REVIEW

# Fungal-contaminated grass and well water and sporadic amyotrophic lateral sclerosis

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## Abstract

Fungi are important infectious disease-causing agents, but are often overlooked as environmental factors in disease. We review several lines of evidence that point to a potential fungal origin of sporadic amyotrophic lateral sclerosis (ALS), the most common form of motor neurone disease. Approximately 90% cases of ALS are sporadic, and the aetiology of sporadic ALS is still unknown. We have previously postulated that grass or soil-associated fungal infections may be a leading cause of sporadic ALS. Herein we extend this proposal to water-associated fungi. A wide variety of fungi have been reported in drinking water including *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma*. Some of these are known to produce neurotoxic mycotoxins. Despite this, drinking water is not routinely monitored for fungal contamination. Fungal contamination could explain the close correlation between distribution of well water and cases of sporadic ALS in the United States. We propose several mechanisms by which an opportunistic fungal infection from environmental exposure (to water, soil or plants) can lead to long term neuronal degradation resulting in the hallmarks of ALS. If confirmed, the association between fungal infection and sporadic ALS could lead to novel treatment strategies for this progressive and fatal disease.

***Key Words:*** *amyotrophic lateral sclerosis; fungi; motor neurone disease; mycotoxins; neurotoxins; ALS; well water; sporadic ALS*

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## What Causes Sporadic Amyotrophic Lateral Sclerosis?

Amyotrophic lateral sclerosis (ALS) is the most common motor neurone disease (MND) in adults and is characterized by degeneration of motor neurons in the brain and spinal cord. ALS affects the upper and lower motor neurons. In its early stages, upper motor neuron degeneration generally causes muscle tightness and cramps, while lower motor neuron degeneration causes muscle weakness, muscle atrophy and persistent muscle twitching. As the disease progresses, most voluntary muscles become paralysed, and speech and breathing become very difficult. Mobility is extremely limited, and help is needed in caring for most personal needs. The incidence of ALS worldwide is around 2 cases per 100,000 persons annually, with the prevalence being approximately 5 cases per 100,000. The average age of onset is currently 58–60 years and the average survival from diagnosis to death is 2–4 years. Only 5–10% of patients survive beyond 10 years.

Sporadic ALS accounts for the overwhelming majority (> 90%) of cases, whilst < 10% are familial ALS, with a defined genetic basis. Sporadic ALS is suspected to be caused by one or more environmental factors. There have been many candidates suggested for these environmental factors, including viruses, bacteria, biotoxins, minerals, heavy metals, pesticides, physical damage through occupational or sporting hazards, military service and even body mass index (Bozzoni et al., 2016). Both incidence and prevalence of ALS are greater in men than in women. Reasons for this are thought to include different exposures to environmental toxins, different biological responses to exogenous toxins, and possibly underlying differences between the male and female nervous systems and different abilities to repair damage (McCombe and Henderson, 2010).

We propose that an opportunistic neurotoxic fungal infection as a result of prolonged contact with plant or ground water that becomes a chronic systemic infection could be the cause for many sporadic ALS cases. Evidence for this hypothesis comes from a variety of sources. We have performed an extensive PubMed literature search of articles from 1990 to 2019 using keywords including fungi, mycotoxins, neurotoxins and ALS, as well as sporadic ALS and environmental agents.

## What Neurotoxins Are Found in Plant-Associated Fungi?

Mycotoxins are toxic secondary metabolites of diverse structure that are produced by a variety of fungi commonly found in the environment that are capable of causing disease and death in humans and other animals (Bennett and Klich, 2003). Mycotoxins can be classified into a variety of types, including aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids. Many of these (in particular, macrocyclic trichothecenes, alkaloids, fumonisin B and ochratoxin A) are known to induce neurotoxicity.

## What Neurodegenerative Diseases Are Fungal Neurotoxins Known to Cause?

A degenerative neurological condition, ryegrass staggers, affects horses, cows and other grass-feeding animals. The symptoms include muscle tremors, stiffness, twitching, tetany and ataxia. It is caused by infection of perennial ryegrass pastures with the endophytic fungi *Neotyphodium (N.) lolii* that produces indole-based alkaloid tremorgenic neurotoxins, predominantly lolitrem B (di Menna et al., 2012). *N. lolii* forms a symbiotic relationship with ryegrass and produces very high amounts of fungal superoxide dismutase 1 (SOD1). Feeding camels straw infected with lolitrem B toxin for 8 weeks resulted in neurological deficits (ataxia) and systemic effects not only on the brain but also on the liver and kidneys (Alabdouli et al., 2014).

Other neurologic disorders in animals are also known to be caused by a variety of grass or plant-associated endophytic fungi.

Leukoencephalomalacia in horses is caused by ingesting fumonisin B, which is produced by *Aspergillus niger* and *Fusarium moniliforme*, common endophytes on many crops, including corn, rice, oats, wheat, maize, barley and soybeans. Symptoms include ataxia, tremor, twitching, circling, inability to swallow and death (Nielsen et al., 2009; Vendruscolo et al., 2016).

Ochratoxin A (OTA) is a mycotoxin produced by secondary metabolism of many filamentous species of *Aspergillus* and *Penicillium* (el Khoury and Atoui, 2010). OTA has complex mechanisms of action including stimulation of oxidative stress, inhibition of protein synthesis, and production of single-strand breaks in DNA. Oxidative DNA damage is significantly increased in the brain following exposure to OTA, and has been implicated in Parkinson's disease (Sava et al., 2006a, b).

In a recent paper we reviewed several lines of evidence that strongly point to an association between environmental fungal infection and ALS (French et al., 2018). We proposed that the fungi that may be responsible for inducing ALS are those that are associated with grass and some other plants and produce neurotoxic mycotoxins. These fungi can infect patients, most likely initially through inhalation and then passing into the blood stream where they overcome the immune system and thus persist long term at a chronic subclinical level in motor neurons. Another possibility is that colonization of neurons could arise from ingestion of fungi. Gut microbiota can pass through the gastrointestinal mucosa and reach the blood stream. Subsequently, the fungi would be disseminated to other organs and tissues of the human body, including the nervous system (Alonso et al., 2018).

It is interesting to note that the most common and potent immunosuppressive drugs derive from fungal toxins (*e.g.*, cyclosporine A and antamides). Thus a mixed fungal infection could suppress the immune system, allowing other, neurotoxic fungi to chronically infect the nervous system. Support for involvement of plant-associated fungi in the aetiology of ALS comes from cases of the disease in clusters of athletes (especially soccer players) who play sport on grass (*e.g.*, Chio et al., 2009), and from epidemiology that points to an increased incidence in agricultural and regional communities, with a high incidence among farmers (amongst other occupations). Direct evidence comes from two clinical studies in Spain showing the presence of a range of fungi in the central nervous system of ALS patients, but not in brains from patients without the disease (Alonso et al., 2015, 2017). They were unable to identify a common fungal species between patients, possibly indicating that a variety of fungi can induce symptoms of sporadic ALS, or that a mixed fungal infection may be more toxic. However, infected plants may not be the only source of environmental fungal exposure that leads to the development of ALS. Fungi may also be found in well water and other water sources, along with cyanobacteria. Cyanobacteria are found in algal blooms in a variety of freshwater, and marine habitats and produce several toxins that have adverse effects on human health. One of them is beta-N-methyl-amino-L-alanine, a neurotoxic toxin (Cox et al., 2018). Beta-N-methyl-amino-L-alanine has been found to be concentrated in the brains of both ALS and Alzheimer's disease patients, but not in most controls (Pablo et al., 2009).

## How Does the Strong Association Between Well Water Usage in the United States and Mortality From Amyotrophic Lateral Sclerosis Fit With a Fungal Origin?

Two studies reported that in the United States, mortality from MND was significantly associated with well water use, both at the state level ($P = 0.022$; Schwartz and Klug, 2016a, b) and at the county level ($P < 0.0001$; Schwartz et al., 2017). (They analysed mortality rates for MND as a surrogate for ALS because ALS does not have a unique International Statistical Classification of Diseases and Related Health Problems (10$^{th}$ Revision) – ICD-10 – code.)

Well water use is obviously significantly associated with living in rural areas, which, if the water is a cause of MND, could explain why ALS is higher in rural and regional areas. This (rural association) was one line of evidence we used to implicate fungi in the disease process (French et al., 2018). In the original study (Schwartz and Klug, 2016b), the initial culprit was suspected to be residential radon exposure, as radon had been implicated in an earlier UK study. However, when state records were examined, radon was not found to be correlated with MND mortality (although it was found to be correlated with chronic lymphocytic leukaemia in a separate study from the same group – Schwartz and Klug, 2016a). Instead, a strong correlation with well water as a source of drinking water was observed. This was not the first report incriminating drinking water as a cause of sporadic ALS. An Italian study reported that inhabitants in rural Italy who consumed drinking water with high levels (≥ 1 μg/L) of inorganic

selenium had an increased risk of ALS (Vinceti et al., 2010). The high selenium content water came from well water, and the possible correlation with selenium generally has not been confirmed. Schwartz and Klug (2016b) did not identify the agent in well water that potentially induced ALS, but they proposed that it could be *Legionella* bacteria.

In a follow-up study, the researchers more closely examined the data of well water use and rates of age-adjusted mortality from MND at the United States county level (Schwartz et al., 2017). They examined data from 923 United States counties and found a significant correlation between mortality and the prevalence of well water in those counties ($P < 0.0001$). Furthermore, foci, or 'hot spots' of MND mortality were significantly associated with 'hot spots' of well water use ($P < 0.0005$). Once again, they hypothesized that the association of MND mortality rates with well water use reflected contamination of wells with *Legionella*. However, we would propose that an alternative explanation is that well water is known to be frequently contaminated with fungi, some of which can produce neurotoxic mycotoxins. It is also possible that *Legionella* plus fungi act additively or even synergistically to effect neuronal degradation.

## What Is Known of Fungal Contamination of Drinking Water?

The presence of a wide variety of fungi has been reported in water intended for human consumption, including surface, ground and tap water (Al-Gabr et al., 2014). Common filamentous fungi detected in drinking water include *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma* (Babič et al., 2017). As noted above, some of these genera produce mycotoxins that are neurotoxic. Despite this, drinking water is not routinely monitored for fungal contamination. The standard microbial drinking water test organisms (*Escherichia coli*, *Enterococci*, *Clostridia*) are not indicative of fungal contamination. Fungi and their mycotoxins, as residues in water, may be aerosolized when showering or inhaled when being sprayed for various purposes such as crop irrigation.

## What Is the Mechanism by Which Chronic Fungal Infection Can Induce Amyotrophic Lateral Sclerosis?

Fungal infections are usually chronic and progressive if undetected and untreated. It is known that neurons respond to some fungal neurotoxins by increasing glutamate production *in vivo* (Bradford et al., 1990). Excessive glutamate secretion from neurons is one of the hallmarks of ALS and has been linked to the destruction of motor neurons as a result of excessive activation of glutamate receptors (Foran and Trotti, 2009). Glutamate also significantly increases the toxicity of mutant Cu/Zn-SOD1 in familial MND. SOD1 mutations may act synergistically with environmental mycotoxins to enhance glutamate secretion inducing motor neurotoxicity. This links both sporadic and familial cases of ALS. Fungal SOD1 aids survival of fungi within macrophages, providing resistance of pathogenic fungi to the oxidative stresses induced by the human immune system (Cox et al., 2003). This may help to maintain long-term subclinical infections leading to long-term, chronic release of the mycotoxin and gradual motor neuron degeneration. Evidence for a delayed manifestation of symptoms comes from studies of military servicemen involved in Operation Desert Storm. It has been suggested that the reported excess incidence of ALS manifesting years later in these veterans was due to a weaponised mycotoxin. Sava et al., 2006 concluded that, "the potential for delayed neurotoxic effects of low doses of mycotoxins should not be overlooked".

There are only a few reports of the effects of mycotoxins on neuronal tissues *in vivo*. Macrocyclic trichothecenes, fumonisin $B_1$ and OTA are known to have the potential to induce neurotoxicity in rodent models (Cox et al., 2003). Macrocyclic trichothecenes induce neuronal apoptosis and inflammation in the olfactory system. Fumonisin $B_1$ induces neuronal degeneration in the cerebral cortex. The acute effects of OTA include DNA damage in most mouse brain regions and oxidative stress throughout the brain as evidenced by increased lipid peroxidation and superoxide dismutase (Sava et al., 2006b).

A second link between fungal neurotoxin exposure, increased glutamate levels and sporadic ALS comes from the finding that elevation of glutamate *in vitro* causes a translocation of 43 kDa transactive response DNA-binding protein (TDP-43; the major pathological protein in sporadic ALS) out of the nucleus of cholinergic neurons, which is a hallmark of sporadic ALS. In sporadic ALS, abnormal C-terminal fragments of TDP-43 accumulate as cellular inclusions in neurons and do not exhibit the normal nuclear localization of TDP-43 (Mackenzie and Rademakers, 2008).

## Why Single Out Fungi As a Primary Cause of Amyotrophic Lateral Sclerosis?

Fungi are opportunistic, infectious microbes found in a wide variety of environments – in the soil, on plants and crops, in water and in the air. Fungi produce toxins with demonstrated neurodegenerative activity in animals, including humans, which makes them a strong candidate as a causative organism of ALS. There is a diversity of clusters of sporadic ALS widely reported from around the world, which clearly point to an environmental cause of this disease. A fungal origin could possibly explain much of this diversity.

Humans are exposed to environmental fungi on a daily basis, and clearly in most cases there is no short term or even longer term adverse effect. However, long term chronic exposure to neurotoxic fungi (*e.g.*, in environments where grass- or water-associated fungi are prevalent such

as on sporting fields, on farms and in settings where well water is the primary water source) increases the chances that fungal exposure corresponds to a temporary loss of immune competence. In these circumstances, the invading fungus can establish a chronic infection and begin its neurodegenerative action. It is possible that genetic mutations such as in SOD-1 and TDP-43 predispose the individual to a greater risk of such infection, thus bringing together the genetic and sporadic forms of ALS.

Clearly, further studies are needed before being able to reach a definite conclusion regarding the potential role of fungi as a cause of sporadic ALS. However, if these studies confirm the association, there are several safe and effective broad-spectrum anti-fungal therapies available that may be effective in treating this disease.

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