# Acetylated alpha-tubulin and neurofilament light chain as clinical plasma biomarkers of Charcot-Marie-Tooth Disease

**John Kealy[1], Aimée Freeburn[1], Disha Disha[1], Tomohiro Onishi[2], Kentaro Otake[2], Maiko Tanaka[2], Hiroki Iwashita[2], Atsushi Suzuki[2], Zara Togher[3], Sinead M. Murphy[3], Massimiliano Bianchi[1]**

[1]Ulysses Neuroscience Limited, Trinity College Institute of Neuroscience, Lloyd Institute, Trinity College Dublin, Ireland.
[2]Neuroscience Drug Discovery Unit, Research, Takeda Pharmaceutical Company Limited, Fujisawa, Japan
[3]Department of Neurology, Tallaght University Hospital, Tallaght, Dublin 24, D24 NROA, Ireland.

ULYSSES NEUROSCIENCE

Charcot-Marie-Tooth disease (CMT) comprises a group of progressive genetic disorders affecting the peripheral nervous system and represents the most common inherited neuropathy, affecting 2.6 million patients worldwide. Limited treatment options are available, and diagnosis is dependent on neurophysiological and neurological examination of the patient, with no fluid-based biomarkers available. This creates a challenge for drug discovery in CMT as there is no validated biomarker to track disease progression or any potential benefits derived from a novel therapy. Dysfunction in microtubule (MT) dynamics have been shown to lead to altered neuronal signalling and impaired neuronal health. MTs undergo cycles of dynamic change called post-translational modifications (PTMs) which regulate MT dynamics – such as acetylated alpha-tubulin (Acet-Tub)[1]. Neurofilament light chain (NfL) is a neuronal cytoplasmic protein that is abundant in neuronal axons, and is released into the blood proportionally to the degree of axonal damage in a variety of neurological disorders[2,3]. We investigated whether Acet-Tub and NfL are suitable biomarkers to track CMT severity and progression, either on their own or as a composite.

**Methods:** Plasma was collected from CMT patients (n=13) with a mutation in either *PMP22* (duplication; CMT1A; n=10) or *MFN2* (CMT2A; n=3). Comparisons were made with hereditary neuropathy with liability to pressure palsies (HNPP) patients, caused by *PMP22* deletion (n=5), and healthy volunteers (n=16). Acet-Tub was quantified by Infrared Western Blotting using the Li-Cor Odyssey CLx; and expressed as a ratio of Total-Tubulin ('Acet-Tub/Total-Tub'). NfL was quantified using the Meso-Scale Discovery (MSD) Quickplex SQ 12 system. All work was performed following ethical approval from Tallaght University Hospital, Dublin.

## Neurofilament Light Chain is increased in the plasma of CMT and HNPP patients

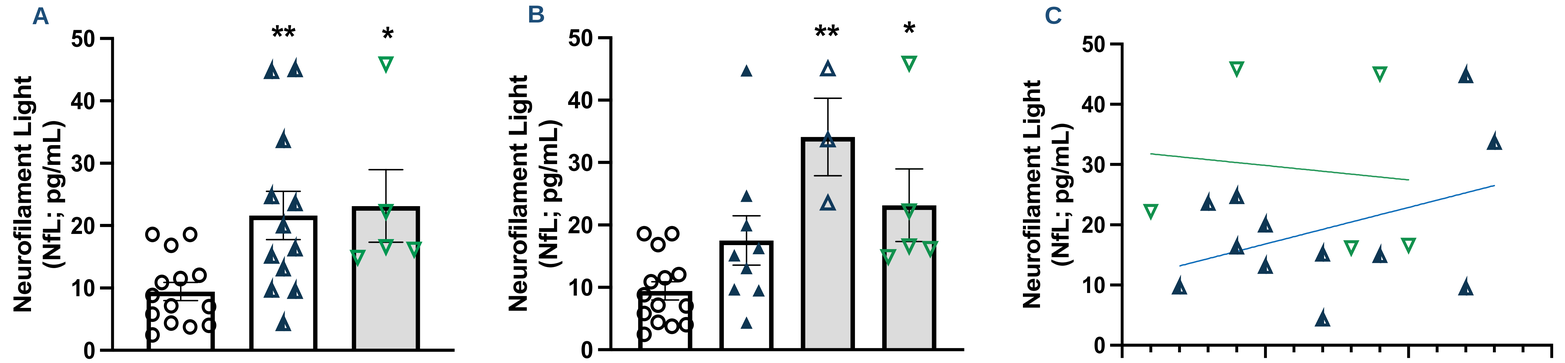


**Figure 1. (A)** NfL was increased in the plasma of all CMT patients combined and in HNPP patients compared to healthy controls ($F_{(2,28)}$ = 5.686, $p$=0.0085). **(B)** When analysed by genotypes, NfL was significantly increased in the CMT2A and HNPP genotypes, but not CMT1A ($F_{(3,27)}$ = 6.88, $p$=0.0014). **(C)** Analysis using the CMTES showed non-significant tendencies towards a positive correlation between NfL ($r$= 0.41; $p$=0.19) in CMT1A and 2A, while HNPP showed little correlation between CMTES and NfL ($r$= -0.121; $p$=0.85). Data are expressed as mean ± SEM, n=3-15 per group. One-way ANOVA with Fisher's LSD analysis and Pearson's correlation coefficient. *$p$<0.05, **$p$<0.01 vs Control.

## Acetylated alpha-tubulin is decreased in the plasma of CMT and HNPP patients

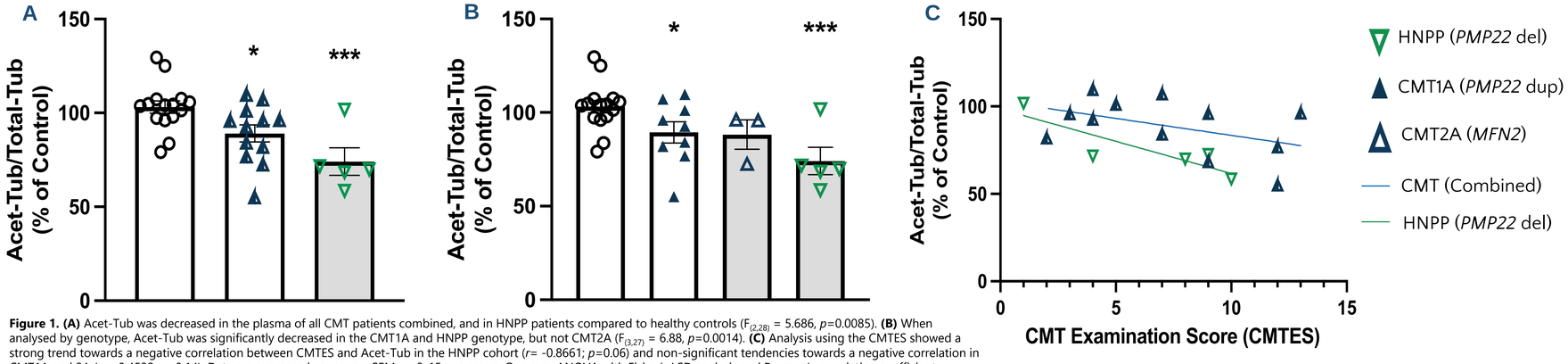


**Figure 1. (A)** Acet-Tub was decreased in the plasma of all CMT patients combined, and in HNPP patients compared to healthy controls ($F_{(2,28)}$ = 5.686, $p$=0.0085). **(B)** When analysed by genotype, Acet-Tub was significantly decreased in the CMT1A and HNPP genotype, but not CMT2A ($F_{(3,27)}$ = 6.88, $p$=0.0014). **(C)** Analysis using the CMTES showed a strong trend towards a negative correlation between CMTES and Acet-Tub in the HNPP cohort ($r$= -0.8661; $p$=0.06) and non-significant tendencies towards a negative correlation in CMT1A and 2A ($r$= -0.4532; $p$=0.14). Data are expressed as mean ± SEM, n=3-15 per group. One-way ANOVA with Fisher's LSD analysis and Pearson's correlation coefficient. *$p$<0.05, ***$p$<0.001 vs Control.

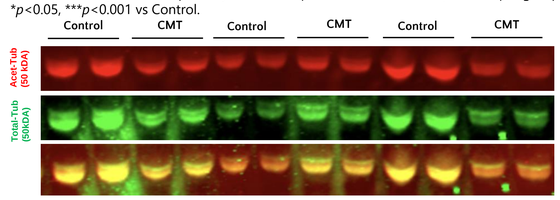


**Figure 3.** Representative infrared western blot for the analysis of acetylated alpha tubulin (Acet-Tub) and total alpha tubulin (Total-Tub).

This study suggests that plasma-based biomarkers may be of benefit to the drug discovery process in CMT and related neuropathies:

- Acet-Tub was altered in demyelinating CMT1A.
- Neurofilament light chain was altered in axonal CMT2A.
- HNPP patients showed simultaneous changes in both biomarkers.

[1]Janke, C. (2014) 'The tubulin code: Molecular components, readout mechanisms, functions', *Journal of Cell Biology*, 206(4), pp. 461–472. doi: 10.1083/JCB.201406055. Parésys, L., Hoffmann, K., Froger, N., Bianchi, M., Villey, I., Baulieu, E.[2]Pareyson, D., & Shy, M. E. (2018). Neurofilament light, biomarkers, and Charcot-Marie-Tooth disease. *Neurology*, *90*(6), 257–259. https://doi.org/10.1212/WNL.0000000000004936 [3]Sandelius, Å., Zetterberg, H., Blennow, K., Adiutori, R., Malaspina, A., Laura, M., Reilly, M. M., & Rossor, A. M. (2018). Plasma neurofilament light chain concentration in the inherited peripheral neuropathies. *Neurology*, *90*(6), e518–e524. https://doi.org/10.1212/WNL.0000000000004932