News Release

Title

Autoantibodies against dihydrolipoamide S-acetyltransferase (DLAT) in immune-mediated neuropathies
－Anti-DLAT antibodies are potential biomarkers of sensory neuropathy－

Key Points

•Chronic inflammatory demyelinating polyneuropathy (CIDP) is an immune-mediated peripheral nerve disease wuchi, in its chronic course, causes limb weakness and sensory impairment.
•There are several subtypes of CIDP linked with distinct pathology, and they show different responses to therapies. Therefore, identification of biomarkers associated with the pathology is an urgent need.
•The objective of this study was to identify novel autoantibodies in CIDP.
•We identified autoantibodies against dihydrolipoamide S-acetyltransferase (DLAT) by combining immunoprecipitation and mass spectrometry using CIDP patient sera and mouse nerve tissues.
•Reactivity to DLAT was confirmed in 18% of the patients with CIDP and 10% of patients with sensory neuropathies with ELISA and Western blotting, but not in Guillain-Barré syndrome, hereditary neuropathies, or healthy normals.
•DLAT is highly expressed in the dorsal root ganglia, a cluster of sensory neurons. Anti-DLAT antibodies thus is a potential biomarker for sensory-dominant neuropathies including CIDP.

Summary

A group of researchers, Dr Yuki Fukami and Prof Masahisa Katsuno (Department of Neurology, Nagoya University Graduate School of Medicine) revealed that autoantibodies against dihydrolipoamide S-acetyltransferase (DLAT) are associated with sensory impairment in patients with immune-mediated neuropathies including chronic inflammatory demyelinating polyneuropathy (CIDP). The findings were published in the online edition of Neurology Neuroimmunology & Neuroinflammation on 5 January 2024.

CIDP is an immune-mediated peripheral neuropathy, a chronic process of peripheral nerve inflammation that causes limb weakness and sensory impairment, but its pathogenesis remains unclear. There are various subtypes of CIDP, that show different responses to therapies. Although these CIDP subtypes are thought to have different pathologies, there are no useful

biomarkers that reflect the pathology in clinical practice, making the search for such biomarkers an urgent issue. The identification of biomarkers is expected to be useful in stratifying patients with similar clinical features, pathogenic mechanisms and treatment responsiveness, and developing treatments accordingly.

In this study, disease-associated autoantibodies in the serum of CIDP patients were validated and their clinical characteristics were investigated. As a result, autoantibodies against DLAT were identified and 18% of CIDP patient sera were reactive to DLAT, many of whom had sensory ataxia. Antibodies against DLAT were also found in some patients with peripheral neuropathies other than CIDP, mainly sensory disorders. DLAT was also found to be highly expressed in dorsal root ganglion cells, a cluster of sensory neurons.

The results of this study suggest that anti-DLAT antibodies is a biomarker for sensory-dominant immune-mediated peripheral neuropathy including CIDP.

Research Background

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an immune-mediated peripheral neuropathy causing limb weakness and sensory impairment in a slowly progressive or relapsing course. Its etiology and pathogenesis remain unknown, but it is thought to be caused by demyelination due to autoimmune abnormalities in peripheral nerve components. The diagnosis of CIDP requires electrophysiological evidence of demyelination at multiple sites on nerve conduction studies. However, the diagnosis of CIDP is difficult and often misdiagnosed due to the presence of atypical variants, making the search for useful biomarkers that reflect the pathology a pressing issue. The identification of biomarkers could help stratify patients with similar clinical features, pathogenesis, and response to treatment.

We have previously shown that serum levels of neurofilament light chains are associated with disease activity in CIDP and electrophysiological and pathological indices of neuroaxonal degeneration. In this study, we validated disease-related autoantibodies in the serum of CIDP patients in search of further biomarkers and investigated their clinical characteristics.

Research Results

In this study, serum samples from 78 CIDP patients and five healthy subjects were initially tested for reactivity with mouse brain tissue extracts by Western blotting under non-reducing conditions. As a result, a 60-70 kDa protein band was detected in 10% (8 subjects) of the CIDP samples. Further glycosidase

treatment was performed to confirm whether it was a glycoprotein, indicating that this antigen protein was not glycosylated. To identify the protein in this band, immunoprecipitation of patient serum and mouse brain extracts was conducted and analyzed by mass spectrometry, identifying it as dihydrolipoamide S-acetyltransferase (DLAT), which was further confirmed with a monoclonal anti-DLAT antibody, showing a 60-70 kDa antigen band region (Fig. 1).

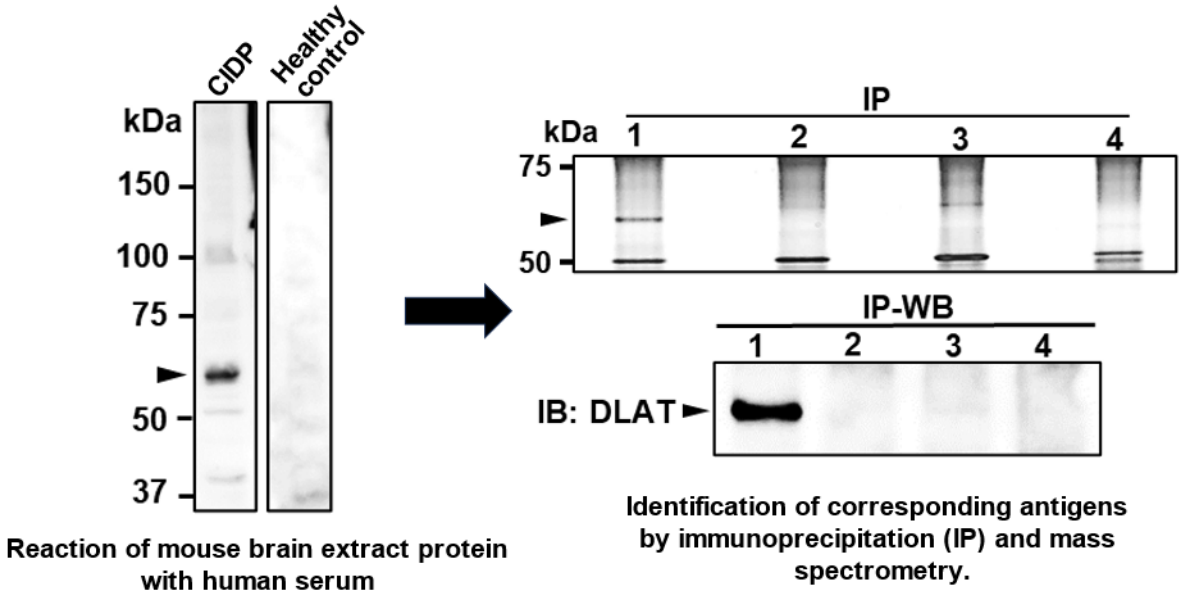

Figure 1. Identified autoantibodies to DLAT

Next, an ELISA using recombinant protein was established to verify reactivity to DLAT, using a large number of samples, which was further confirmed by Western blotting. Large-scale screening confirmed the presence of antibodies in 29 of 160 (18%) patients with CIDP and 6 of 58 (10%) patients with immune-mediated sensory neuropathy. In contrast, only 2 out of 140 (1%) were detected in controls, including patients with multiple sclerosis, Guillain-Barré syndrome, other neurological diseases, and healthy serum (Fig. 2).

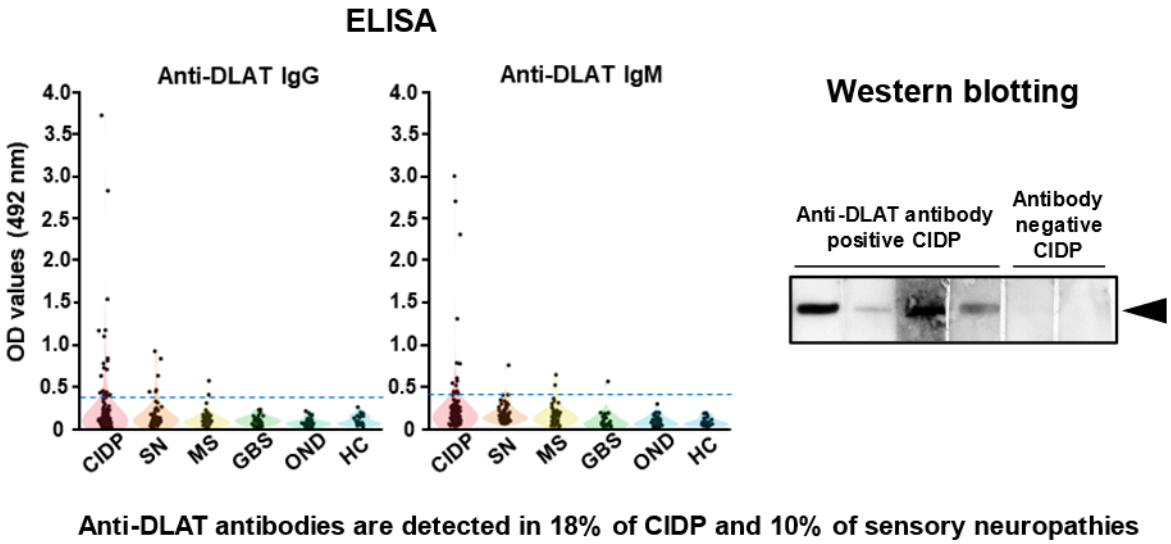

Figure 2. anti-DLAT antibodies are detected by CIDP

Furthermore, high expression of DLAT was observed in human autopsy dorsal root ganglion cells, confirming the reactivity of patient serum with mouse dorsal root ganglion cells. Reactivity with patient serum was also confirmed in immunostaining studies using HEK293 cells overexpressing DLAT and mouse dorsal root ganglia (Fig. 3). However, experiments using primary cultured mouse dorsal root ganglion cells did not show clear cytotoxicity, suggesting a T-cell-mediated mechanism or other factors may be underlying.

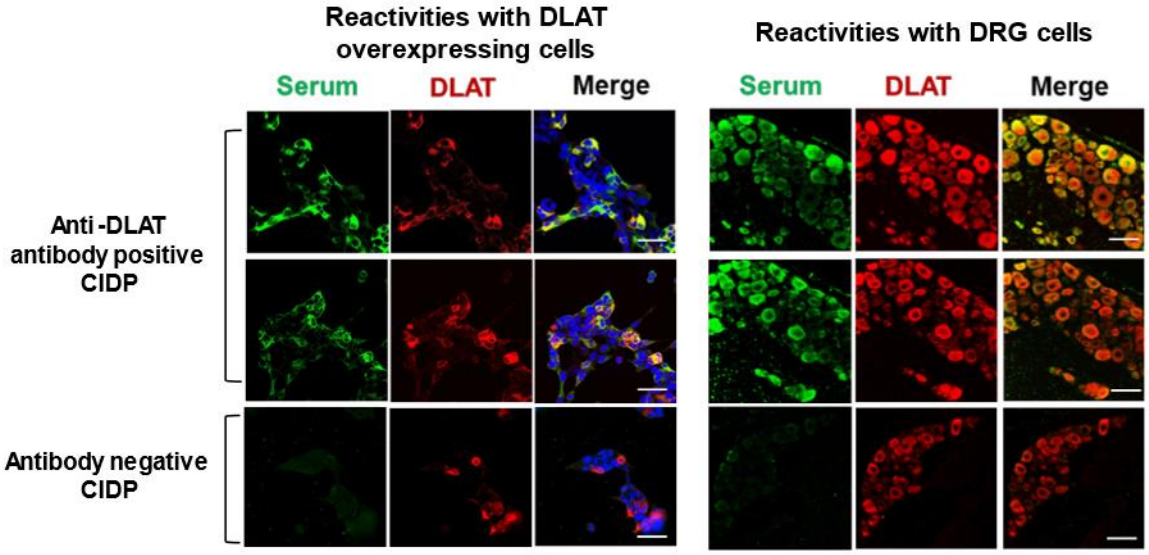

Figure 3. Anti-DLAT antibodies react with dorsal root ganglion cells.

The clinical features of 29 anti-DLAT antibody-positive CIDPs, compared with 131 antibody-negative CIDPs, showed a higher proportion of patients with sensory ataxia (69% vs. 37%), cranial nerve damage (24% vs. 9%), and malignancy (20% vs. 5%).

In summary, anti-DLAT antibodies were detected in the sera of patients with the sensory-disorder-driven type of CIDP; these antibodies were also frequently found in patients with sensory-disorder-driven neuropathies other than CIDP. DLAT is highly expressed in dorsal root ganglion cells, a cluster of sensory neurons, suggesting that it is a biomarker for sensory-dominant immune-mediated peripheral neuropathies (Fig. 4).

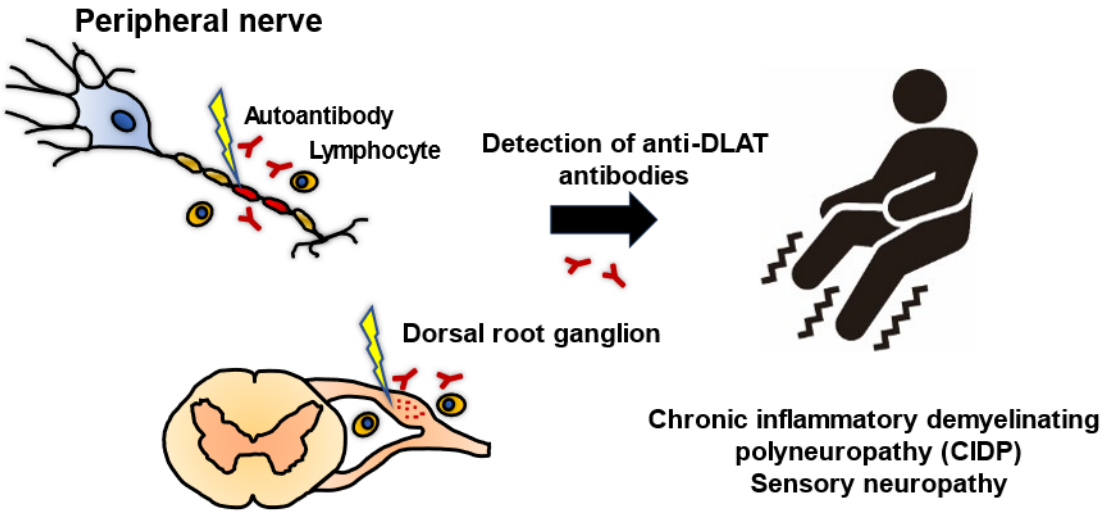

Figure 4. Anti-DLAT antibodies are biomarkers associated with sensory disorders.

Research Summary and Future Perspective

This study demonstrates that anti-DLAT antibodies is a potential diagnostic biomarker for sensory-dominant immune-mediated peripheral neuropathy. Further studies focusing on the clinical features of patients with anti-DLAT antibodies could help better define the disease. In addition, the lack of evidence of auto-antibody cytotoxicity suggests that direct involvement in the pathogenesis of the disease may be underpinned by other factors such as T-cell-mediated mechanisms, which require further validation.

Publication

Yuki Fukami, Masahiro Iijima, Haruki Koike, Satoru Yagi, Soma Furukawa, Naohiro Mouri, Jun Ouchida, Ayuka Murakami, Madoka Iida, Satoshi Yokoi, Atsushi Hashizume, Yohei Iguchi, Shiro Imagama, Masahisa Katsuno
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