News Release

Title

Ratio of urinary N-terminal titin fragment to urinary creatinine is a novel biomarker for amyotrophic lateral sclerosis

Key Points

• Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease showing a rapid progression.

• Biomarkers for monitoring disease progression of ALS have not been established.

• Our findings indicate that urinary N-terminal titin fragment is a non-invasive measure of muscle damage in ALS, which could be applied in disease monitoring and prediction of disease progression.

• Urinary N-terminal titin fragment can be used in clinical practice and clinical trials.

Summary

A group of researchers, headed by Prof. Masahisa Katsuno, Department of Neurology, Nagoya University Graduate School of Medicine have revealed that urinary levels of titin N-terminal fragment normalised with urinary Cr which can be repeatedly measured reflect motor function and could be a marker for survival of patients with ALS. This work was published online in *Journal of Neurology, Neurosurgery, and Psychiatry* on March 19, 2021.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by rapid progression of upper and lower motor neuron loss, which causes paralysis and death within an average of 3-4 years from disease onset. After trials and testing spanning over half a century on over 150 different therapeutic agents or strategies, only riluzole and edaravone, which have beneficial effects in a limited population of patients with ALS, have been approved. This could be attributed to the lack of sensitive, non-invasive biomarkers that reflect human pathophysiology.

In this study, compared with healthy controls (HC), patients with ALS had significantly increased urinary levels of titin N-terminal fragment normalised with Cr (titin/Cr), which were correlated with the scores of the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R). A Cox proportional hazards model demonstrated that the high urinary level of titin/Cr was a survival predictor in patients with ALS. Multivariate analysis of prognostic factors showed that the urinary titin/Cr and serum NfL were independent factors for poor prognosis.

Our findings indicate that urinary N-terminal titin fragment is a non-invasive measure of muscle damage in ALS, which could be applied in disease monitoring and prediction of disease progression.

Research Background

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by rapid progression of upper and lower motor neuron loss, which causes paralysis and death within an average of 3-4 years from

disease onset. Most ALS cases are sporadic with unknown aetiology.

Well-designed studies have reported negative results of several candidate drugs for ALS therapy, which could be attributed to several reasons. Disease progression before motor dysfunction onset attenuates the effects of disease-modifying therapies that are beneficial in preclinical studies. Great variations in the clinical courses of ALS could impede the assessment of the drug effects on disease progression. Moreover, there is a lack of reliable, less invasive outcome measures for monitoring of ALS progression, evaluating treatment efficacy, and exploring potential molecular targets of therapy development.

Therefore, biomarker which predict severity, disease course, and prognosis of ALS are warranted to develop biomarkers based on the results of clinical studies in patients with sporadic ALS.

Research Results

Skeletal muscle titin, which is also known as connectin, is the largest human protein that works in a spring-like mechanical fashion to connect the Z-disc to the M-line in the sarcomere. Mutations in *TTN* have been reported in various myopathies. Moreover, titin degradation is reported in muscle biopsies obtained from patients with ALS.

We found that urinary levels of titin N-terminal fragment normalised with urinary Cr levels (titin/ Cr) were significantly increased in patients with ALS compared with HC (figure 1A). To examine the utility of urinary titin N-terminal fragment as a biomarker that reflects ALS severity, we investigated the association of urinary levels of titin/Cr with the total score of the ALSFRS-R in the patients with ALS. Urinary levels of titin/Cr were strongly correlated with the total score of ALSFRS-R (Figure 1B).

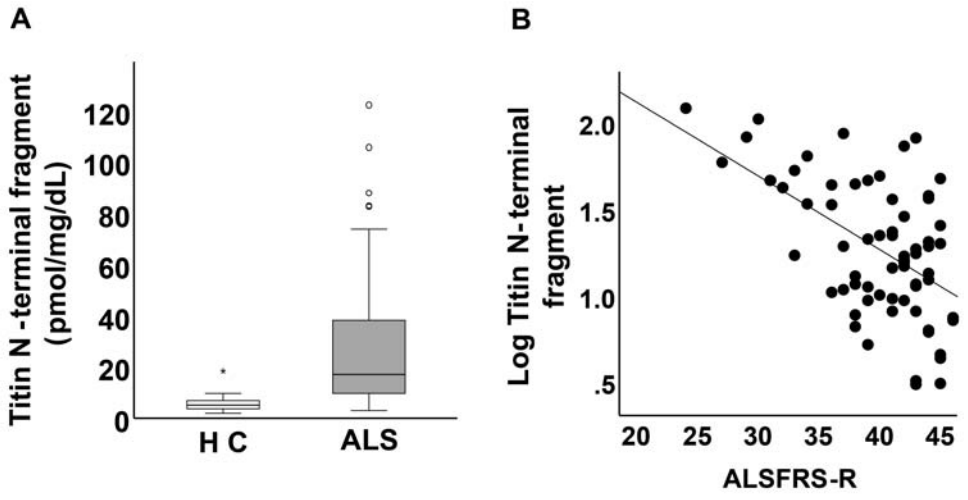

Figure 1. N-terminal titin fragment in urine and its relation to ALSFRS-R

Subsequently, we examined whether urinary N-terminal titin fragment could predict prognosis in patients with ALS. Compared with the high titin/Cr group, we found that the low titin/Cr group had longer median survival (Figure 2). The Cox regression analysis also identified urinary titin/Cr that were associated with survival period from baseline. These findings indicate that the baseline level of urinary titin/Cr is prognostic markers for ALS.

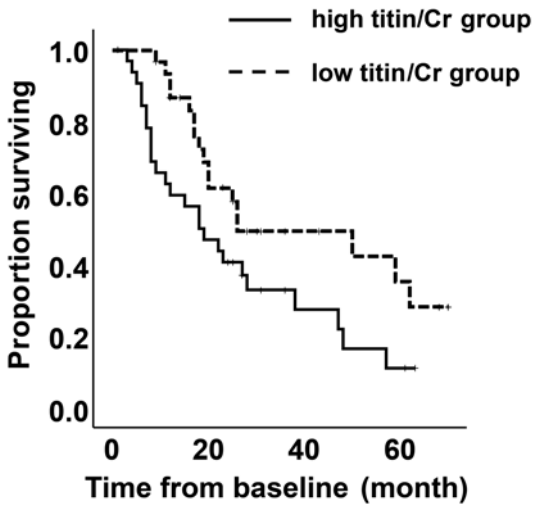

Figure 2. Urinary N-terminal titin fragment predict survival in patients with ALS

Next, we also assessed urinary $p75^{ECD}$ and serum NfL, which are recently developed ALS biomarkers. There was a correlation of motor function measures with the urinary titin N-fragment and $p75^{ECD}$ levels, but not with serum NfL. These findings suggest that urinary titin, as well as urinary $p75^{ECD}$, reflects ALS severity. ROC curves indicated that the urinary N-terminal titin fragment and serum NfL could discriminate patients with ALS from healthy controls with a high probability (Figure 3).

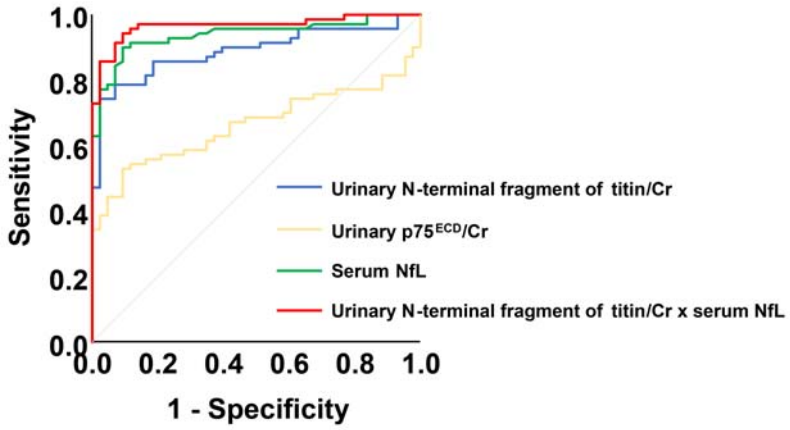

Figure.3 ROC analysis of the urinary titin/Cr, urinary p75ECD/Cr, serum NfL, and the products of urinary N-terminal titin fragment and serum NfL to discriminate patients with ALS

Research Summary and Future Perspective

Our findings indicate the potential of urinary titin N-terminal fragment normalised with urinary Cr as a non-invasive biomarker for evaluation of disease severity and prognosis in ALS and other motor neuron diseases. Future prospective, multicentre, longitudinal studies are warranted to confirm our findings and to examine whether urinary titin N-terminal fragment can predict the biological effect of the drug therapeutics

for ALS.

Publication

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