in genetic diseases as a gain of function. It is speculated that these abnormal proteins eventually lead to the death of nerve cells, but exactly how these changes lead to ALS or FTD has not yet been determined[13].

Inheritance

The *C9orf72* repeat expansion is inherited in an autosomal dominant manner, meaning that the mutation needs only to be present on one chromosome to create disease. Autosomal dominant

mutations cause disease in successive generations, and as such, any child of a parent with the expansion has a 50% chance of inheriting the expansion. In nearly every case that has been documented, there is evidence that the mutation was inherited from a parent, and any possible children of the carrier would have a risk of inheriting the gene. This has held true even when the carrier does not report a family history of disease.

Penetrance

The proportion of people with a genetic mutation who develop the signs and symptoms of the disease associated with it is known as the gene's penetrance. Even if one inherits the *C9orf72* repeat expansion, it does not appear to be fully penetrant; meaning that not all people who carry the C9orf72 expansion will develop signs and symptoms of ALS or FTD in their lifetime. The first medical literature that attempted to calculate *C9orf72* penetrance was published in 2017 and stated that penetrance approaches 100% by age 83[14]. However, the authors speculated that 90% penetrance was possible given there are known cases of *C9orf72* carriers dying at old age without a diagnosis of ALS or FTD. A more recent study using a different calculation was published, but estimated the penetrance for ALS alone[15]. There are some compelling observations that would make it unlikely that the penetrance of *C9orf72* is as high as 90%. It has been documented that a large number of *C9orf72* carriers diagnosed with ALS or FTD do not report any family history of disease; i.e. they have sporadic cases[16]. Others have discovered higher than expected numbers of *C9orf72* repeat expansion carriers in broad population screens, which would further support a lower penetrance[17]. Despite these observations, no further attempts at arriving at a penetrance estimate have been reported in the medical literature. Unfortunately, the full understanding of penetrance of C9orf72 has not been decided and it may not be settled for some time. It should be noted that many genes associated with adult-onset diseases, like the hereditary breast and ovarian cancer genes *BRCA 1/2,* also do not have complete (100%) penetrance, however the presence of these genes are still understood to be serious risks.

2024 Update: In late 2023 a study[24] looking at families of C9orf72 ALS patients in the Netherlands estimated that broadly the risk for ALS alone was about 30% for most people in families with C9orf72 expansions. They also found some families with so much disease that the risk of ALS alone must be higher, as much as 60%. They did not attempt to see the rate of FTD in the families studied. In 2024 a geneticist at Oxford published a paper[25] sharing a calculation for family-specific penetrance for C9orf72, with a strong family history bestowing a higher penetrance, and low or no family history of disease bestowing a low risk. Authors of both of these papers shared their observations in End the Legacy webinars, which are available here.

Age of Onset/Diagnosis

The *C9orf72* repeat expansion causes disease in an age-dependent manner, meaning that disease occurs more often when age increases; cases are diagnosed in adulthood, most often diagnosed in

late-middle age or even old age in some. Two large studies of thousands of *C9orf72* ALS or FTD cases arrived at an average age of diagnosis for a carrier of 58 years old[18,19]. They both show a bell curve pattern of diagnosis: about half of cases are diagnosed approximately between the ages 50 and 65 with a fewer number of cases diagnosed earlier (rarely as young as in the 20's) and later (carriers over 90 have been diagnosed). It is important to note that the average age of diagnosis is made up of those who are diagnosed with the disease. It does not capture the people who are not diagnosed in their lifetime. As disease manifests in an age-dependent manner, there are many more asymptomatic carriers at any one time than there are carriers with an active diagnosis.

Biomarkers

A biomarker is a term referring to biological measurements that provide information on a disease course. In some cases, biomarkers may be able to signal disease activity before there are serious symptoms. Some promising biomarkers in *C9orf72* are:

- NfL(neurofilament light chain): A marker of neuronal damage that can be found in both spinal fluid or in blood. Complicating NfL is the fact that levels increase across the lifespan in everyone, but in *C9orf72* it has been found that NfL increases faster over time, and the absolute measurements are higher than in unaffected individuals even 40 years before symptom onset[21]. NfL tests have recently become commercially available in the United States via LabCorp.

- DPRs (dipeptide repeat proteins): Products produced by the *C9orf72* repeat expansion, and not by people without the expansion. The role of these DPRs (also sometimes referred to as RAN proteins) in the disease process is currently unknown, and could be pathological or incidental[22]. Researchers have found these DPRs in spinal fluid, however these tests are not available commercially and are only being tested in a research setting.

- Brain MRI (magnetic resonance imaging of the brain): Studies have repeatedly shown that *C9orf72* repeat carriers have less estimated brain volume on MRI than their unaffected siblings as early as young adulthood and up to 40 years before diagnosis[21]. MRI machines are available in most major medical centers, but when used for clinical care, they tend to have high costs for patients to access them and these data have only been evaluated in a research setting.

- TDP-43 Biomarkers: TDP-43 is a protein that is found in all cells. TDP-43 aggregates in brain and spinal cord are present on autopsy in over 90% of ALS cases and about 50% of FTD cases[23]. These aggregates are also present in *C9orf72* ALS and FTD. There are currently no validated biomarkers for measuring TDP-43 aggregates in living people. Recently, attention has been paid to the possibility that the loss of full function of TDP-43 is part of the disease process and may precede TDP-43 aggregation. A new biomarker of

this loss of function has been discovered in the CSF of presymptomatic *C9orf72* carriers, but this study has not yet undergone peer review.

- ETL Webinar on the topic

Research

Many people would like to study *C9orf72* repeat expansion carriers both pre-symptomatically and those with diagnosed disease!

A list of studies looking to observe us (and provide access to free and often anonymous genetic counseling and time with genetic ALS and FTD experts) is here. *Please note that, while we make every effort to be sure the status of clinical trials and studies is accurate and up-to-date, we cannot make guarantees. You will need to go to the websites directly in order to be informed of all current studies and trials.

There are no interventional trials recruiting presymptomatic C9orf72 expansion carriers at this time.

For anyone with the C9 mutation diagnosed with ALS or FTD there is one gene-targeted trial testing the use of the drug Metformin out of the University of Florida.

C9orf72 carriers who have been diagnosed with ALS may also be eligible for the many drug trials for ALS patients, though these drug trials do not target *C9orf72* specifically.

There are no general FTD drug trials recruiting *C9orf72* FTD patients at this time.

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