PNAS COMMENTARY

Linking ALS mutations to the disruption of the MHC-II pathway

Zhe Zhang[a] and Shuying Sun[a,b,c,1]

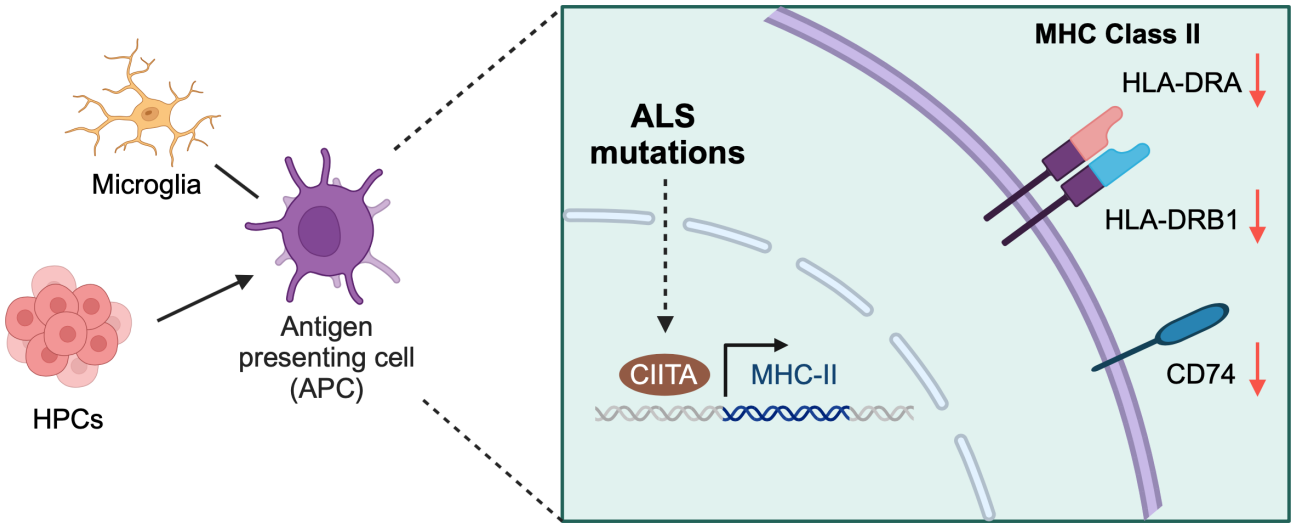

Fig. 1. ALS mutations result in the decreased expression of components in the MHC-II pathway. Microglia serve as the antigen-presenting cells in the central nerve system, and HPCs can give rise to antigen-presenting cells in the peripheral system. In both cell types, FUS and C9ORF72 mutations lead to decreased expression of HLA-DRA, HLA-DRB1, and CD74, the key components of the MHC-II pathway through the regulation of transcriptional factor CIITA. Such alterations in the pathway could interrupt immune response. Created with BioRender.

Amyotrophic lateral sclerosis (ALS), commonly known as Lou Gehrig's disease, is a progressive neurodegenerative disorder that primarily affects motor neurons in the brain and spinal cord. As the disease progresses, the affected neurons degenerate, leading to muscle weakness and eventually paralysis and death by catastrophic respiratory failure (1). ALS is characterized by its clinical heterogeneity. Genetic mutations play a significant role in the etiology of ALS, which contributes to approximately 10% of all ALS cases (2).

Immune response is broadly recognized to influence the progression of ALS, supported by patient samples and animal models (3). However, the direct connection between genetic causes and the impaired immune system was still elusive. In this issue of PNAS, Chi et al. reported that several ALS-associated RNA/DNA-binding proteins and C9ORF72 repeat expansion influence the gene expression of components in the major histocompatibility complex II (MHC-II) antigen presentation pathway (4). This possibly leads to immune system dysfunction which can contribute to neurodegeneration in ALS.

There have been lots of discussions about the involvement of the immune system in the onset and progression of ALS. Neuroinflammation in the central nervous system (CNS), evidenced by the activation of microglia, astrocytes, and the alteration of cytokines, is recognized as a hallmark of ALS (5, 6). On the one hand, as the primary immune cells of the CNS, microglia are activated and exhibit a neuroprotective effect by producing anti-inflammatory cytokines and clearing cell debris (6). On the other hand, they also release proinflammatory cytokines and reactive oxygen species that damage neurons during disease progression (6). In addition to the increase of activated microglia in postmortem tissues, dysregulated inflammatory cytokines and elevated active state of T cells have been identified in the cerebrospinal fluid (CSF) of patients, suggesting the changes of immune response in the CNS (5).

There is also compelling evidence supporting altered peripheral immune response in ALS. Analyses of blood samples from ALS patients showed altered levels of cytokines, such as TNF-α, IL-6, and IL-1β (7). Moreover, a reduction in $CD4^+$ T cell numbers and an elevation in the $CD4^+/CD8^+$ T cell ratio have been observed in patient blood samples (8).

Author affiliations: [a]Department of Physiology and Brain Science Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205; [b]The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205; and [c]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

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[1]To whom correspondence may be addressed. Email: shuying.sun@jhmi.edu.

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A subset of $CD4^+$ T cells, Tregs, which suppresses immune response has also been found to be dysregulated in ALS, potentially leading to reduced ability to control inflammation (5, 8). Additionally, there is evidence suggesting the interaction of the central and peripheral immune system (9). The active T lymphocytes, especially $CD4^+$ and $CD8^+$ T cells, as well as natural killer (NK) cells have been reported to penetrate the blood–brain barrier in ALS patients, indicating an adaptive immune response (10–12).

> **"Chi et al. identified the MHC-II antigen presentation as a common pathway influenced by multiple ALS-linked mutant genes."**

Despite numerous observations of immune response changes in ALS, the direct link between genetic mutations and impaired immune functions remains elusive. Recently, accumulating evidence revealed a correlation between *C9ORF72* repeat expansion and immune changes, especially due to the loss of function of the C9ORF72 protein (13). Two independent *C9orf72* knockout mouse lines consistently exhibited deficits in the innate immune system, resulting in splenomegaly, lymphadenopathy, and elevated proinflammatory cytokines (14, 15). Within the CNS, C9ORF72 is highly expressed in microglia (15). Loss of C9orf72 results in the accumulation of lysosomes and increased proinflammatory microglia in mice (15). Although the absence of C9orf72 is not sufficient to induce neuronal loss or impaired motor function, it can exacerbate pathological and behavioral phenotypes in transgenic mice expressing the expanded repeats (16). This indicates that the immune defects caused by C9ORF72 haploinsufficiency can contribute to neurodegeneration.

Chi et al. previously revealed that a group of ALS-associated RNA/DNA-binding proteins, including FUS, EWSR1, TAF15, and MATR3, are components of U1 snRNP and RNAP II machinery (17). Many studies focused on their functions in neurons (18), although these proteins are expressed broadly in many cell types, including immune cells. In this work, Chi et al. found that many immune-related proteins were down-regulated after knocking out FUS, TAF15, or MATR3 in HeLa cells using quantitative mass spectrometry analysis (4). Among the down-regulated genes affected by all the three RNA/DNA-binding proteins, a significant portion has functions related to antigen presentation. Interestingly, HLA-DRA and HLA-DRB1, the major components of the HLA-DR (Human Leukocyte Antigen–DR isotype) complex which functions in MHC-II antigen presentation (19), were the top down-regulated proteins. CD74 was known as the HLA-DR gamma chain, which stabilizes HLA-DR and chaperons the complex to the endosomal system for antigen processing (20). Related to the reduction of HLA-DR components, the CD74 mRNA level was also decreased in all the lines knocking out FUS, TAF15, or MATR3. The authors further validated the findings in the human HMC-3 microglia cell line. The downregulation of the MHC-II pathway was evident in cells with FUS, EWSR1, TAF15, or MATR3 knockdown. Altogether, this indicates that these RNA/DNA-binding proteins play important roles in regulating HLA-DR mediated MHC-II antigen presentation to T cells. The authors further demonstrated that the reduction in the components of the MHC-II pathway is due to the down-regulation of CIITA, the major transcription factor regulating gene expression of HLA-DR and related factors (Fig. 1).

To further study the effect of disease-causative mutations on the MHC-II pathway, Chi et al. used the FUS^{R495X} mutation as an example. Two cell types were used: HMC-3 microglia and human embryonic stem cell (ES)-differentiated hematopoietic progenitor cells (HPCs) that can give rise to various types of immune cells. The R495X heterozygous mutation was introduced to endogenous FUS by CRISPR editing in both systems. The authors found a reduction of HLA-DRA, HLA-DRB1, and CD74 expression levels along with the decreased CIITA in FUS^{R495X} mutant cells. Interestingly, they did not find similar changes in the FUS^{R495X} ES cells before differentiation to HPCs, suggesting that the reduction of MHC-II-associated genes is cell type specific. Additionally, Chi et al. found the reduction of the same panel of genes in induced pluripotent stem cell (iPSC)-differentiated HPCs derived from C9ORF72-ALS patients. This suggests that the disruptions in the MHC-II pathway in HPCs could be a common feature in ALS associated with FUS and C9ORF72 mutations. This finding also unveils a novel potential mechanism underlying altered immune responses in C9ORF72-ALS/FTD.

Chi and colleagues have identified MHC-II antigen presentation as a common pathway influenced by multiple ALS-linked mutant genes. This study reveals novel molecular mechanisms on how disease-causative mutations can lead to perturbed gene expression related to immune cell function. The disruption of the MHC-II pathway will likely influence the immune response and therefore contribute to the observed neuroinflammation in both mouse models and patients. The findings of this research underscore the importance of potential non-cell-autonomous toxicity in neurodegeneration and warrant more consideration for therapy design. The finding also sparks new intriguing questions. For instance, how do mutations in these diverse genes all lead to alterations in the MHC-II pathway? Can similar defects be detected in animal models or patient samples? What is the functional outcome of the reduced MHC-II pathway in immune cells and how this affects neurons? Is the restoration of CIITA expression sufficient to rescue the immune cell defects induced by the disease-associated mutations? Answering these questions could further improve our understanding on the molecular mechanism of immune dysregulation and its contribution to the disease etiology of ALS and provide insights on the development of biomarkers and therapeutic strategies.

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