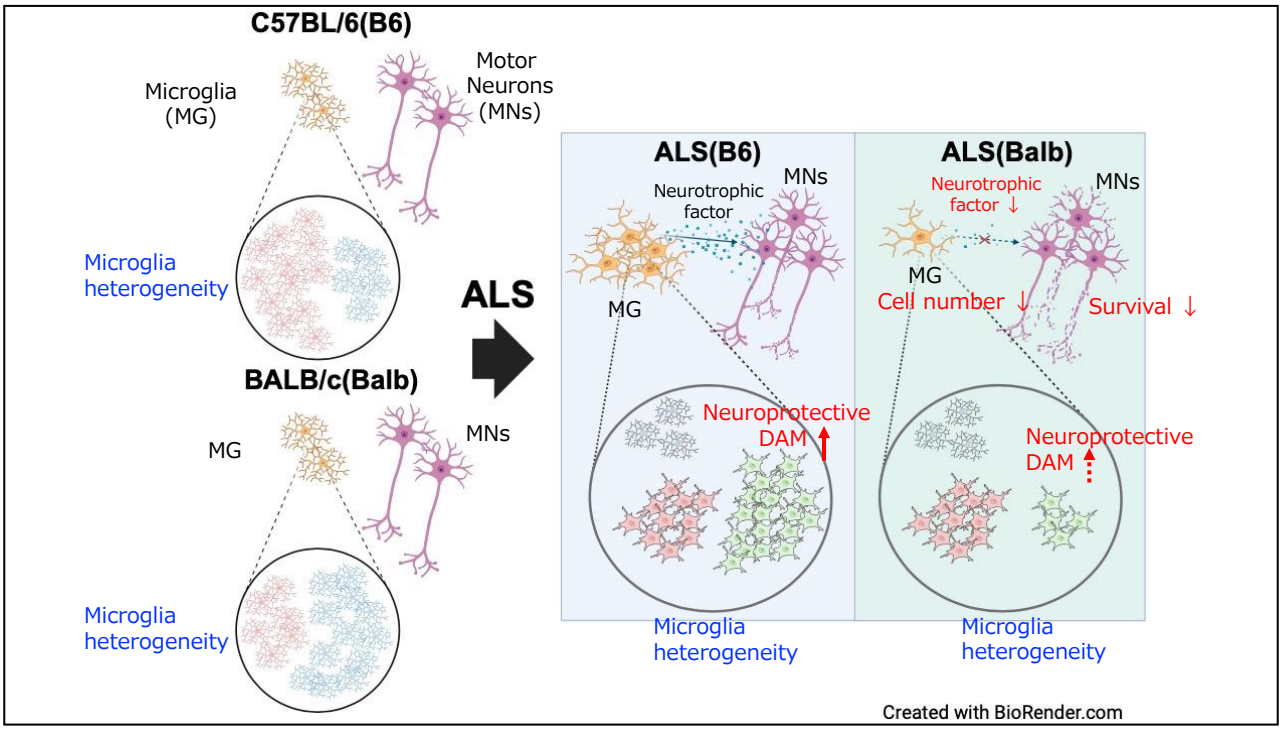
News Release

Title

Genetic background variation impacts microglial heterogeneity and disease progression in amyotrophic lateral sclerosis model mice

Key Points

•The widely used inbred mouse strains, C57BL/6 and BALB/c show different microglia heterogeneity.
•The change in genetic background from C57BL/6 to BALB/c affected microglial heterogeneity and ALS pathology and its progression, likely due to the defective induction of neurotrophic factor-secreting Disease-associated microglia (DAMs) and the decreased number of microglia.
•The environmental factors derived from peripheral immune cells may affect disease progression by regulating microglial heterogeneity, survival, and DAM induction.



Summary

Recent single-cell analyses have revealed the complexity of microglial heterogeneity in brain development, aging, and neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). Disease-associated microglia

(DAMs) have been identified in ALS mice model, but their role in ALS pathology remains unclear. The effect of genetic background variations on microglial heterogeneity and functions also remains unknown.

The research group led by Dr. Okiru Komine (first author) and Prof. Koji Yamanaka (Department of Neuroscience and Pathobiology, Research Institute of Environmental Medicine (RIEM), Nagoya University), in collaboration with Prof. Tomoo Ogi (Department of Genetics, RIEM, Nagoya University) and Associ. Prof. Kunihiko Hinohara (Department of Immunology, Nagoya Univesity Graduate School of Medicine) revealed that genetic background variation impacts microglial heterogeneity and disease progression in ALS model mice.

To examine the effect of genetic background variation on microglia heterogeneity and ALS disease, they established and analyzed two mice models of ALS with distinct genetic backgrounds of C57BL/6 and BALB/c (ALS(B6) and ALS(Balb)). They observed that the change in genetic background from C57BL/6 to BALB/c affected microglial heterogeneity and ALS pathology and its progression, likely due to the defective induction of neurotrophic factor-secreting DAMs and the decreased microglia number. Single-cell analyses of ALS mice and RNA sequencing analyses of microglia also revealed a possible association between microglial heterogeneity and systemic immune environments.

The researchers expect their finding provides new evidence that genetic diversity may influence microglial heterogeneity, their responses, and disease progression in ALS. Furthermore, the findings will contribute to a better understanding of the complicated pathomechanism of motor neuron disease and may lead to the identification of new biomarkers and new therapeutic approaches to regulate the microglia heterogeneity and their neuroprotective functions through regulating the immune system.

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Research Background

ALS is a devastating neurodegenerative disease characterized by the selective loss of motor neurons, resulting in skeletal muscle weakness and death within 2–5 years after onset. Recent single-cell analyses have revealed the complexity of microglial heterogeneity in brain development, aging, and neurodegenerative diseases such as ALS. DAMs have been identified in ALS mice model, but their role in ALS pathology remains unclear. The effect of genetic background variations on microglial heterogeneity and functions also remains unknown.

Research Results

To examine the effect of genetic background variation on microglia heterogeneity and ALS disease, the researchers established and analyzed two mice models of ALS with distinct genetic backgrounds of C57BL/6 and BALB/c (ALS(B6) and ALS(Balb)) and performed single-cell RNA sequencing analysis in the spinal cords of ALS(B6), ALS(Balb), and both wild-type mice (WT(B6) and WT(Balb)). They found that both wild-type strains showed different microglia heterogeneity (Fig 1, lower left). They also observed that ALS(B6) microglia were found to exhibit increased ratios of a part of DAM, whereas ALS(Balb) microglia displayed slightly increased ratios of it (Fig 1, upper left). The number of microglia also decreased in ALS(Balb) mice (Fig 1, right).

To investigate the effect of altered microglial heterogeneity on ALS pathogenesis in mice, they next performed survival analyses on ALS(B6) and ALS(Balb) mice. ALS(Balb) mice exhibited a significantly shorter survival time than ALS(B6) mice, despite having comparable onset times, indicating that ALS(Balb) mice accelerated the disease progression (Fig 2).

Therefore, to elucidate the mechanism of the accelerated disease progression in ALS(Balb) mice, they compared the gene expressions of the DAM, whose induction is weak in ALS(Balb) microglia, among all strains. They found that *Igf1* (Insulin-like growth factor-1), a neurotrophic factor, was highly expressed and distributed in the DAM (Fig 3).

Finally, to determine whether a cell-autonomous (intracellular) or non-cell-autonomous (extracellular) factor significantly affects microglial phenotypes, we compared the gene expression profiles of microglia isolated from all strains and primary cultured microglia from WT mice (in vitro (B6) and in vitro (Balb)). Principal component analysis (PCA) revealed that the contribution ratio of PC1 likely derived from multiple environmental effects was highest (57.6%), while those of PC2 likely derived from differences between healthy (normal) and ALS disease (with mutant SOD1) and PC3 likely derived from a cell-autonomous effect caused by the clear separation of each primary cultured microglial cell were 16.4% and 10.5%, respectively (Fig 4). These results indicate that cell-autonomous differences in gene expression alone cannot account for all microglial phenotype variations *in vivo*. Notably, inbred C57BL/6 and BALB/c strains usually exhibit biased peripheral helper T-cell responses, Th1, and Th2, respectively. At the disease end-stage, we found altered ratios of peripheral immune cell populations and Th1- or Th2- biased peripheral immune responses between ALS(B6) and ALS(Balb) mice (Data are shown in the original paper.), indicating that the peripheral immune environment may affect disease progression by regulating microglial heterogeneity, survival, and DAM induction.

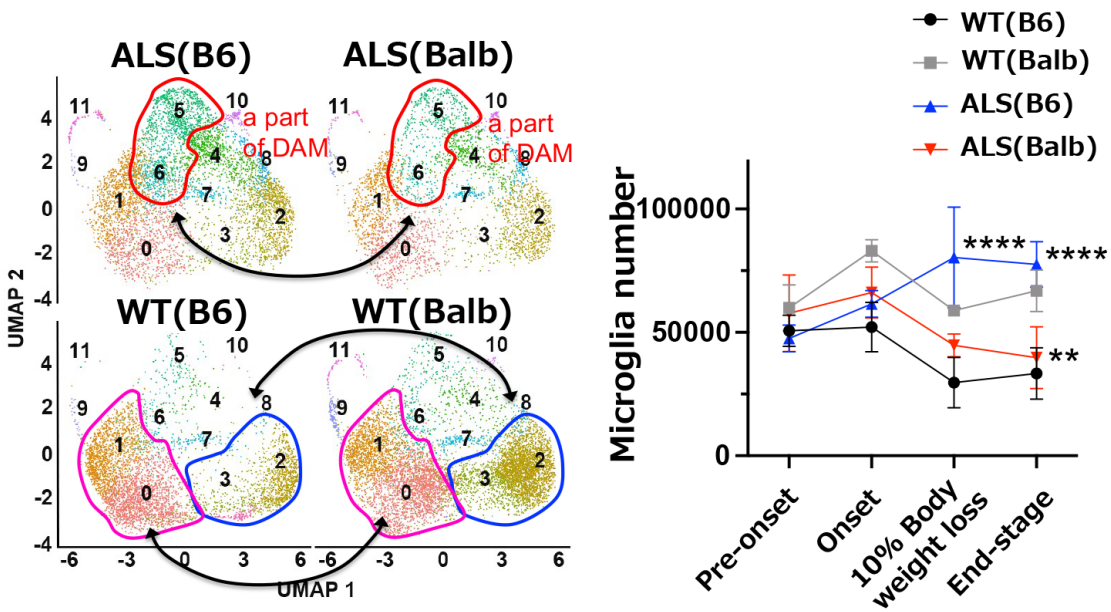

Fig 1 Single-cell analysis of microglia revealed that microglial heterogeneity was different between WT(B6) and WT(Balb) and a part of DAM slightly increased in ALS(Balb) (left). The microglia number also decreased in ALS(Balb) (right).

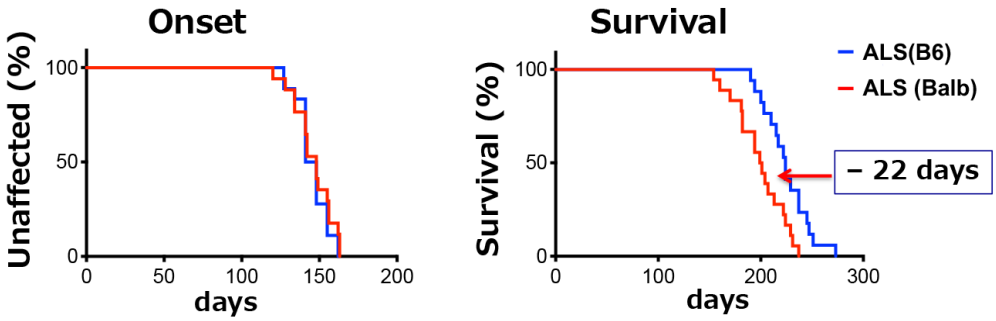

Fig 2 ALS(Balb) mice displayed faster disease progression.

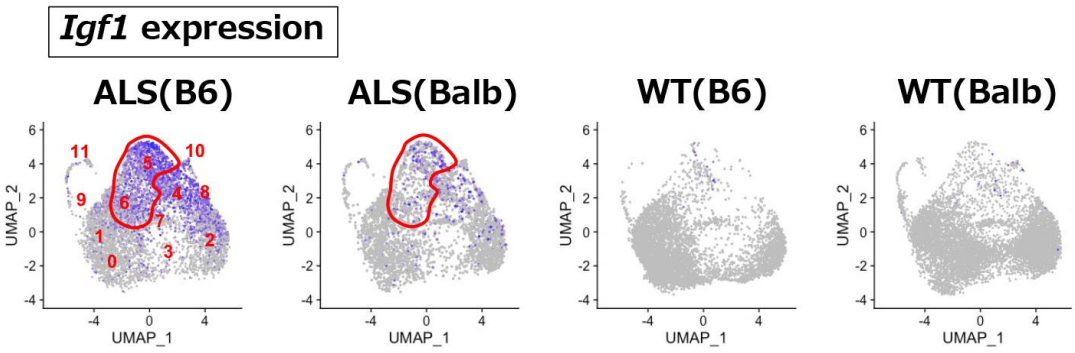

Fig 3 *Igf1* was highly expressed and distributed in the DAM, whose induction is weak in ALS(Balb) microglia.

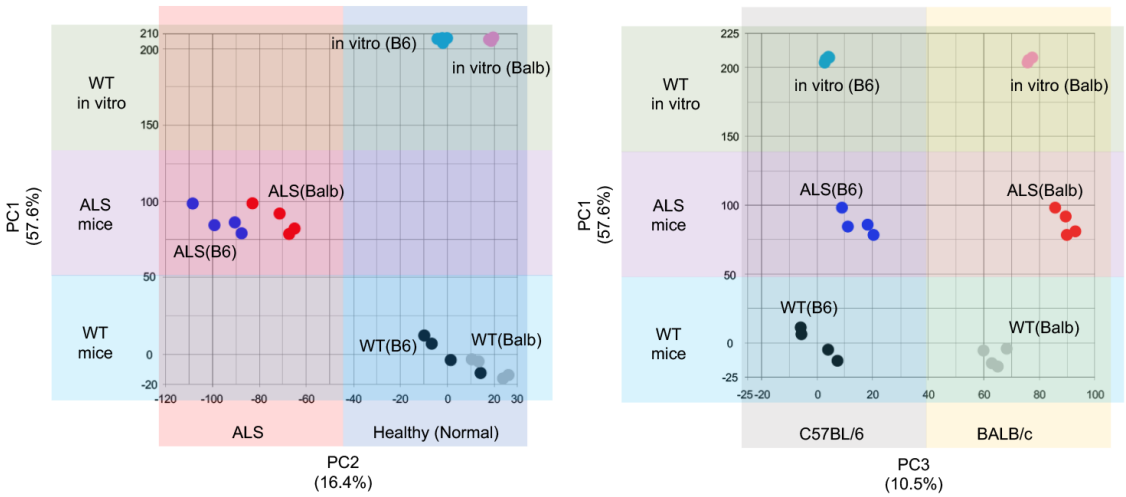

Fig 4 Principal component analysis (PCA) of the gene expression profiles of microglia isolated from all strains and primary cultured microglia from both wild-type mice

Research Summary and Future Perspective

This study establishes for the first time that genetic background variations affect microglia heterogeneity, their responses, and disease progression in ALS model mice. The finding will contribute to a better understanding of the complicated pathomechanism of motor neuron disease and may lead to the identification of new biomarkers and new therapeutic approaches to regulate the microglia heterogeneity and their neuroprotective functions through regulating the immune system.

Publication

Genetic background variation impacts microglial heterogeneity and disease progression in amyotrophic lateral sclerosis model mice
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