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The amyotrophic lateral sclerosis exposome: recent advances and future directions

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal disease of motor neuron degeneration with a typical survival of only 2 to 4 years from diagnosis. The causes of ALS are multifactorial. Known genetic mutations only account for around 70% of familial ALS and 15% of sporadic cases lacking family history. Moreover, heritability estimates range from 8% to 61%, indicating additional causes beyond genetics. Consequently, interest has grown in environmental contributions to ALS risk and progression, and hypotheses regarding exposures. The first, the gene–time–environment, posits that ALS onset occurs from an interaction of genes with environmental exposures during aging. The second, the multistep model of ALS, suggests that several “hits”, potentially environmental, trigger ALS onset, even in the presence of highly penetrant ALS mutations. Studies have sought to better characterize the ALS exposome, defined as the lifetime accumulation of environmental exposures that raise disease risk and affect progression. Identifying the full scope of environmental toxicants that enhance ALS risk unlocks the potential prospect of making ALS more preventable by eliminating or mitigating exposures. This critical review will summarize the evidence to date for an ALS exposome along with epidemiological studies characterizing contributions from various sources, highlighting study strengths and limitations. Finally, the review will cover potential mechanisms of exposure-mediated toxicity, ending with the future directions necessary to further advance ALS exposome science.

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Competing interests

SAG, G-G J, ELF are employees of the University of Michigan. MGS and JH are employees of the University of North Dakota. SAG is listed as an inventor on a patent, issue number US10660895, held by the University of Michigan titled “Methods for Treating Amyotrophic Lateral Sclerosis” that targets immune pathways for use in ALS therapeutics. SAG has served on a DSMB and served as a medical adviser for an ALS documentary. ELF is listed as an inventor on a patent, issue number US10660895, held by the University of Michigan titled “Methods for Treating Amyotrophic Lateral Sclerosis” that targets immune pathways for use in ALS therapeutics. ELF has consulted for Biogen.

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Keywords

Environment; environmental exposure; environmental pollutants; gene–environment interactions; motor neuron disease

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease,[1] with a typical survival of only 2 to 5 years from diagnosis[1,2]. The disease presents with limb weakness in spinal onset ALS or difficulty speaking or swallowing in bulbar onset ALS; however, muscle weakness spreads over time and death is usually from respiratory failure[3]. ALS pathophysiology is complex and incompletely understood; however, protein inclusions of the protein TAR DNA binding protein (TDP-43) are almost universally present. Additionally, impaired RNA metabolism, cytoskeletal or trafficking defects, oxidative stress, and mitochondrial dysfunction, along with neuroinflammation are recurrent themes[4].

The causes of ALS are multifactorial. Genetics plays a role and ALS can be inherited, as in familial ALS in 15% of cases, but may occur sporadically in the remaining 85%[4]. Around 70% of patients with familial ALS harbor a known ALS gene, whereas this number is only 15% in sporadic ALS. Moreover, heritability estimates, which are population-specific, range from 8% to 61%. Incomplete heritability across these diverse populations suggests additional ALS causes. Increasingly, the environment is thought to play a role in ALS risk and progression. This focus has led to various hypotheses to explain ALS risk based on environmental contributions. The first, the gene–time–environment hypothesis of ALS, posits that disease onset occurs from an interaction of genes with environmental exposures during aging[5]. The second, the multistep model of ALS, suggests that several “hits” are needed to trigger ALS, possibly environmental in origin, even in the context of a known ALS mutation[6].

Studies of environmental contributions to disease can be examined through the lens of the exposome. The exposome is the sum of all environmental exposures over the lifespan. The name derives from the totality of all exposures and draws parallels with other system-wide entities[7], such as the genome, transcriptome, and epigenome. Exposures are classified as internal, *e.g.*, microbiome, inflammasome, or external, *e.g.*, pollutants in the environment[8]. Exposure occurs through lifestyle (*e.g.*, diet, exercise, smoking), ecosystems (*e.g.*, dwellings, geographic location), social interactions, and contact with physical, chemical, or biological materials[9].

Current studies are underway to better characterize the ALS exposome and pinpoint environmental exposures that raise the risk of ALS onset and affect disease progression. Identifying the full scope of environmental toxicants unlocks the potential prospect of making ALS preventable by eliminating or mitigating exposures. This critical review will first provide an overview of the evidence to date for an ALS exposome. It will then summarize the literature seeking to characterize aspects of the ALS exposome, highlighting study strengths and limitations. The review will then cover potential mechanisms of

exposure-induced toxicity and outline the path needed to make more significant advances in ALS exposome science.

Concept of the ALS exposome

The exposome concept can be applied at the population level (Figure 1A). Large studies query exposures with surveys and/or quantify exposures in biofluids in persons with ALS. Comparing questionnaire responses or exposure levels in ALS cases versus controls identifies environmental risks for ALS, shaping our understanding of the ALS exposome. Moreover, large genome-wide association studies (GWAS) datasets can define ALS polygenic risk scores, facilitating studies of exposome–gene interactions. The exposome concept can also be applied to the individual, chronicling exposures that occur over the lifespan of a specific person born with a predetermined genetic burden (Figure 1B). Individuals experience distinct dynamic exposures, which vary spatiotemporally[10]. The exposome can cause a "biography-to-biology transition[8], as exposures an individual experiences over time shifts them from a state of health to one of disease, based upon interactions with their specific genetic susceptibilities. It is anticipated that generating a deeper understanding of the ALS exposome and exposome-gene interactions from population studies will help assess individual risk to a specific person, *i.e.*, personalized prediction, ultimately paving the way to personalized prevention[11].

Evidence for an ALS exposome

Several lines of evidence support environmental contributions to ALS risk. The first is the occurrence of spatial clusters, and the premise that populations living in certain geographic locations have an elevated risk of ALS due to the presence of environmental toxicants. Another thread of evidence is incomplete heritability, which suggests factors beyond genetic mutations increase ALS risk.

Spatial clusters

ALS incidence varies worldwide; standardized incidences are comparable across Europe (1.89 per 100,000 in Northern Europe, 1.71 per 100,000 in Western Europe, 1.75 per 100,000 Southern Europe), which are similar to North America (1.79 per 100,000) but higher than in South America (1.59 per 100,000) and Asia (0.83 per 100,000 in East Asia, 0.94 per 100,000 in West Asia, 0.73 per 100,000 in South Asia)[12]. The highest standardized incidences are in Oceania (based on a 1985 to 2006 New Zealand study) (2.56 per 100,000) and North Africa (based on a 1980 to 1985 Libya study) (2.03 per 100,000). Previous areas with high incidence may have resulted from a history of ALS "hotspots" across Guam and New Guinea and parts of Japan linked to an early theory of cycad ingestion as an environmental cause of ALS in tandem with parkinsonism dementia complex (ALS/PDC)[13,14]. Overall, the varying impact of ALS across the globe could be related to environmental causes, genetics, or availability of population registries with accurate count cases[15].

The occurrence of ALS spatial clusters have been investigated across populations (Supplementary Table S1)[16]. Odds of an ALS cluster increases with well water use[17] and

proximity to a body of poor-quality water, *e.g.*, with cyanobacterial blooms and elevated levels of the neurotoxin β-N-methylamino-L-alanine (BMAA) (see Neurotoxins in ALS section)[18]. Evidence of an ALS spatial cluster needs to account for genetic background to eliminate genetic contributions, *e.g.*, local founder effects. There are additional challenges to identifying genuine spatial clusters. Ideally, residential history from ALS patients must be derived spanning years rather than just at diagnosis since earlier exposures at a different location may have triggered the illnesses. Moreover, surveys frequently obtain residential addresses, but exposures may have occurred during occupational work. Technological advances, including remote sensing and spatial information of exposures, *e.g.*, water quality or air pollution via satellite readings, along with analytical tools can further refine discovery.

ALS genetic architecture and incomplete heritability

The genetic architecture of ALS is complex and primarily driven by rare monogenic variants[4]. GWAS of large ALS populations catalyze discovery of ALS genes; nevertheless, a genetic cause is known in only about 70% of familial ALS cases and 15% of sporadic ALS cases. Mutations to *C9orf72*, *TARDBP*, *SOD1*, and *FUS* are the most common[4], and their relative frequency vary by populations. For instance, mutations to *SOD1* are more prevalent than to *C9orf72* in populations of Asian versus European descent[19]. Nevertheless, even for these highly penetrant mutations, ALS onset occurs through a multistep process of “hits” (see Gene–environment interactions in ALS section). Furthermore, some ALS genes, such as *ANG*, *ATXN2*[20], and *DCTN1*, do not necessarily induce illness in some populations, but rather confer enhanced risk. These “risk” genes may potentially interact with the environment to trigger ALS[21,22].

In addition to monogenic inheritance, oligogenic and polygenic models of ALS have emerged. In the oligogenic model, risk arises from the presence of two or more potential ALS variants, which may, for example, lower onset age[23]. In the polygenic model, risk arises from a profile of numerous single nucleotide polymorphisms (SNPs), which increases opportunity for interactions with the environment (see Gene–environment interactions in ALS section). Indeed, analyzing polygenic risk in ALS through GWAS suggests shared risk with environmental factors, such as smoking status and exercise intensity[24], suggesting Mendelian randomization of GWAS may be useful in understanding exposures.

Heritability estimates for ALS vary from 8% from large GWAS datasets[25] to 61% from twin studies[26]. In one large GWAS study, significant genome-wide loci contributed only 0.2% to heritability, meaning most of the 8% heritability arose from rare SNPs below genome-wide significance[25], indicating many ALS genetic risk variants have yet to be identified. Yet, heritability is incomplete, suggesting that environmental exposures and gene–environment interactions contribute to ALS risk.

Gene–environment interactions in ALS

The concept that non-genetic factors contribute to ALS risk was first formulated by a hypothesis opinion in 1973, which stated that secondary factors intersect with genetic factors to influence ALS risk[27]. When and how an exposure or set of exposures occurs to trigger disease remain critical questions. The gene–time–environment hypothesis proposes that ALS

onset occurs from environmental exposures superimposed on a person's genetic profile over time (Figure 2a–b)[5]. In this model, an individual's genetic burden is predetermined at birth, either through monogenic, oligogenic, or polygenic risk. Genetic burden contributes to disease pathology at the cellular level with aging, coupled with environmental burden (dose and frequency of exposures). In the model, genetic burden interacts with environmental burden over time. At a constant environmental burden, individuals with a higher genetic burden will attain the threshold for disease earlier than an individual with a lower genetic burden (Figure 2a). Vice versa, at a constant genetic burden, individuals exposed to a higher environmental burden will meet the threshold for disease at an earlier age than an individual with a lower environmental burden (Figure 2b). Thus, ALS onset is dictated by both genetic and environmental burden with aging[5].

A similar concept is raised by the multistep model of ALS, which postulates that multiple "hits" or "steps", possibly of environmental origin, occur to initiate ALS (Figure 2c)[6,28–30]. This ALS model is based on the Armitage-Doll model for cancer[31], which derives the number of steps by plotting the log of incidence against the log of age, based on the premise that incidence is proportionate to the product of risks for undergoing the first step and ensuing steps. The first ever reported multistep analysis for ALS calculated 6 steps for disease onset[28], similar to subsequent studies of mostly sporadic ALS in Australian[29,30] and Japanese[30] populations and 5 steps in South Korean[30] populations. The differing number of steps for sporadic ALS in different populations may reflect distinct genetic backgrounds, environmental exposures, or both. Analyzing ALS cases with known mutations finds that multiple steps are needed to trigger ALS, even for penetrant mutations, such as *SOD1* (2 steps), *C9orf72* (3 steps), and *TARDBP* (4 steps)[6].

If environmental factors, such as pesticides and heavy metals, contribute to ALS risk, it is possible that SNPs to detoxifying enzymes, *e.g.*, *PON1*, *ALAD*, may increase ALS risk. To date, there is no clear concordance across studies, possibly because they considered genetics separately from exposures, and both need to be assessed simultaneously if ALS arises from gene–environment interactions[32].

Aspects of the ALS exposome

Studies of the ALS exposome are rooted in early work in the Western Pacific based on putative exposure to the neurotoxin BMAA[14]. Numerous investigations have since built on this foundation, identifying potential environmental exposures that raise ALS risk. At present, the selection of environmental exposures to study in relation to ALS is often driven by clinical observations or by easily studied variables[5,33], which results in bias in potentially relevant risk factors. Future studies using newer technologies will facilitate untargeted analysis in an agnostic manner of both later and earlier exposures, since early exposures may play a role in neurodegenerative disease much later in life[34].

We will frame our discussion of risk factors with the strongest evidence to date, based on recent systematic reviews and meta-analyses[16,35]. The emphasis will be on epidemiological studies in defined populations across various exposure types over the past 5 years (Figure 3, Supplementary Table S2). Meta-analyses highlight the same risks[35,36], suggesting that these

environmental exposures may be universal, and enhance the odds of developing ALS across diverse populations.

Pesticides

A recent meta-analysis reported that pesticides increased ALS risk with an odds ratio (OR) of 1.48, pooled across 10 studies[35], which largely estimated exposures from retrospective reports or likelihood of exposure in occupational settings. To overcome limitations from recall or ascertainment bias, some groups have estimated exposures by directly measuring pesticide levels in ALS biosamples.

Direct analysis of plasma for persistent organic pollutants, defined as organohalogen compounds with long half-lives, found higher levels in ALS versus control participants, especially among organochlorine pesticides[37]. However, persistent organic pollutant levels in cerebrospinal fluid (CSF) did not differ in another study of ALS and control participants[38]. Direct measures of pesticide exposures are not without limitations. Depending on pesticide half-life, direct measures might only reflect a current exposure, without assessing prior exposures, which may be critical triggers within a susceptible exposure window. Moreover, direct pesticide measures are largely targeted, and will not capture exposures to all possible compounds during the life course. Directly assaying persistent organic pollutants from biosamples is new to the ALS field and will require further study in additional cohorts.

An alternative strategy is to capture pesticide exposures through a surrogate marker, such as proximity to a confirmed source of pollution. In a population-based study in Italy, this approach found no association between ALS cases and living in areas of high crop density, a surrogate for potential pesticide exposures[39]. On the other hand, analyzing ALS cases pulled from a claims database with residential pesticide exposure estimates found that herbicides, 2,4-dichlorophenoxyacetic acid and glyphosate, and the insecticide carbaryl chlorpyrifos associated with ALS[40]. Additional analysis of the same dataset concluded risk from polychlorinated biphenyls, such as heptachlorobiphenyl, mostly derived from certain power plants burning biomass and industrial boilers[41].

Overall, recent studies using different assessment strategies are consistent with the published meta-analysis indicating that pesticide exposures are important ALS risk factors[35], which require further research to understand how they trigger disease onset.

Occupation and occupational exposures

Evaluating exposures in the occupational setting is one approach to understanding ALS risk factors[42,43]. Occupations that increase ALS risk include production work[42], agricultural occupations[44], mechanics[45,46], painting[45,46], construction[45,46], precision tool manufacturing[47], and glass, pottery, and tile work[47]. ALS risk is also associated with occupational exposures to metals[42,44,48,49], lead[50–52], silica, organic dust[53], diesel[53,54], formaldehyde[55], solvents[51], pesticides[51], and polycyclic aromatic hydrocarbons[53]. Moving forward, it will be important to understand what aspects of the above noted occupations increase ALS susceptibility, how these occupational exposures trigger disease, and whether these exposures are relevant to persons that do not share similar occupational histories.

Military service is considered an ALS risk factor, but the data are not always consistent. Different studies of the same Gulf War veteran population report variable associations with ALS risk[56]. These inconsistencies may arise from the methods used to identify cases, *e.g.*, hospitalizations, self-reports, death records[56], or that individuals serving in the military more recently have not yet reached the age of peak ALS incidence. Highlighting this point, analysis of a national prospective cohort found service in World War II, but not the first Gulf War, increased ALS risk[57]. In contrast, a more recent report found United States veterans deployed post-9/11 had an ALS prevalence of 19.7 per 100,000 people[58], far higher than the global crude prevalence of 4.4 per 100,000[59]. ALS prevalence was especially striking for air force personnel (33.2 per 100,000), tactical operation officers (51.8 per 100,000), and health care workers, scientists, and professionals (29.1 per 100,000)[58]. The causes of increased ALS risk in military personnel are unknown, but research directions include head injury, physical exertion, and exposures to chemicals, metals and burn pits[56].

Sports and physical activity

Another investigated ALS risk is professional sports, especially of football or soccer[60], and is linked to both occupation and physical activity. Standardized ALS incidence ratio is 3.59 per 100,000 among all professional National Football League players from 1960 to 2019[61], versus the global standardized incidence of 1.68[12]. Standardized ALS incidence ratio is 1.91 for all players and 4.66 for players diagnosed under 45 years of age among all professional Italian soccer players from 1959 to 2018[62]. An appealing hypothesis for the association between professional athletics and ALS is repetitive trauma, especially head trauma. However, analysis of Scottish professional soccer players found greatest risk for neurodegenerative disease in defenders but lowest risk in goalkeepers, suggestive of anaerobic activity as a critical factor[63] versus trauma, at least in soccer and for neurodegenerative disease broadly. This concept is upheld by a Swedish study of long-distance cross-country skiers with 39 ascertained ALS cases, which only found risk for ALS among elite, not recreational, skiers[64]. By contrast, the National Football League study, with a similar 38 ascertained ALS cases, did not find differences in risk by primary playing position, i.e., non-speed (linemen) versus speed positions (all other positions)[61]. Thus, overall, whether risk from sports arises from a specific athletic activity or instead reflects intense physical activity, trauma (see Trauma section), some other exposures on field (see Microbiome and infectious agents section), or a combination of factors requires further study.

Systematic review of epidemiological case-control studies suggests physical activity may increase ALS risk[65], although there are inconsistencies across reports[33], possibly from differing methodology and confounding from genetics (see Gene–exposome studies in ALS section). Moreover, the association between physical activity and ALS risk is complex and depends on exercise intensity and duration, with neuroprotection from light activity and harm from intense activity[24]. Further support comes from a study showing genetic liability to the trait “frequent and strenuous leisure-time exercise” associates with ALS risk and that *C9orf72* carriers with greater historical physical activity had earlier disease onset[21]. Physical activity could possibly link athletics to ALS risk through several potential mechanisms, including oxidative stress, dysregulated energy metabolism, excitotoxicity, neuromuscular

junction remodeling, stress to fast twitch muscle fibers, and altered growth factor signaling (Figure 4)[65].

Metals in biosamples

Although metal exposures are linked to ALS risk based on meta-analysis of multiple mostly retrospective studies[35], there are similar caveats with these analyses as those encountered with pesticides. Retrospective studies of metals exposures using questionnaires are subject to recall and ascertainment bias. To avoid these limitations, some researchers have directly measured trace metal levels in biosamples. A 2020 report found that increased chromium, manganese, nickel, tin, and zinc exposures, measured in teeth, within a specific time frame in early childhood are linked to ALS upon aging[66]. In adults, analysis of nails suggests that elevated mercury and zinc enhance ALS risk, while manganese, arsenic, selenium, copper, cadmium, and lead show no disease association[67,68]. A nested case-control study of a prospective European cohort of 107 ALS versus 319 control participants found elevated concentrations of cadmium and lead in the erythrocyte fraction of blood from individuals that later developed ALS[69]. In contrast, analysis of CSF from 38 ALS cases showed no significant difference in metal concentrations versus controls[70]. Despite the advantages, directly measuring trace metal exposures in biosamples has its own set of challenges, including absorption kinetics, tissue distribution and optimal analysis tissue, and elimination half-life[71].

Another approach to studying the link of metals exposure to ALS comprises geospatial analysis, as performed for pesticides. A geospatial study in Ireland failed to find an association between ALS cases (n=1,701) to metal content in soil samples[72]. Given the number of potential limitations to this method, replication efforts in additional cohorts would be beneficial.

Moving forward, more studies are needed that assay metal levels from ALS biosamples. However, it will be necessary to determine the optimal tissue based on metal metabolism, biodistribution, and half-life elimination.

Air pollution

There is increasing evidence that air pollutants associate with ALS risk, including elemental carbon[73], ultra-fine particle matter (PM; $PM_{2.5}$, $PM_{10}Si$, PM_{10}, subscript indicates micron size)[74–76], NO_x[75], and NO_2[74,77]. $PM_{2.5}$, NO_2, and NO_x are primarily traffic-related pollutants, while coarse PM_{10} particles are from road dust and agricultural and construction industries. Recent studies suggest that the association between long-term air pollution exposure and ALS is driven primarily by motorized traffic-related pollutants[74–76]. In addition to increasing ALS risk, there is a positive association between air pollution exposure and an increased number of hospital stays and emergency room visits by persons with ALS[78,79]. Interestingly, concentrations of some of these pollutants were still below World Health Organization air quality guidelines, suggesting that additional tightening of regulatory guidelines may be beneficial[74].

Not all studies report, however, an association between air pollution and ALS. A hospital-based case-control study in a heavily polluted area of Italy reported no link between

vehicular pollution, measured by PM_{10} levels, and ALS risk[77]. These differences may be due to sample sizes, uncertainty in air pollution estimates, analysis of distinct pollutant size, *e.g.*, $PM_{2.5}$ versus PM_{10}, and composition, *e.g.*, carbon, silicon, and lack of pollutant exposure data prior to 1993. Additionally, it is important to account for air pollution exposure in both residential and occupational settings. Diesel exhaust is a primary source of traffic-related air pollution and, as noted above (Occupation & occupational exposures section), occupations such as truck drivers, bus drivers, and machine workers and operators all associate with increased ALS risk[43,49,50].

Trauma

The premise behind trauma as a risk factor is that mechanical injury to the nervous and surrounding tissues may trigger histopathology, such as protein aggregation or stress granule formation[80,81], predisposing to future ALS. Trauma may occur to the head (*i.e.*, brain, brain stem) or trunk/limbs (*i.e.*, spinal cord), and be accidental or arise from occupations, *e.g.*, intensive sports, active-duty military service. US Veterans deployed post 9/11 have higher ALS rates, but independent of traumatic brain injury[58]. Pathological hallmarks of chronic traumatic encephalopathy, a condition associated with repeated head injury, are present in postmortem ALS brains with and without history of head trauma[65]. Analysis of 155 postmortem brains from the Veterans Affairs ALS Brain Bank found only nine had chronic traumatic encephalopathy (CTE). Those with CTE had a history of traumatic brain injury with distinct ALS phenotypes including bulbar onset and behavioral/mood changes[82].

Studies have attempted to dissect the role of repetitive trauma in sports from increased physical activity levels (see Sports and physical activity section). A 2019 meta-analysis examined ALS risk based on 16 studies of professional and nonprofessional sports of varying close-contact intensities, *e.g.*, soccer, American football, marathon running[83]. Organized competitive sports correlated with increased ALS rate ratio, which tended to favor participants in professional versus nonprofessional sports. Moreover, professional sports prone to repetitive concussive head and spine trauma correlated with greater ALS risk versus nonprofessional sports prone to similar trauma. Another systematic review noted that studies of nonprofessional athletes does not support increased ALS risk[84] but advocated for larger studies that account for confounding effects. Overall, findings underscore the complex interplay between head trauma and physical activity.

Some studies suggest trauma is not the initial risk factor but rather ensues from reverse causation in early, pre-symptomatic ALS by predisposing patients to falls. This potential confounding parameter may be eliminated by examining the risk to ALS from trauma occurring earlier than 5 years before diagnosis. Any instance of “Head trauma or concussion that caused you to black out or lose consciousness” correlated with ALS (OR 1.60) in a case-control study in northern New England and Ohio, which was most significant in the 10 years prior to diagnosis (OR 1.64)[45]. These findings were echoed by Italian and European studies, with OR 2.61 and 1.54, respectively, for head trauma on ALS risk when excluding participants injured less than 5 years before diagnosis[85 86]. Meta-analysis reinforces risk from head or trunk injury, which is highest within 5 years of diagnosis, possibly from predisposition to falls[87]. Although epidemiological evidence supports trauma as a potential

ALS risk factor, larger well- phenotyped prospective cohorts with a diverse range of physical activity, occupations, ages, and sex are needed to better understand the time sequence and linkage between trauma and disease.

Electromagnetic exposure

Electromagnetic fields (EMFs) occur on a spectrum of ultra-high to extremely-low frequency (energy). Although present naturally from the earth, electricity and communications demands have starkly increased the level of EMFs in the environment[88]. Exposures can occur to an individual from towers near their dwelling or workplace. It is speculated that EMFs interfere with synaptic transmission and neurotransmitter homeostasis[89]. Several epidemiological investigations have assessed the impact of ultra-high to extremely-low frequency EMFs on ALS risk. Analysis of Global System for Mobile Antennas in France found a significant trend in ultra-high frequency EMFs exposure gradients to ALS incidence, with a relative risk of 1.83 in the non-exposed versus highest exposed categories[90].

On the other end of the spectrum, a large European study reported an unadjusted ALS risk OR of 1.16 with occupational extremely-low frequency EMFs, which did not remain significant with adjustment[91]. A Dutch study found occupational extremely-low frequency EMFs linked to ALS mortality, with a HR of 2.19 for males who had held a job with high exposure[92]. Moreover, electric shock may be linked to ALS (adjusted OR 1.19 in discovery cohort, no significant correlation in the replication cohort)[91].

Attempting to examine EMF in a non-occupational setting, an Italian study examined relative location of ALS case residence to high-voltage power lines, finding no correlation[93]. This was reiterated by a 2021 meta-analysis, which found no correlation between residence proximity to a high-voltage power line to ALS risk[94]. Overall, EMFs may contribute to ALS risk, particularly in the occupational setting, but replication and validated methods of estimating EMF exposure are needed for larger cohort studies.

Microbiome and infectious agents: lessons from human studies

The gut microbiome has emerged as a potential contributor to ALS risk (Supplementary Table S3)[95]. Multiple studies report gut microbiome composition differences in ALS cases versus healthy controls or participants with non-ALS neurodegenerative diseases[96–100]. For instance, ALS cases have lower levels of butyrate-producing bacteria[96,99], which could compromise gut integrity and immune homeostasis and exacerbate inflammation, worsening the disease course. Indeed, a study that examined the correlation between dietary fiber, the substrate for butyrate-producing bacteria, based on a 24-hour diet recall, found that participants in the highest tertile of vegetable fiber intake had the longest survival[101]. Moreover, the microbiome correlates with disease characteristics. In ALS participants, microbiome richness may differ by survival[102] and composition may change with disease progression[97,99]. Regarding onset segment, gut dysbiosis may increase with worsening symptoms in spinal onset ALS, whereas oral dysbiosis may be correlated to bulbar onset ALS[100].

In addition to immune-modulation, the microbiome can influence host metabolism, which is perturbed in ALS[103]. Indeed, a gut microbiome-fecal metabolome analysis found changes in microbial composition in ALS cases associated with altered gene function in metabolic pathways and specific metabolites[104]. Another study found 41 fecal metabolites that differed in ALS cases versus controls, mostly lipids along with peptides, nucleic acids, and other metabolites[105]. Studies involving Mendelian randomization to evaluate potential causal relationships between gut microbiota, metabolites, and neurodegenerative diseases have revealed significant links between certain gut microbiota and metabolites to increased ALS risk[106,107].

Though the primary focus has been on environmental contributions from the host microbiome, some studies have proposed the involvement of various infectious agents, *e.g.*, viruses[108,109], and fungal neurotoxins[110] on ALS risk. A theory of *Mycobacterium avium* subspecies *paratuberculosis*, present in the soil of sports fields, has been proposed as a causative ALS factor in outdoor sports players[111]. However, evidence to date remains relatively sparse.

Overall, while studies to date provide valuable insights into putative roles of the microbiome on ALS risk through immune-modulation and metabolism, there is discordance across studies and uncertainty regarding the most relevant bacterial phyla and genera. Investigations uniformly exclude participants on probiotics, antibiotics, or other drugs affecting microbiome, yet most do not account for dietary habits, which can impact microbiome structure. Additionally, reports generally do not state the disease stage samples were collected at, which could differ between participants of the same study and between studies. Future carefully designed and fully adjusted studies are needed, which may lead to new potential therapeutic avenues and personalized interventions[95].

Energy intake and dietary composition

ALS is characterized by altered metabolome, hypermetabolism, and impaired energy homeostasis[112,113]. Individuals with ALS typically display lower body mass index (BMI) during the presymptomatic phase prior to diagnosis[114], which associates with poor survival[115,116]. However, higher BMI during the presymptomatic phase may also correlate with shorter survival[116], possibly dependent on genetic background[115]. These findings are supported by reports that lipids impact ALS risk and survival. Intake of n-3 polyunsaturated fatty acids is linked to lower ALS risk[117]. In persons who develop ALS, there is higher intake of total fat, saturated fat, trans-fatty acids, and cholesterol prior to disease onset[114,115]. With respect to survival, elevated triglycerides associate with improved survival while higher serum high-density and low-density lipoprotein cholesterol levels associates with poor survival[118]. These relationships may, in part, be genetically and epigenetically mediated; Mendelian randomization suggests hyperlipidemia may be a causal risk factor for ALS[24]. A genome-wide study of DNA methylation identified multiple differentially methylated loci enriched in pathways related to metabolism and cholesterol biosynthesis[119].

Studies have also examined correlations between various types of foods and micronutrients with ALS risk[112,113]. A 2020 survey found an inverse association with overall fish intake and ALS risk but lacking a dose-response relation[85]. An Italian food-frequency

questionnaire reported no relation of ALS risk to fish or total energy intake, however red and processed meats linked to higher risk while vegetables, citrus fruits, and vitamin E linked to lower risk[120]. Two Asian surveys similarly found detrimental effects from meat and fast foods and protective effects of vegetables and fruits[121,122], as well as of beta-carotene and vitamin A[121]. A meta-analysis found no associations between caffeine, coffee, and tea intake on ALS mortality[123], whereas a systematic review concluded micronutrients likely affect disease risk[124] further supported by a mendelian randomization study finding links to linoleic acid and vitamins D and E[125].

It is important to note that many of these studies did not adjust for variables that may impact findings, including genetics[24,119], lifestyle choices, and medication use affecting metabolic profiles, *e.g.*, statins. For example, healthy, protective diets are more frequently adopted by individuals who engage in regular exercise[126], preferences that could influence study outcomes. Future studies are warranted and need to account for these important variables before we can fully understand the intersection of diet and ALS risk.

Lifestyle and socioeconomic status

Several lifestyle, clinical, and socioeconomic factors are implicated in ALS risk[33,127]. It has long been known that socioeconomic status and education correlate with ALS incidence[128]. Moreover, studies now show shared polygenic risk between ALS and several traits, such as negative correlations to higher cognitive performance and higher educational attainment[24]. These results suggest that some genetic factors underlying mental ability and ALS may overlap. It will be important to explore how cognitive performance protects against neurodegeneration and identify the relative contribution and function of these overlapping genetic variants. Epidemiological studies have explored a wide variety of other lifestyle factors that may influence ALS risk. For example, alcohol consumption and smoking have been associated with ALS risk, while reading, duration of education, and retirement have been identified as protective[115,129,130]. However, results can be contradictory[131] and further studies are needed. Importantly, relationships are complex, and it may be necessary to account for lifestyle pattern or timing of the habit. For instance, regarding smoking on ALS risk, higher pack-years and longer duration may be more important contributors to risk than intensity of smoking, whereas longer time-since-quitting appears protective, indicating smoking exert effects earlier in the life course[132].

Exposome-phenotype considerations

Most studies of environmental exposures have focused on ALS onset with few assessing impact on disease phenotype and heterogeneity. A South-East England study suggested geographic location impacts survival[133], which was not recapitulated by an Irish study[134]. Nonetheless, the English study raised the possibility that factors, like social deprivation or air pollution, could affect ALS survival. Michigan studies indicated that higher exposure to persistent organic pollutants[135] and prior work in Production Occupations and self-reported occupational exposure to pesticides shortened ALS survival[42]. A small US study, however, did not show dependency of survival on agricultural chemical occupational exposure[50]. The Michigan study also found that the exposome affected onset segment, with self-

reported radiation exposure linked to bulbar onset and various occupations and self-reported exposures to metals, particulate matter, volatile organic compounds, combustion and diesel exhaust, and electromagnetic radiation linked to cervical onset[42]. In contrast, bulbar onset associated with construction occupations in a Maltese study[136] and agricultural workers in Brittany, France[137]. Bulbar onset is also more prevalent in professional Italian soccer players[138,139] and Israeli triathletes[140], but not in Spanish soccer players[141]. Overall, while current studies suggest that exposures may lead to unique phenotypes, definitive conclusions are limited by small sample sizes and differences in study data collection. However, if exposure history influences ALS phenotype, this information could be useful to predict clinical outcomes, stratify clinical trial participants, and identify disease biomarkers. This is an important topic, and more work is needed to enhance the precision of these findings.

Targeting modifiable risks in ALS

Environmental exposures are potentially modifiable, and, thus, suggest a possible path towards preventing onset or mitigating progression in ALS, advocating a paradigm shift from treatment to prevention[11,142]. Although we remain in the early stages of assessing the feasibility of ALS prevention, targeting modifiable risk factors is presently accessible. Several clinical trials and observational studies have been conducted to examine the impact of modifying diet and the microbiome on ALS disease course (Supplementary Table S3).

Regarding diet, a small, early double-blind, placebo-controlled, randomized phase 2 clinical trial demonstrated safety and tolerability of high caloric enteral nutrition[143], suggesting feasibility of dietary intervention. The LIPCAL-ALS study evaluated the efficacy of a high-caloric fatty diet on ALS survival, but discerned no benefits, although *post hoc* analysis suggested some salutary effects in fast-progressing participants[144]. Further analysis of LIPCAL-ALS using neurofilament light chain as a biomarker of disease progression indicated levels were lower in participants on high-caloric fatty diet versus placebo[145]. An upcoming pilot trial will assess safety and reproducibility of a normocaloric ketogenic diet in ALS participants[146], given its ability to reduce hyperexcitability and modulate neuroinflammation, as well as its therapeutic impact in animal models[147]. Additionally, clinical trials of various supplements that influence bioenergetics have been tested, such as acetyl-L-carnitine[148] and coenzyme Q10[149], but none to date have been shown to effectively improve ALS disease course.

Clinical studies modifying the microbiome are limited, though supported by preclinical evidence[150,151]. A probiotic treatment comprised of five lactic acid bacteria altered the gut microbiota of ALS participants, but not towards biodiversity levels commensurate with control participants; moreover, the probiotic did not slow disease progression[97]. FETR-ALS will examine the feasibility of fecal microbiota transplant from healthy donors to ALS participants in a randomized, double-blind multicenter study[152]. A single case report of washed microbiota transplant from a healthy donor to an ALS patient suggested slowing of disease progression[153].

The ketogenic diet may shape the microbiome[154,155], and constitutes a potential link between diet and gut in ALS. Moreover, the gut microbiome influences host immunity,

which is dysregulated in ALS[4], though clinical trials have yet to identify effective candidates[156]. Indeed, the FETR-ALS trial of fecal microbiota transplant will examine its impact on immunological features, such a regulatory T cells[152]. Overall, these examples serve to highlight the complex and multi-faceted interplay of environmental forces in ALS, and the challenges and opportunities of targeting modifiable risks to alter disease course."

Gene–exposome studies in ALS

Despite growing evidence of both genetic and environmental contributions to ALS, and the tenets set forth by the gene–time–environment hypothesis, few studies have examined gene-exposome interactions in ALS. The presence of SNPs in detoxifying enzymes lends further credence to the importance of examining gene–environment interactions in ALS[32], since discordance across studies may have arisen because SNPs were assessed without taking environmental exposures into considerations.

Of studies that examined or hypothesized gene–environment interactions, one analyzed the impact of lifestyle factors in the context of *C9orf72*[115]. The study found a higher daily energy intake of 712 kJ in *C9orf72* carriers and 497 kJ in non-carriers versus controls. Furthermore, in the presymptomatic stages, median BMI was lower and physical activity similar in *C9orf72* carriers versus controls whereas median BMI and physical activity were higher in non-carriers versus controls. Since *C9orf72* carriers had lower BMI and similar activity to controls, but higher energy intake, it suggests they also have elevated energy expenditure, possibly related to the *C9orf72* mutation and gene–environment interactions[115]. Moreover, a methodology, instrumental variable analyses, suggested a causal effect from higher BMI at a younger age and of alcohol consumption and smoking on ALS onset in participants lacking mutant *C9orf72*, further indicating that factors predisposing to neurodegeneration may be genotype-dependent[115].

Another study of Mendelian randomization demonstrated a causal link between ALS and physical exercise[21]. Exercise-induced transcriptomic changes were enriched in ALS genes, including *C9orf72*. Further examination of the *C9orf72*-exercise correlation found onset age was inversely proportional to historical physical activity in *C9orf72* carriers versus non-carriers. Moreover, physical activity level was more consistent across *C9orf72* carriers versus non-carriers and controls. Overall, the study concluded that *C9orf72* expansions particularly predispose to exercise-induced ALS[21]. Mendelian randomization, a method that leverages a population's genetic variation to evaluate the effect of environmental modifiers on disease risk, has suggested multiple lifestyle risks in ALS[127], and constitutes a tool to further understand gene–environment interactions.

Although most gene–environment interaction studies have examined mutant *C9orf72*, as the most common ALS mutation, one small investigation assessed levels of selenium species in CSF from 9 ALS participants harboring various mutations versus controls (n=42)[22]. Although selenium levels were generally elevated in ALS CSF, they were especially high in the single participant harboring mutant *TUBA4A*, coding a tubulin subunit. Given the small sample size, the authors could not draw any conclusions, but noted that selenium compounds also impair tubulin dynamics, and posited potential shared genetic etiology or pathogenic

pathways favoring disease onset in this participant. Finally, preclinical studies demonstrate that methylmercury accelerates disease progression in mutant SOD1^{G93A} mice[157], which was attributed to a “dual hit” from environment and genetics[158].

Potential mechanisms of ALS exposome toxicity

There is a large body of work assessing exposures in ALS, as outlined in the previous sections. By contrast, far fewer studies have been conducted to understand the mechanisms of environmental toxicant-mediated neurodegeneration in ALS, and fewer still of mechanisms of gene–environment interactions. Here, we will review ALS-specific studies and formulate a hypothesis of potential mechanisms by drawing parallels with other neurodegenerative diseases (Figure 4). We also outline knowledge gaps and potential avenues of future investigation.

Metal-mediated mechanisms in ALS

Metals have a long history in ALS due to the connection with *SOD1*, superoxide dismutase 1, an antioxidant copper-zinc metalloprotein, which destroys free superoxide radicals. How environmental metal exposure contributes to ALS pathogenesis, on the other hand, has been less investigated. One putative mechanism is through metal-induced protein aggregation (Figure 4a). Inclusions of wild-type TDP-43 are an almost universal pathology in ALS patients, which impairs multiple downstream pathways, such as RNA splicing[4]. Several metals can induce TDP-43 aggregation *in vitro*, such as lead and mercury[159,160], which could trigger ALS pathology as supported by epidemiological studies indicating certain metals as risk factors (see Metals in biosamples section). However, it is unknown whether the metal concentrations required to induce TDP-43 aggregation *in vitro* are physiologically relevant and commensurate with levels *in vivo* in people with ALS.

In vivo, exposure to methylmercury accelerates symptom onset, *e.g.*, poor rotarod performance, in SOD1^{G93A} but not wild-type mice[157]. Coincident with symptom onset, methylmercury increases intracellular calcium concentrations in brainstem motor neurons mediated by glutamate receptors. Metal-induced neuroinflammatory processes may also occur *in vivo*; in a model of methylmercury motor dysfunction, the anti-inflammatory and antioxidant acetyl-11-keto-beta-boswellic acid partially rescues grip strength and locomotion and brain inflammation, oxidative stress, apoptosis, and demyelination[161]. Lastly, there are mechanisms of metal-induced toxicity more generally that have not been investigated in the context of ALS[162].

Neurotoxins in ALS

Several potential environmental neurotoxins have been proposed as causative agents in ALS (Figure 4a). The most widely investigated are BMAA, linked to cyanobacteria in bodies of water with low quality, and methylazoxymethanol, the aglycone of cycasin, found in *Cycas* spp. (cycads)[163]. Misincorporation into proteins based on the structural similarity of BMAA to α-amino acids has been investigated as a disease mechanism, coupled with exposure studies in rodents and primates[13]; however, the relevance of administered doses in animal studies to exposure of humans with ALS from the environment is uncertain.

Methylazoxymethanol causes DNA damage and epigenetic changes and is proposed to cause latent progressive neurodegenerative disease through genotoxicity during early *in utero* exposure[163]. A small sporadic ALS cluster in the French Alps was also proposed to occur through exposure to genotoxic *Gyromitra gigas* (false morel mushrooms)[164]; however, genetic screening was limited to *SOD1* and *C9orf72* in most cases.

Proposed pollution- and pesticide-mediated mechanisms of injury in ALS

Although epidemiological studies have suggested air pollution as ALS risks, no studies to our knowledge examined mechanisms through the lens of ALS. However, it is possible to draw hypotheses based on exposure studies combined with known ALS pathophysiology. Air pollution enhances systemic inflammation and disrupts the blood-brain barrier and cerebrovascular function, also activating innate immune responses in the brain, *e.g.*, microglia[162] (Figure 4a). Additionally, air pollution increases lipid peroxidation and oxidative stress. Importantly, male sex may predispose to more severe air pollution-induced neurotoxicity[165]. Similarly, ALS patients are characterized by perturbed blood-brain and blood-spinal cord barriers[166] and altered immune profiles, peripherally and centrally within the brain and spinal cord, including microglial activation[4]. Immune dysregulation is sex dependent, and more ALS patients are male[167]. Thus, air pollution and inflammation in ALS may represent a point of convergence, but research is needed to test this possibility.

In exposure studies, pesticides and persistent pollutants similarly induce oxidative stress, neuroinflammation, and neuronal apoptosis[162] and inhibit critical enzymes related to neurotransmitter activity, such as cholinesterases[168]. A 2022 preclinical study of cis-chlordane found it was particularly toxic to human stem-cell-derived motor neurons compared to other cell types, altering action potential dynamics[169]. *In vivo*, in zebrafish larvae, cis-chlordane triggers motor neuron and neuromuscular junction degeneration, impairing motor deficits in a touch-evoked escape response.

Overall, few studies have investigated the mechanism of exposure-meditated pathophysiology leading to ALS, posing significant knowledge gaps in the field.

Microbiome mechanisms in ALS: lessons from preclinical models

The microbiome acts through the gut–brain axis and regulates host immunity and metabolism in health and disease[170]. Accumulating evidence suggests the microbiome plays a role in ALS pathogenesis. At the intestinal barrier, $SOD1^{G93A}$ mice are characterized by intestinal junction and Paneth cell defects and enhanced IL-17 and intestinal permeability and $SOD1^{G93A}$ aggregation[171,172] (Figure 4b). Moreover, ALS mice exhibit a shift in fecal microbiome, with fewer bacteria producing the beneficial short-chain fatty acid butyrate[171], which promotes immune tolerance and host metabolism[173]. Intervention with butyrate rescues the ALS phenotype, including improved weight, intestinal permeability, muscle and enteric neuromuscular function, and survival[172,174]. Longitudinal analysis shows early microbiome changes, prior to symptom onset, linked to autoimmunity, inflammation, and metabolism[174]. Overall, these findings suggest potential participation of the microbiome in early pathological changes through immune and metabolic mechanisms.

Indeed, in the same SOD1^{G93A} mouse model, blocking lipid metabolism by pharmacologically inhibiting or genetically ablating carnitine palmitoyl transferase 1, which imports fatty acids into mitochondria for breakdown by β-oxidation, slows disease progression, lowers inflammation and oxidative stress, and improves mitochondrial function[175,176]. These metabolic changes are mediated, in part, by diet, epigenetics, and microbiome changes. Another study similarly reported microbiome-mediated changes in specific metabolites, such as nicotinamide[150], a component of the cofactor NAD, necessary for energy metabolism. Microbiome-derived nicotinamide metabolism is decreased in ALS[150].

Regarding neuroinflammation, correlation analysis found that various microbes are linked positively or negatively to brain and spinal cord microglial activation in mutant SOD1^{G93A} mice[177]. Furthermore, perturbing the microbiota of SOD1^{G93A} mice by antibiotic treatment alters microglial transcription, favoring expression of genes linked to neurodegeneration[178]. Importantly, antibiotic treatment may exert gene-dependent effects; in an ALS mouse model with *C9orf72* loss of function mutation, antibiotic treatment promotes myeloid cell infiltration and microgliosis in the spinal cord[151]. The effect in mutant mice was ascribed to loss of *C9orf72*-mediated suppression of gut inflammation. Overall, these studies suggest a potential immune-modulating effect of the gut microbiome in ALS. Since dysregulated inflammatory activation is a recurrent theme in ALS[4], impaired immune and metabolic homeostasis through the gut–brain axis in ALS are feasible mechanisms of disease progression. Indeed, fecal transplant studies show protective effects of specific microbiota in ALS[150,151], but studies to date have relied solely on genetic murine models of ALS.

Epigenetic restructuring in ALS

Molecularly, environmental factors can trigger disease pathology through epigenetic restructuring, which is a central tenant of exposome studies[8], for example through metals[179], pesticides[180,181] or microbiome[182]. Evidence does suggest differential epigenomes in ALS cases versus controls, *e.g.*, genome-wide methylation[183], although the precise causes for epigenetic restructuring in ALS are likely multifactorial, *e.g.*, of microRNAs through TDP-43 (Figure 4b). A small study of twins discordant for ALS status found older epigenetic age in the affected twin, and differentially methylated CpGs in genes regulating GABA signaling[184]. Accelerated epigenetic age in ALS cases was noted in another larger cohort of ALS cases, asymptomatic carriers, and healthy controls[185]. Differentially methylated CpGs colocalized with regions containing ALS-associated SNPs, coding for sodium channels *SCN9A* and *SCN7A*. A classifier model based on methylation marks correctly classified all but one of the 17 presymptomatic mutation carriers as healthy, suggesting that the presence of disease better correlates with blood DNA methylation than genetic status, indicative potentially of environmental contributions. Moreover, epigenomic changes can modify age at ALS onset in *C9orf72* carriers[186], also aligned with the view of an “epigenetic age” in ALS.

Advancing ALS exposome studies

As outlined in the “Aspects of the ALS exposome”, numerous studies have examined potential contributions from various external and internal exposures on ALS risk and progression, which have added to our growing understanding of the ALS exposome. Nevertheless, many studies had significant limitations, such as retrospective design that did not fully adjust for all potential confounders. Importantly, most studies relied solely on questionnaires, which are subject to recall bias, especially in a retrospective design, and lacked validation of exposure levels in biosamples. The challenges faced to accurately assess exposures are significant (Box 1), as can be seen from the study limitations outlined in the tables (Supplementary Tables S1–S3). Moreover, few studies have examined genetic profiles in addition to environmental contributions to ALS risk to assess gene–environment interactions. A 2022 systematic review using the Bradford Hill criteria to assess causality between environmental exposures to ALS identified key gaps in ALS exposome studies[16]. These included specificity, dose-response, mechanistic plausibility, and coherence (*i.e.*, overlap between findings from animal models and human epidemiology). We similarly identified lack of mechanistic plausibility studies in ALS, as outlined in the previous section.

To advance understanding of exposome risks, and, further, determine whether knowledge can be leveraged to prevent ALS, large consortia are needed to launch prospective, longitudinal, and long-running studies (Figure 5, Box 2). Indeed, an important priority of the NIH ALS Strategic Plan is forming clinical consortia to complete natural history studies to better delineate ALS risk factors. These consortia will need to enroll persons with a family history of ALS, asymptomatic carriers of known penetrant ALS mutations[6,142], and other at-risk groups along with large numbers of healthy individuals. Large prospective nationwide health studies, such as the UK Biobank, NIH All of Us, or the National Health and Nutrition Examination Survey, could also shed insight into ALS risks among the general population. Consortia participants would be deeply phenotyped longitudinally to prospectively capture exposures that can be linked to disease onset to identify the earliest presymptomatic signs of ALS, *e.g.*, elevated neurofilament light chain[187], and then the earliest ALS symptoms to identify risk factors associated with phenoconversion to ALS.

Future prospective studies will also benefit from the availability of biofluid samples from people well before developing ALS to investigate early biomarkers of disease[188,189] and to identify omic signatures that predict populations most at risk. Such an omic risk profile could be further used to enrich population studies or screen those most at risk of developing disease. Advanced models will also be needed to evaluate certain types of exposures, such as to EMFs. Additionally, genetic profiles and a polygenic risk score of ALS will need to be developed to examine gene–environment interactions. Studies will also need to account for numerous potentially confounding factors, *e.g.*, sports to trauma and to soil exposure[111], military to trauma and to toxicant exposure[56], and carefully and fully adjust to identify independent risk factors for ALS.

Prospective design could potentially facilitate identifying crucial windows of exposure when at-risk individuals are especially susceptible to toxin-mediated damage, unlocking the possibility of preventing ALS. Proof that an environmental intervention can lower ALS

incidence is perhaps best supported by ALS/PDC in the Western Pacific, which declined precipitously when exposure to cycad seeds was reduced[34]. Lastly, mechanistic insight is also needed, which may lead to rational, mechanism-based interventions or preventative measures. These endeavors will require better models of sporadic ALS, or so called “dual hit” models of genetic mutations aggravated by environmental exposures[157,158], analyzed by agnostic multiomics platforms integrating exposomic science[190] to yield knowledge of disease pathophysiology.

Conclusion

Studies of the ALS exposome have advanced significantly since the earliest indications that risk and progression are subject to environmental influences. Replicate studies and meta-analyses have now accrued substantial evidence that persistent organic pollutants and occupation and occupational exposures, among various other factors, contribute to ALS risk and progression. Most studies have relied on questionnaire and survey instruments in a retrospective design, but analysis in biosamples, to quantify exposure directly *in situ* in ALS tissue, especially of analytes with long half-lives, is a crucial way forward, ideally prospectively. Moreover, whereas most studies have focused solely on environmental exposures, increasingly, the trend is to examine gene interactions as well. Research has shed insight into potential mechanisms of environmental exposure-mediated pathophysiology. However, overall, mechanistic studies are sparse but are an important and necessary future direction. It is anticipated that fully elucidating the scope of the ALS exposome may facilitate the prospect of making ALS more preventable by reducing exposure to the most risk-inducing environmental toxicants. Mechanistic studies may further suggest potential therapeutic routes by identifying pathways amenable to therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Dual hit model
A model of both genetic mutation(s) and environmental exposure(s)

Familial ALS
Heritable ALS occurring in individuals with a family history of the illness

Sporadic ALS
ALS occurring in individuals without a family history of the illness

Monogenic inheritance
Inheritance of a trait attributable to a single gene

Polygenic inheritance
Inheritance of a trait attributable to several genes

Gene penetrance
Extent that a gene trait manifests in an individual. Mutations with high penetrance are likely to manifest phenotypically; mutations with low penetrance are less likely

Heritability
Extent that a trait can be explained by inheritance

Exposome
Accumulation of environmental exposures over a lifetime

Gene–time–environment hypothesis of ALS
Posits that disease arises from an interaction of genetic burden with environmental burden over the life course

Microbiome
A community of microorganisms that dwell within a specific habitat. In the context of the human microbiome, the habitat compromises organs, e.g., the gut microbiome, skin microbiome *etc*

Microbiota
Comprises all living microorganisms of a microbiome

Multistep model of ALS
Posits that multiple “hits”, presumably environmental in origin, trigger disease onset, even in carriers of highly penetrant mutations

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Key points

- Amyotrophic lateral sclerosis (ALS) is a fatal disease of motor neuron degeneration, with both genetic and environmental contributions to risk and rate of disease progression.
- The gene–time–environment hypothesis of ALS posits that disease arises from an interaction of genetic burden with environmental burden over the life course.
- The multistep model of ALS posits that multiple “hits”, presumably environmental in origin, trigger disease onset, even in carriers of highly penetrant mutations.
- Epidemiological studies suggest potential contributions to the ALS exposome from pesticides, occupational exposures, sports and physical activity, metals, air pollution, trauma, electromagnetic fields, microbiome, diet, and lifestyle factors.
- Mechanisms remain incompletely understood but may revolve around neurotoxicity from specific environmental toxins, microbiome-mediated changes, epigenetic restructuring, systemic and central inflammation, and excitotoxicity.
- Most studies of the ALS exposome have a retrospective design using questionnaires, prone to recall bias and other limitations. Future studies will require prospective, longitudinal designs quantifying exposures in biosamples in addition to questionnaires.

Box 1.

Summary of current methods/considerations in ALS exposome research. Related to Supplementary Tables S1 and S2.

Study participants

Population-based case-control study:

Can be based on ALS registries or other databases, *e.g.*, death registries, drug prescription. *Advantages*: Less selection bias since cases are recruited from larger registry, compared to recruitment from clinics. If exposures are modeled, then most cases can be included in the analysis, especially for registries/databases with high level of completeness in case ascertainment. Controls can be selected from the population the registry is derived from and matched to cases, ensuring similar characteristics between cases versus controls. *Disadvantages*: In places where ALS is not reportable, registry may be incomplete. If using other databases, case diagnoses may not be fully verified, although engaging a neurologist to validate cases can address this potential issue. Information on cases may be limited to that available in the registry. If cases-controls will be queried, participation rate may be incomplete, leading to potential selection bias.

Cohort case-control study:

ALS cases can be recruited from clinic with informed consent. *Advantages*: Cases seen by neurologist, so diagnoses are clinically confirmed. Cases that consent to participate can be directly queried and clinical information obtained from electronic medical records. *Disadvantages*: Potential for selection bias; cases limited to distinct geographic and population, limiting generalizability. Difficulty selecting controls and ensuring sufficient matching, which may lead to differing characteristics in cases versus controls.

Study design

Retrospective:

Studies designed using historical data. Cases identified from registries, databases, clinics, and referrals, relatively straightforward for a rare disease since cases are specifically recruited to the study. *Advantages*: Datasets are readily available. Modeling exposures or measuring exposures in prospectively banked biosamples not subject to recall bias. *Disadvantages*: Estimating exposure by questionnaires or job exposure matrices may be subject to recall bias.

Prospective:

Studies designed to capture future ALS cases. *Advantages*: All exposure assessments can be prospectively and accurately captured. *Disadvantages*: ALS is relatively rare, and a large cohort would have to be followed prospectively to capture sufficient ALS cases; however, this can be addressed to some extent by selecting a population at-risk of developing ALS.

Assessing exposures

Questionnaires or surveys:

Format can span self-administered or interviewer/investigator-led, by mail, telephone, online, either of the ALS case or their caregiver and of controls. *Advantages*: Can query timeframe of exposure, duration of exposure, intensity of exposure and at the same time collect other demographic, occupational, avocational, residential, environmental, and lifestyle information to fully adjust analyses. *Disadvantages*: Subject to recall bias, subject to selection bias if less progressive cases are more likely to participate. Recall bias can be address by using a prospective study design. If using interviewer led questionnaires, the investigator will not be blinded and possibly inadvertently overemphasize traumatic events for cases. If using self- administered questionnaires, ALS cases may have spent more time thinking about events that may have caused their illness; however, this can be addressed to some extent by including controls with other neurologic diseases.

Job exposure matrices:

Job exposure matrices (JEMs) can be calculated based on job codes for occupations held by cases and controls. *Advantages*: JEMs may be less subject to recall bias than questionnaires that query detailed exposures. *Disadvantages*: JEMs broadly categorize exposures and do not account for possible exposure differences within the same job type. Jobs held need to be coded to calculate JEMs.

Modeling:

Leverage a dataset of geographic exposures, *e.g.*, EMFs, air pollution, and geocoded case and control addresses to model exposures. *Advantages*: Can use large population-based datasets and model over large geographic areas to increase generalizability. *Disadvantages*: Most exposures are characterized by many variables, which are hard to fully account, making it hard to model exposure estimates. Cases and controls may move addresses or may split their time between residences; thus, there are many variables for the participants that are hard to account in the modeling of exposure estimates.

Remote/satellite sensing:

Technology that assesses geographic exposures remotely. *Advantages*: Obviates the need for field sampling. *Disadvantages*: Access to the technology may be limited and generally requires specialized instrumentation.

Biosamples:

Measuring exposures in biosamples is amenable to prospective design. *Advantages*: Directly quantifies exposures in biosamples from cases versus controls. *Disadvantages*: Logistics may be challenging because samples need to be collected, either in clinic, which is easier, but possibly in the field, which is harder. Moreover, collected biosamples need to be appropriately banked. Assessing exposures from biosamples might not capture lifetime exposure, especially depending on tissue turnover and exposure half-life. Moreover, the optimal biosample for analysis remains uncertain. Finally, measuring exposures generally requires specialized equipment.

Box 2.

Outstanding directions in ALS exposome research. Related to Figure 5.

Epidemiological study design

Prospective study design of a large cohort at elevated risk of developing ALS to capture sufficient cases, by collaborating with consortia dedicated to studying the ALS exposome or with large nationwide health studies.

Longitudinal monitoring of the prospective cohort for development of ALS.

Longitudinal questionnaires of prospective cohort participants

Longitudinal banking of tissues/biosamples from prospective cohort participants.

Quantify exposures directly *in situ* in tissues/biosamples and generate genetic profiles from participants with ALS.

Identify crucial windows of exposure.

Investigate gene–environment interactions in participants that develop ALS versus those that do not.

Mechanistic studies

Utilize current ALS models[192].

Generate models of sporadic ALS. Presently, most research relies on mutant $SOD1^{G93A}$. Although some patients with sporadic ALS harbor mutant *SOD1*, this occurs in only 1 to 2%. Patient-derived induced pluripotent stem cells are a potential tool[193].

Generate “dual hit” models of environmental and genetic factors.

Leverage omics platforms, as well as exposomic science, to agnostically interrogate pathophysiology in ALS, including in “dual hit” models.

Generate knockout or overexpression models of potential targets of interest.

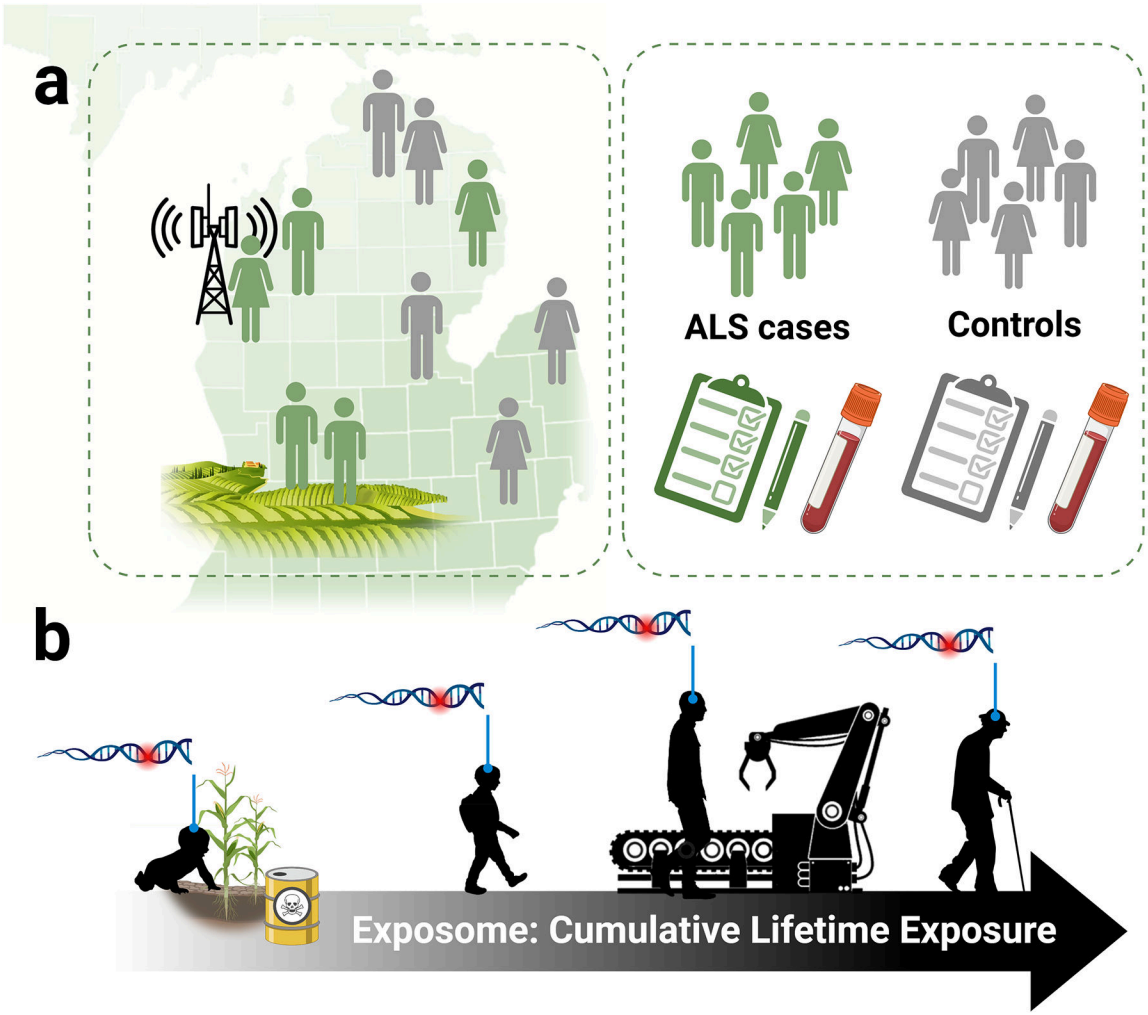

Figure 1. Studying the ALS exposome.

(**a**) Concept of the exposome applied at the population level. Left: Population-based and cohort case-control studies performed by approximating exposures, *e.g.*, to pesticides by proximity to agriculture, to electromagnetic fields by proximity to towers, based on residential address (here in Michigan, as an example) of ALS (green) versus controls (grey) to identify environmental risks. Right: Population-based and cohort case-control studies can query, *i.e.*, by questionnaire, or quantify exposures, *i.e.*, from biofluids, in ALS cases (green) versus controls (grey) to identify environmental risks. Biofluids can also be used to develop genetic profiles. Polygenic risk scores derived from genome-wide association studies can be coupled to exposome studies to facilitate studies of gene–environment interactions. (**b**) Concept of the exposome applied at the level of an individual with a genetic burden (red sphere), predetermined at birth, which interacts with personal exposures over the life course. In this example, the individual was exposed to pesticides as an infant and took up an occupation in production. In the future, a thorough understanding of the ALS exposome and gene–exposome interactions from population studies will help assess individual risk to a specific person, *i.e.*, personalized prediction, with the eventual aim of developing personalized prevention. Created, in part, with BioRender.com.

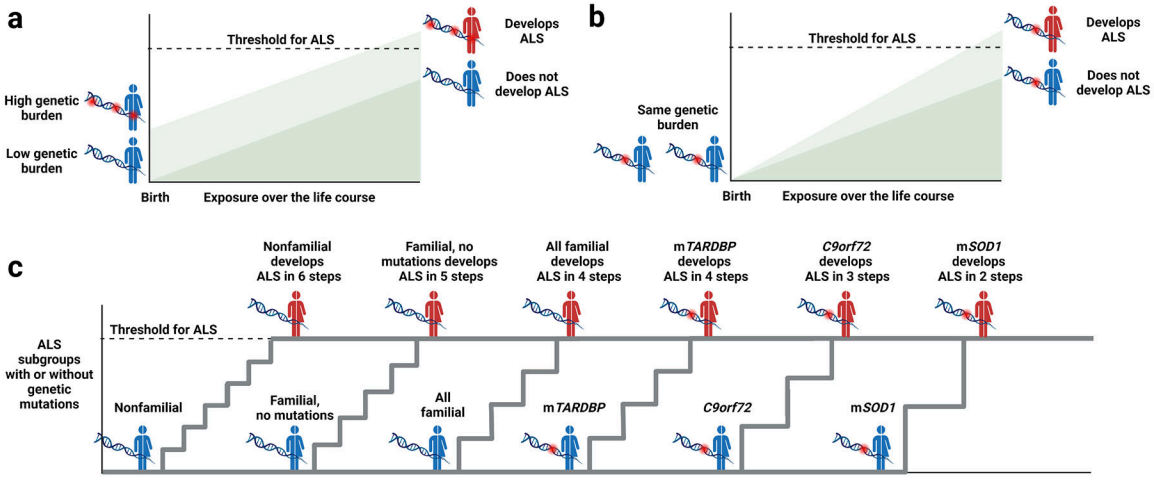

Figure 2. Gene–environment interactions in ALS.

(**a-c**) The gene–time–environment hypothesis of ALS[5]. An individual's genetic burden (blue DNA strand) is predetermined at birth, either by the presence of a single (or few) known penetrant and pathogenic ALS variant(s) (red spheres) or by polygenic risk from multiple single nucleotide variants (red spheres). An individual's environmental burden (green shapes) accumulates during the life course. (**a**) At a constant environmental burden (same slope), individuals with a higher genetic burden (starts with higher risk) will attain the threshold (horizontal dashed line) to develop ALS earlier (red figure) versus an individual with a lower genetic burden [starts with lower risk, (blue figure)]. (**b**) At a constant genetic burden, individuals exposed to a higher environmental burden (steeper slope) will meet the threshold (horizontal dashed line) to develop ALS (red figure) earlier versus an individual with a lower environmental burden (blue figure). (**c**) In the multi-step model of ALS, even carriers of ALS mutations require several steps, possibly from environmental exposure, to trigger disease. For example, carriers with mutant superoxide dismutase 1 (m*SOD1*) require 2 steps, whereas carriers of mutant TAR DNA binding protein (m*TARDBP*) require 4[6]. Created with BioRender.com.

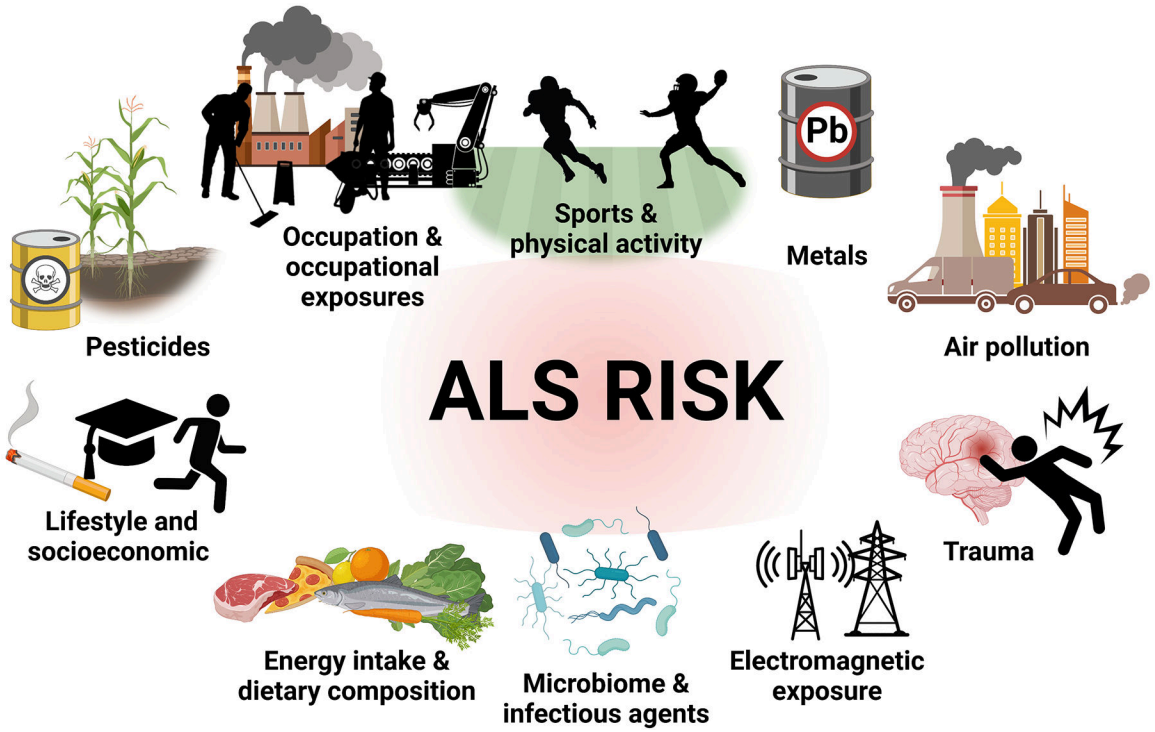

Figure 3. The ALS exposome.

The exposome is comprised of the summation of external, *i.e.*, present in the surroundings, and internal exposures, *e.g.*, microbiome, arising from lifestyle, ecosystem, and interactions with physical, chemical, and biological materials in the environment. In ALS, there is evidence to suggest potential contributions to the exposome from persistent organic pollutants (POPs), including pesticides, occupation and occupational exposures, sports and physical activity, metals, air pollution, trauma, *e.g.*, head/brain, electric shock, electromagnetic field exposure, microbiome, energy intake and dietary composition, and various lifestyle and socioeconomic determinants, *e.g.*, smoking, exercise intensity, education level. Created, in part, with BioRender.com.

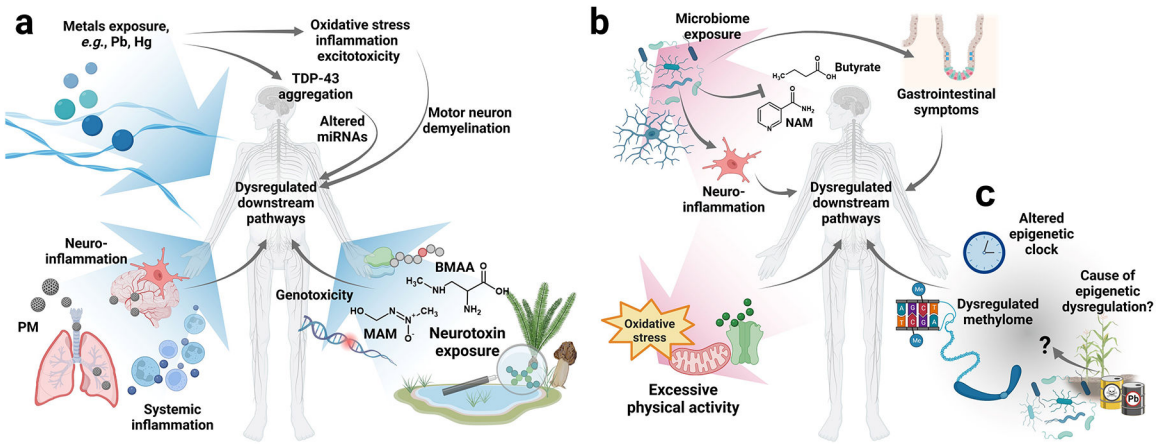

Figure 4. Potential exposome-mediated mechanisms of ALS pathophysiology.

(**a-c**) Few studies have examined exposure-mediated pathophysiology in ALS. (**a**) Potential mechanisms of external exposures in ALS, through metals, neurotoxins, and air pollution. Metals may induce wild-type TDP-43 aggregation in ALS, which would impair miRNA homeostasis, in addition to other possible modes of injury, *e.g.*, oxidative stress, inflammation, excitotoxicity, motor neuron demyelination. Potential neurotoxins are external exposures present in the environment, such as β-N-methylamino-L-alanine (BMAA) and methylazoxymethanol (MAM) from cyanobacteria, in *Cycas* spp. (cycads) and water bodies. The putative mechanism for BMAA toxicity is misincorporation into proteins, whereas MAM causes DNA damage and epigenetic changes. Poisonous mushrooms may also contain neurotoxins. Proposed mechanism of pollution-mediated toxicity in ALS. Particulate matter (PM) enters the lungs and activates systemic inflammation. PM also disrupts the blood-brain barrier and activates central microglial. (**b**) Potential mechanisms of internal exposures in ALS, through microbiome and intensive physical activity. The microbiome in SOD1^{G93A} mice correlates with brain and spinal cord microglial activation[177]; loss of *C9orf72* leads to gut inflammation[151]. The level of beneficial microbiome-derived metabolites may be lower in ALS, *e.g.*, short-chain fatty acids (butyrate)[172,174], nicotinamide (NAM)[150,191], along with intestinal junction, Paneth cell, and enteric neuromuscular defects leading to gastrointestinal symptoms. Proposed mechanism of excessive exercise-mediated toxicity in ALS, *e.g.*, oxidative stress, dysregulated energy metabolism, excitotoxicity. (**c**) The epigenome is altered in ALS, although the causes are likely multifactorial, and may arise from external, *e.g.*, pesticides, metals, or internal exposures[179–181], *e.g.*, microbiome[182], as well as from other pathologies. Epigenetic restructuring occurs in ALS with differentially methylated CpGs, for example to genes regulating GABA signaling and sodium channels. ALS accelerates epigenetic aging (clock). Created, in part, with BioRender.com.

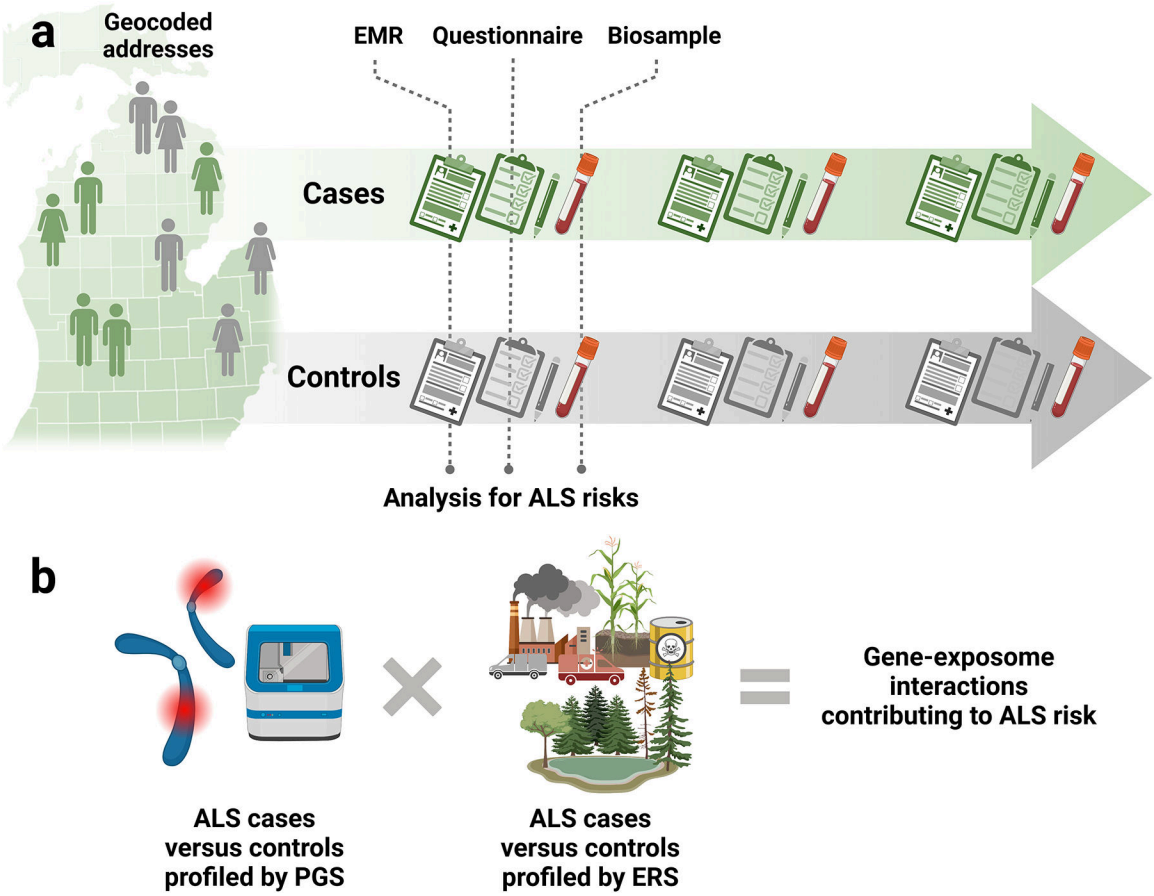

Figure 5. Study design for future ALS exposome studies. Related to Box 1.

(**a**) Advancing ALS exposome science will require longitudinal study of large, prospective, deeply phenotyped case-control cohorts of participants at-risk of developing ALS, *e.g.*, with family history and occupation linked to ALS risk. Electronic medical records (EMR), detailed questionnaires, and biosamples for banking can be collected prospectively and longitudinally over time. Biosamples can be assessed using untargeted exposome-wide methods and exposures can be quantified by environmental risk scores (ERS)[135]. (**b**) Additionally, genetic profiles and ALS polygenic risk scores (PGS) will need to be developed to examine gene–environment interactions. Created, in part, with BioRender.com.