Unraveling the complex interplay between genes, environment, and climate in ALS

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Summary

Various genetic and environmental risk factors have been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). Despite this, the cause of most ALS cases remains obscure. In this review, we describe the current evidence implicating genetic and environmental factors in motor neuron degeneration. While the risk exerted by many environmental factors may appear small, their effect could be magnified by the presence of a genetic predisposition. We postulate that gene-environment interactions account for at least a portion of the unknown etiology in ALS. Climate underlies multiple environmental factors, some of which have been implied in ALS etiology, and the impact of global temperature increase on the gene-environment interactions should be carefully monitored. We describe the main concepts underlying such interactions. Although a lack of large cohorts with detailed genetic and environmental information hampers the search for gene-environment interactions, newer algorithms and machine learning approaches offer an opportunity to break this stalemate. Understanding how genetic and environmental factors interact to cause ALS may ultimately pave the way towards precision medicine becoming an integral part of ALS care.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease that primarily affects motor neurons leading to paralysis and death from respiratory failure. With a prevalence of 4.9 – 12 cases per 100,000 population1 and an incidence of 3.1 cases per 100,000 person-years,2 ALS is a relatively rare disease. It is also a disease of aging, with symptoms typically commencing around age 65.3 These characteristics (a fatal, rare disease of the aged) hint at the underlying etiology and suggest a roadmap to search for ALS risk factors. Today, we know the genetic causes of approximately 20% of cases. The remaining cases are likely caused by a mixture of multiple genetic and environmental factors4 (Figure 1).

Here, we aim to summarise what is known about the environmental and genetic ALS risk factors. We also explore how these factors potentially interact to trigger motor neuron death.

ALS phenotypes

ALS clinical presentation is highly variable across patients. For example, several aspects of the ALS phenotype vary widely, such as the onset age, the extent of damage to the upper or the lower motor neuron, the most affected body region, progression rate, and the occurrence of non-motor symptoms, the most frequent being cognitive impairment.5–7 Despite this, most studies examining the cause of ALS treat patients as a monolith by assuming that the same risk factors are operating in each case. The more significant numbers analyzed by this approach increase the statistical power. However, the assumption is not necessarily valid and different environmental and genetic causes may operate within
Mendelian randomization studies

Most studies looking for ALS etiology are observational in nature. As such, many issues potentially limit the validity of their results, such as selection bias, reverse causality, and confounders. These issues are not unique to ALS and are present in the epidemiological study of any rare disease.

More recently, Mendelian randomization has emerged as a valuable tool to overcome such hidden confounders. Mendelian randomization studies rely on the random assortment of genes from parents to offspring during conception, as described by Mendel’s second law. Since the inheritance of one trait will be independent of the inheritance of other traits, genetic variants related to one exposure will not be linked to those related to potential confounders and can be used as a proxy. Single nucleotide polymorphisms (SNPs) related to a particular exposure are retrieved from the summary statistics of previous GWA studies and then used as a separate outcome GWA to assess the relationship between the exposure and the outcome.

However, Mendelian randomization studies are not free from limitations, and three major assumptions must hold for the results to be valid: (i) genetic variants should be associated with the exposure (relevance assumption); (ii) they must not be associated with confounders (independence assumption); and (iii) they must not influence the outcome through other pathways than the exposure considered (exclusion restriction assumption). The first assumption is usually quantified using the F-statistics, while the remaining two are addressed through sensitivity measures such as the MR-PRESSO and Cochran’s Q test. A practical consideration is that Mendelian randomization requires large datasets to detect meaningful effects, and, ideally, a positive outcome should be replicated in an independent dataset.

Furthermore, the SNPs used as instrumental variables of the exposure are identified based on a specific linkage disequilibrium structure. Hence their validity may not be the same across multiple populations. Ideally, the two GWASes should be selected from the same population to avoid this pitfall. Finally, Mendelian Randomization studies could be limited when considering time-varying exposures, such as behaviours. In these cases, GWAS would be performed at a specific time point while the behaviour could change before and after that point in response to many internal and external stimuli. The confounding effect of time (or aging) is a recurring theme when tackling gene-environment-climate relationships.

Genetic factors

Heritability estimates provide a measure of how much phenotypic or disease risk variance can be explained by genetics. Estimates from twin studies and pedigree studies give higher heritability estimates compared to those from population-level genome-wide SNP data. The difference reflects the contribution of rare variants captured in family studies and perhaps the relatively small numbers of cases involved in twin and pedigree studies yielding wide confidence intervals. With only 30 genes reliably identified to cause or modify ALS risk, a significant proportion of heritability in ALS is still missing.

Despite this knowledge gap, the known genes are already converging on multiple functional pathways. These pathways include global protein and RNA homeostasis dysfunction, oxidative stress and inflammation, mitochondrial dysfunction, impaired cytoskeletal integrity, and altered axonal transport dynamics (summarized in Table 1). Interestingly, many of these pathways are reactive and responsive to exogenous influences. For example, exposure to pesticides, cigarette smoke, traumatic brain injury, and physical activity can induce oxidative stress and inflammation, which could have a knock-on effect on cellular pathways with pre-existing lower tolerance to dysregulation due to predisposing genetic factors. In a recent Mendelian randomization study, physical activity was correlated with disposing genetic factors. In a recent Mendelian randomization study, physical activity was correlated with disposing genetic factors.
changes resulting in altered gene expression and triggering abnormal inflammatory response. 22,23

Taken together, there is a theory of evidence that suggests that the risks conferred purely by genetics, especially by variants with minor effects operating in sporadic ALS, are not sufficient to cause disease. Instead, they require an external trigger to push the system into an irreversible cascade of cell death and loss. This theory could usefully explain some of the variability in penetrance observed from genetic studies.

### Environmental factors

Given that the environmental exposure of an individual (the “exposome”) is essentially infinite, it is perhaps not surprising that a plethora of environmental factors has been implicated in ALS pathogenesis. 4,25,26 These hypotheses predominantly arose from anecdotal cases, patients’ medical history, risk factors identified in other diseases, spatial epidemiology studies, 27 and biological plausibility based on our incomplete knowledge of the molecular mechanisms involved in neurodegeneration. 9 A common criticism of these suspects is that they arise from a “streetlight’s effect,” reminiscent of Captain Louis Renault “rounding up the usual suspects” in Casablanca, rather than being data driven. Clearly, many environmental factors remain to be explored, and there is an unmet need to develop methods to investigate the exposome systematically. 28

In contrast to genetic studies, evidence nominating an environmental factor is seldom successfully replicated. These discordant findings could be due to the patients’ different ancestral origins leading to differential susceptibility to that environmental factor. It could also be driven by environmental exposures being local, circumscribed events within a geographical area. More often, however, methodological differences account for most of this discordance. Selection bias could yield a false relationship or hide a true one. The use of prevalent cases in these studies is concerning, as detected relationships may be driven by survival rather than by risk. Confounders could differentially influence the

| Pathways                          | Genes                                      | Molecular impact on disease                                                                 |
|-----------------------------------|--------------------------------------------|---------------------------------------------------------------------------------------------|
| Protein homeostasis               | C9orf72, SQSTM1, TBK1, CCNF, FUS, TARDBP, OPTN, UBQLN2, VCP, CHMP2B, SOD1, VAPB, SIGMAR1 | Protein aggregation and accumulation due to mutation-induced protein misfolding and/or aberrant out-of-frame protein production hampered by impaired removal via autophagy or ubiquitin-proteasomal pathway. |
| RNA homeostasis                   | C9orf72, SQSTM1, TBK1, CCNF, FUS, TARDBP, ATXN2, SETX, ANG, hnRNPA1, MATR3 | Altered RNA splicing producing cryptic or aberrantly spliced transcripts and dysregulated transcriptome causing differential and pathogenic expression of genes. Aggregation of abberrant transcripts into RNA foci could act as a sink resulting in depletion of other transcripts resulting in further dysregulation of the transcriptome and proteome. These could have knock-on effects on other cellular processes. |
| Oxidative stress & inflammation   | SQSTM1, TBK1, FUS, TARDBP, UBQLN2, VCP, SOD1 | Free radicals could induce abnormal modification of proteins causing it to aggregate and/or altering protein function, cause DNA and organelle damage. Inflammation induced by microglia could aggravate oxidative stress response. Persistent and uncontrolled oxidative stress response drives the formation of irreversible stress granules that can sequester RNA-binding proteins and accelerate pathogenic aggregation of proteins. |
| Cellular energetics               | CHCHD10, TBK1, OPTN, VCP, SOD1             | Structural and functional mitochondrial abnormalities cause defective energy production, abnormal calcium handling, dynamics, and apoptotic signaling. Mitochondria can be damaged by reactive oxygen species, mitochondrial DNA damage, and impaired fission and fusion. |
| Cytoskeletal integrity & intracellular transport | C9orf72, SQSTM1, CCNF, FUS, TARDBP, UBQLN2, TUBA4A, SOD1, ALS2, SPG11, VAPB, FIG 4, SIGMAR1, PFN1, ANXA11, KIF5A | Intracellular trafficking of organelles within the soma and dendrites are important in maintaining a functional intracellular signaling and cross-talk between neurons at the synaptic junctions. Destabilized cytoskeletal scaffold, impaired machineries for axonal transport and endo-trafficking can affect homeostasis of other cellular processes potentially amplifying pathogenic processes already underway. |

Table 1: Crosstalk of genes in functional pathways impacted in ALS.
results across the studies depending on their adjustment
(known) and distribution (unknown). Reduced sample size could lead to a type II error, hiding genuine differences and exacerbating false-positive associations. Finally, measures chosen to assess an exposure could be differently susceptible to information bias (recall bias being the most common).

Compounding these study design effects, most analyses assume a linear model, but this may not be the case for all environmental factors. Looking at other diseases, the relation between alcohol consumption and acute coronary syndrome is J-shaped, for example. Additional complexity arises from the timing and duration of the exposure, circumstances that are rarely taken into account but could strongly influence the risk and vary across studies. An exposure that happened decades before disease onset (e.g., sports-related head injury as a teenager) will be more challenging to study than one that occurs immediately before onset (e.g., bone fracture). Finally, as mentioned above, differing phenotypes could underlie different etiologies. In conclusion, each epidemiological study collecting environmental study is likely closer to being an entirely new experiment rather than a replication of a previous observation.

It should also be considered that collecting and analyzing information across multiple environmental factors could provide insights into disease pathogenesis that would have been missed by considering single exposures. This reinforces the need to focus on the individual exposome, despite some issues that are difficult to overcome. Firstly, many tools are needed to assess the exposures, both through internal (omics characterization) and external measurements. Furthermore, the collection of many variables will need analysis to employ appropriate dimensionality-reduction approaches. Finally, to characterize an individual’s lifetime exposure would require either multiple measures overtime or a smaller number of measures known to be stable over extended periods. Registries could offer a convenient solution to the issue of collecting numerous, accurate, and sequential data on exposures.

Despite these caveats, several environmental factors have been robustly implicated in the pathogenesis of ALS, and we review the evidence for each here.

Cigarette smoking
At this stage, there is sufficient epidemiological and genetic data indicating that cigarette smoking is a bona fide risk factor for ALS. Studies showed an increased risk ranging from 1.43 to two-fold for individuals who smoked compared to never-smokers. Compellingly, the risk was further increased when comparing current smokers to individuals who never smoked. Furthermore, most studies showed a pack-years dose-response relationship, although the relationship may rely more on the exposure duration than intensity. Some studies also showed that lowering the age of smoking initiation could further increase the risk. Finally, even the number of years since smoking cessation was associated with a decreased risk.

This is not to say that every study has agreed with smoking as a risk factor. Negative and weak data for smoking as an ALS risk factor have also been published. A systematic review based on eighteen papers did not support an overall association between smoking and ALS, though it did show a significantly higher risk among women (RR 1.66). While the ease in assessing smoking exposure has aided in studying this risk factor in ALS, factors associated with a benign cardiovascular profile (physical activity) or occupation may have acted as confounders. However, a recent study showed a shared genetic risk between smoking and ALS, while a Mendelian randomization study suggested that confounders do not influence this relationship. These genetic data underscored the epidemiological data implicating cigarette smoking in ALS pathogenesis and provided an early example of the power of genomics to resolve such lifestyle factors.

How smoking increases the ALS risk remains unclear, though theories abound. A single cigarette smoke puff contains considerable quantities of oxidative molecules. Accordingly, one hypothesis is that smoking could overwhelm the antioxidant capacity of cells. Furthermore, some chemicals present in cigarette smoke (lead and formaldehyde) have a neurotoxic effect. Finally, smoking inhibits paraoxonase (PON), an enzyme able to reduce the damage from oxidative stress. This would explain the increased risk for women for whom oxidation mechanisms seem to be upregulated, leading to an increased level of intermediate oxidant metabolites. The change in the smoking habits among women during the last decades could have contributed to the increase of the ALS incidence reported among this subgroup.

Physical activity
There are multiple lines of evidence pointing to excessive physical activity increasing ALS risk. For example, patients often highlighted their high fitness levels before disease onset, and there are anecdotal reports of prominent athletes, including the eponymous Lou Gehrig after whom the disease is named, developing ALS. Furthermore, patients usually show a favorable cardiovascular profile, which could be interpreted as an effect of engaging in physical activity. The relationship has been further fueled by the evidence of a six-fold higher ALS risk among former Italian professional soccer players and a four-fold higher mortality rate for ALS among what Americans refer to as Football compared to the general population. Finally, the hypothesis could be biologically plausible as strenuous exercise
could place increased strain on the motor system necessary to undertake these activities, possibly through an oxidative stress mechanism.49-50

Similar to smoking, the evidence implicating physical activity in the etiology of ALS has been contradictory to date. Four systematic reviews did not find a relationship51-52 or reported inconclusive data.53-55 Even when considering physical activity types separately (occupational, leisure-time, varsity athleticism, professional sport), the reports remained conflicting.55 Moreover, it is unclear which level of exercise is required to drive ALS risk.56 A questionnaire-led, population-based, case-control study including 636 patients and 2166 controls showed no association for vigorous, occupational or cumulative physical activity. In contrast, leisure-time physical activity was significantly higher among ALS patients (OR 1.08, 95% CI 1.02 - 1.14, p = 0.008).57 The peculiar organization of the motor cortex could favour both the athletic prowess and the risk of developing ALS.4 Thus, performing physical activity (leisure-time) would be associated with ALS while occupational may not. A recent PET study may help unravel this conundrum, as it showed distinct changes in the brain metabolism of ALS patients who practiced sport compared to those who did not. Despite having the same level of disability, the former group showed a more diffuse metabolic change in the cerebellar, frontotemporal, and corticospinal tracts, suggesting that these patients could be coping better with the neurodegenerative process.58

Methodological issues may have contributed to the inconsistency of these results. The studies varied widely in sample size, and most of them were retrospective and prone to recall bias and confounders. Other confounders, such as smoking, cardiovascular comorbidities, body mass index (BMI) and hypercholesterolemia,59 were inconsistently accounted for across studies. There is even no consensus on how to quantify the intensity of physical activity, with various methods being deployed, including questionnaires, information on previous occupations, Metabolic Equivalents, Compendium of Physical Activities, or frequency of sweating.59 Also, it is unclear why other types of professional athletes apart from soccer and football players are not at risk.58 Even among soccer players, only some positions, such as midfielder, showed a higher risk for ALS.58 Though initially greeted with skepticism,59 these data have been borne out.

It has been suggested that the increased risk among soccer and football players could be mediated by exposure to pesticides from the field, head traumas, or the use of performance-enhancing drugs.60 However, genomics seems to suggest that physical activity is indeed involved. A previous study using a linkage disequilibrium score regression showed that strenuous physical activity might contribute to ALS risk. Notably, and in keeping with some aspects of the available epidemiological data, light physical activity was protective against the risk of developing ALS.41 Moreover, a recent Mendelian randomization study found that physical activity is directly linked to the ALS risk, suggesting a causal relationship.41

**Dyslipidemia**

Genetic data have been quite conclusive in asserting that hyperlipidemia is an ALS risk factor. A recent Mendelian randomization study ruled out the role of confounders and reverse causality by showing that increased plasma LDL cholesterol is directly correlated to the risk of developing ALS.49 The finding was confirmed separately in European and Asian populations, adding further weight to this risk factor.61 As a consequence, it has been postulated that lowering blood cholesterol levels could reduce the ALS risk.

In contrast to the genetic data, previous epidemiological data reported conflicting results. While some studies showed higher levels of total cholesterol, LDL and LDL/HDL ratio52-64 and HDL,53 others found no differences.65,66 Some studies even reported an opposite ratio.57 Some recent meta-analysis involving 3291 ALS patients and 3367 controls showed no pooled differences in any of the lipid markers considered except for lower levels of total cholesterol among Asian patients.69 Similar results came from a meta-analysis involving 1930 cases and 3635 controls which did not find significant differences for lipid markers, even when considering Caucasian and Asian ethnicities separately.66 Against this, the most extensive study to date analyzing nearly 700 drugs based on Medicare data that included 10,450 ALS cases and 104,500 controls showed that a broad range of antihyperlipidemic drugs with different mechanisms of action were associated with a lower ALS risk. These data strongly point to hyperlipidemia influencing ALS risk, rather than a secondary effect from any particular drug class.70

However, there are several reasons to trust genetic over retrospective epidemiological studies in this case. First, the lipid profile is part of a more global cardiovascular profile, including BMI,67,68 glycaemia,73 cardiovascular comorbidities, and cigarette smoking. Each of these factors could have acted as a confounder in the retrospective epidemiological studies. Second, ALS is characterized by a hypermetabolic state,74 and abnormal lipid profile observed after patients develop symptoms could merely reflect secondary changes arising from the degenerative process. Such reverse causality could affect epidemiological studies but not properly designed genetic studies. Finally, the epidemiological studies typically employed sample sizes that were several folds smaller than those utilized in genome-wide studies and Mendelian randomization analyses.
Other environmental factors

Many other environmental factors have been proposed as influencing the risk of developing ALS, including heavy metals, pesticides, electromagnetic fields, occupations, drugs, and cyanotoxins. Evidence for these factors has been weaker; however, they could be involved in less common pathogenetic mechanisms (see below) and should not be overlooked.

Climate

The effect of climate change on the environment must be carefully considered. Earth is racing towards at least a 1.5 °C temperature increase over the following decades, resulting in myriad ecological and geopolitical changes. The resultant environmental upheavals will have lasting effects on public health concerning food security and water access, the geographical distribution of vectors responsible for zoonotic infections, exposures to industrial pollutants, and even the pattern of renal, cardiovascular and pulmonary conditions. For example, the environmental conditions conducive for malaria transmission will extend over many areas of the United States, South America, Europe, and Australia by the end of the century.

Neurological disorders should also be included in that rubric, given the relative importance of the local environment in their pathogenesis. Much importance has been placed on the butterfly effect, whereby minor, locally defined events have outsized effects on distant environs. However, we must not forget that the opposite is also true: planetary climate change due to an excessive release of greenhouse gases into the atmosphere will alter local air pollution patterns, a risk factor for many neurodegenerative diseases, including ALS. Shifting industrial bases due to food scarcity and economic forces arising from climate changes could compound these environmental disruptions. Humidity will increase because of the higher rate of water evaporation from the earth’s surface. A previous study showed that the birth seasonality among four ALS cohorts across the two hemispheres correlated with humidity rates during the year. Presciently, the authors argued in 2012 that this aspect could be influenced by an imminent climate change. Finally, a climate temperature increase could directly or indirectly lead to increased oxidative stress, changes in cerebrovascular hemodynamics, excitotoxicity, and microglial activation, all implicated in neurological conditions, such as ALS.

The effect of climate changes may not just be isolated to the environmental aspect of gene-environment interactions. Population displacement due to climate change could cause the exposure of immigrants with certain genetic factors to new external agents to which they had not been previously exposed. Such hazardous materials, neurotoxins and infections could adversely affect their nervous system. While the local population has evolved a tolerance level to their local environment, migrants to the area may lack the same protection, making them more susceptible.

Such considerations are speculative and unexplored at present. However, the 2021 United Nations Change Conference (COP26) highlighted the potentially devastating effects of climate change on the environment. Consequently, it is worth keeping this hypothetical scenario in mind as we could be facing it soon.

Gene-environment interactions

A fundamental conundrum in studying environmental and lifestyle exposures is that they tend to be common in the community (e.g., smoking, exercise, and hyperlipidemia), and yet ALS is ostensibly rare. Gene-environment interactions offer a possible explanation for this and may be particularly relevant when considering rare, sporadic diseases that occur in later life. Under this model, not every person exposed to an environmental factor would develop the disease. Instead, only those individuals with an underlying genetic predisposition would be at risk. Environmental factors alone or genetic factors alone would be insufficient to lead to motor neuron degeneration. Instead, the interactions between these environmental and genetic factors drive the issue. While such interactions could be rare in the general population, they could explain many cases in the ALS subpopulation. In the absence of a deeper understanding of this mechanism, the sporadic occurrence of the disease in the community would look stochastic. In reality, it hints at a much more complex pathogenic system.

Various methodologies have been developed to model gene-environment interactions and are described elsewhere. The most straightforward approach would be examining factors already known to be associated with ALS. For example, the risk exerted by an environmental exposure associated with ALS could vary according to the presence or not of one of the genetic variants listed in Table 1. Epidemiological studies could explore this hypothesis using stratified analysis in which the risk of developing the disease is calculated across the possible combinations of the two factors. If the risk among individuals doubly exposed is greater than the product of the risk given by the two factors individually, then a multiplicative interaction can be inferred. For example, both asbestos and cigarette smoking increase the risk of developing lung cancer, but those exposed to both have a risk that far exceeds either alone. In another example, estrogen use was globally protective against the risk of Alzheimer’s Disease, but the risk was only truly decreased among estrogen-negative women (HR = 0.59; 95% CI, 0.36 – 0.99). The gene-environment interactions may also exist on an additive scale. In this paradigm, the joint exposure
risk would differ from the sum of the single exposures risks.94-95 It is still debated whether the multiplicative or additive measure more truly reflects the underlying biological interactions, and each may be valid depending on circumstances.96 For example, if the etiology involves multiple steps (as is postulated for ALS98,99), two factors acting at the same stage will fit an additive model. In contrast, factors acting at different stages will fit a multiplicative hypothesis.90 If both exposures are needed for the disease to develop, interaction on an additive scale should be searched.91 However, interactions on a multiplicative scale have been more widely used, perhaps for the practical reason that they are prominent in magnitude and easier to detect.95

Our discussion so far has been predicated on the notion that each interacting factor is associated with ALS on its own. However, there are many complex interactions in nature where this is not the case. For example, individuals with glucose-6-phosphate dehydrogenase deficiency are usually asymptomatic as the genetic predisposition exerts no effect on its own. However, these patients develop fever, dark urine, and abdominal pain when they consume fava beans.90 The LIPC gene was associated with high cholesterol levels only after adding dietary fat to the model.101 A polymorphism of the serotonin transporter gene (SLC6A4) promoter moderates the influence of stressful life events on depression while not exerting an effect in the absence of stressors.102 Many other examples come from studies assessing the risk of asthma, lung cancer, type 2 diabetes, and heart diseases.88 These types of interaction are much more difficult to detect, particularly in a rare disease such as ALS.

Genetics could also predispose to an environmental exposure which in turn causes the disease. For example, an individual could be predisposed to commencing cigarette smoking, which will increase the ALS risk. This is not a gene-environment interaction in the proper sense but rather a covariance (or gene-environment correlation). This covariance could also act the other way around, such as the case of older paternal age increasing the risk of mutagenesis103 or somatic mutations accumulating within the brain as the person ages.104 This latter mechanism may be an elegant explanation for the apparent discrepancy between the low heritability estimates and our failure to date to identify significant environmental factors underlying ALS.

Nevertheless, such possibilities should be carefully considered as they could reflect the underlying biology or act as a noise obscuring other findings.105 Gene-environment interactions could also explain the phenotypic variability among siblings carrying the same genetic mendelian mutation. Additional genetic variants or environmental exposures could determine a different age of onset106 or a different disease,107 or even prevent the disease from happening (reduced penetrance).90

To date, there have been a limited number of studies examining gene-environment interactions in ALS. A study involving 143 ALS patients and an equal number of controls found that genetic variants in the PON1 gene increased the risk of ALS among individuals exposed to pesticides.108 A second study explored the interaction of the same gene using population density as a proxy of environmental exposure to pesticides. The authors found a significant interaction between density population and a marker rs854560 (L55M) at the genotypic level.109 However, it is not easy to draw meaningful conclusions from these publications. Instead, these studies highlight the difficulties in designing studies to examine gene-environment interactions studies in ALS.

First, there is a paucity of accurately measured data for both genetic and environmental factors. While genome sequencing has become progressively more affordable,110 the same cannot be said for the exposure, where only limited data are available.24 More recently, spatial data analysis has emerged to infer an environmental effect.6,27,111 Furthermore, metabolomics and epigenetics are increasingly used as proxies of environmental exposures. Particularly in the case of metabolomics, it can be challenging to distinguish an environmental effect precipitating the disease from a secondary effect of the disease itself.28 On the other hand, epigenetics could offer an extra layer to consider when seeking the effect of environmental factors on genetics. Such information would complement the genomic sequence information; environmental factors can alter gene expression through epigenetic modifications (such as DNA methylation, histone modifications, chromatin remodeling) and each can be quantified with appropriate methods.112 Nevertheless, the nature of quantifiable measures continues to improve and may offer an opportunity to understand gene-environment interactions in ALS more completely.

As a second major issue, many samples are required to study ALS. This is due to its rarity, the high number of exposure variables that need to be considered, the low frequency of genetic variants involved, and the minimal effect that the various factors are expected to have. A priori knowledge could partially reduce the sample size needed; data collection could be limited to environmental data known to act on pathways involved in the disease pathogenesis. However, this hypothesis-based approach limits the discovery of new variants and environmental exposures.38 As an alternative strategy, a polygenic risk score could be used to measure genetic predisposition. A recent study investigated the interaction between genetic susceptibility, as assessed by the weighted sum of previously associated loci, and the exposure to household coal for the risk of developing lung cancer among Asian females. Using two different cohorts, the authors found that the risk given by the genetic predisposition was weaker among coal ever-
motor neurons.115 Being the third variable in the ALS equation, time likely interacts with the genetic predisposition, influencing the age of onset.5

Finally, here we focused on gene-environment interactions, but interactions could also occur between two (or more) environmental exposures or genetic variants (epistasis).88 Recent and more sophisticated methods to explore large-scale gene-environment interactions have been developed and could help solve the riddle of dealing with many variables.116,117

Conclusions
Gene and environmental factors could be variably involved in ALS pathogenesis. Exploring their interactions will likely give some critical insights. So far, the field has dealt chiefly with simplistic etiological models of ALS in which nature and nurture were considered as a dichotomy.28 Furthermore, environmental factors are typically studied at diagnosis, whereas their window of action could precede the onset by many years. While simple is not necessarily wrong, studying one factor at a time has potentially hidden effects that will become apparent when multiple factors are considered simultaneously. Knowledge of gene-environment interactions would allow the identification of susceptible individuals who could then be targeted for specific interventions. For example, they could be advised to avoid the culprit exposure during their lives. This precision medicine approach for ALS could lessen the risk of developing the disease or delay onset by decades.28

Outstanding questions
Many questions must be addressed as we travel along this Road to Utopia. Which exposures and genetic variants make the most sense to examine early on? How should we make our selection? While such a candidate-based approach would dramatically lower the need for data, it would hamper discovering new genetic and environmental factors hidden due to our limited knowledge of biological processes. The next question follows from this observation: is it feasible to study gene-environment interactions over many exposures in a rare disease such as ALS? The era of big data, along with the easy-to-access data repositories, offers us perhaps the first real glimmer of hope to undertake such a study. Recently published algorithms can search for interactions on a genome-wide and exposome basis even in the setting of limited sample sizes and power.116,117 Machine Learning techniques may reveal unexpected data-driven correlations and prioritize a smaller number of factors for study.119–121 However, in the end, the data lake will likely need to expand in size dramatically. A concerted effort is required to make environmental and genome-wide data from large population-based settings publicly available.28

Search strategy and selection criteria
Data for the review were identified through searches from PubMed with the following search terms: “Amyotrophic Lateral Sclerosis”, “ALS”, “environment*”, “genetic*”, “interaction*”. Only articles in English with published up to August 2021 were included; references of the collected published studies were also considered. Selection of the most appropriate references was made by the authors.

Declaration of Competing Interest
Rosario Vasta and Ruth Chia declare no conflicts of interests.

Bryan J. Traynor holds the US, Canadian and European patents on the clinical testing and therapeutic intervention for the hexanucleotide repeat expansion in C9orf72. He is an associate editor of Brain and sits on the editorial boards of Neurobiology of Aging, the Journal of Neurology, Neurosurgery, and Psychiatry, and EClinicalMedicine.

Adriano Chiò serves on the editorial advisory board of Amyotrophic Lateral Sclerosis and Neurological Sciences and has received research support from the Italian Ministry of Health (Ricerca Finalizzata), Regione Piemonte (Ricerca Finalizzata), University of Turin and the European Commission (Health Seventh Framework Programme) and serves on scientific advisory boards for Mitsubishi Tanabe, Roche, Denali Pharma, Cytokinetica, Biogen, and Amylyx and has a research contract with Biogen.

Contributors
RV and RC wrote the manuscript and designed and produced the figure and the table. BJT and AC outlined, supervised, and edited the draft and approved of the final version to be submitted. All authors approved the final version of the manuscript.

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