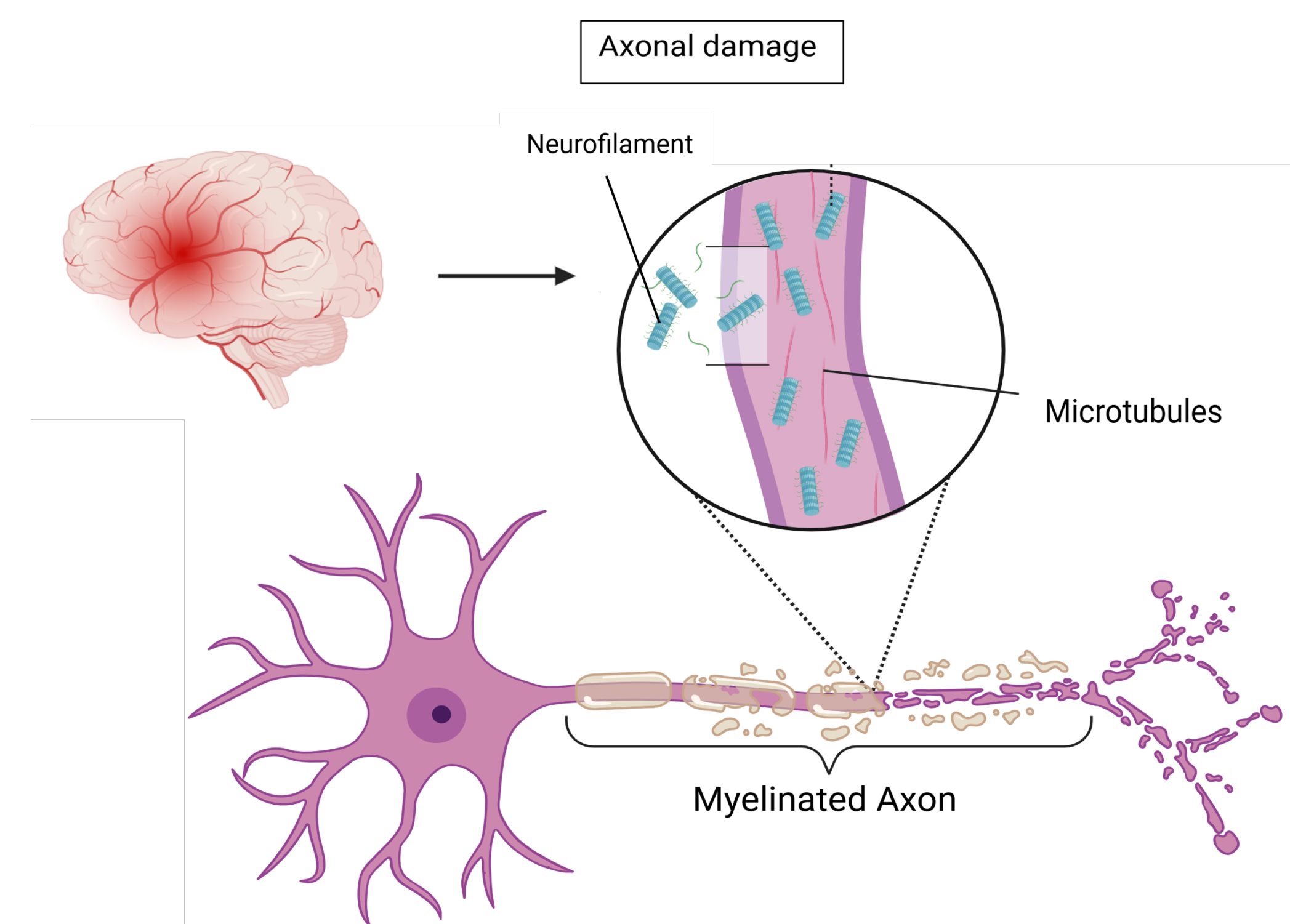


Neurofilament Light Chain Exists As Multiple Truncated Species In Human Brain

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INTRODUCTION

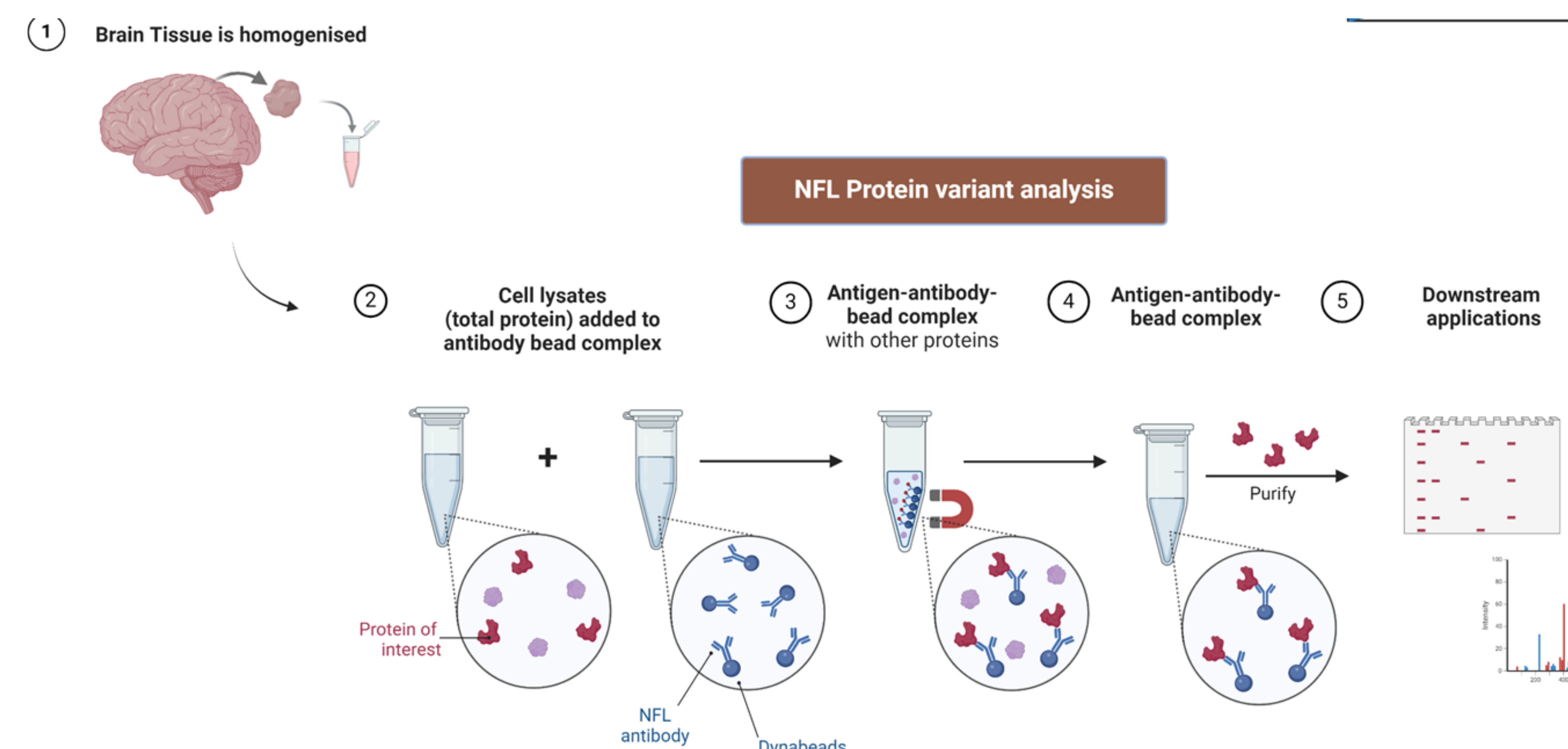


Neurofilament light (NFL) is an established biomarker of neuroaxonal injury, elevated in Alzheimer's disease (AD)¹. Current assays detect the ~68 kDa full-length protein, but multiple NFL variants exist^{2,3}. Their clinical relevance is unknown,

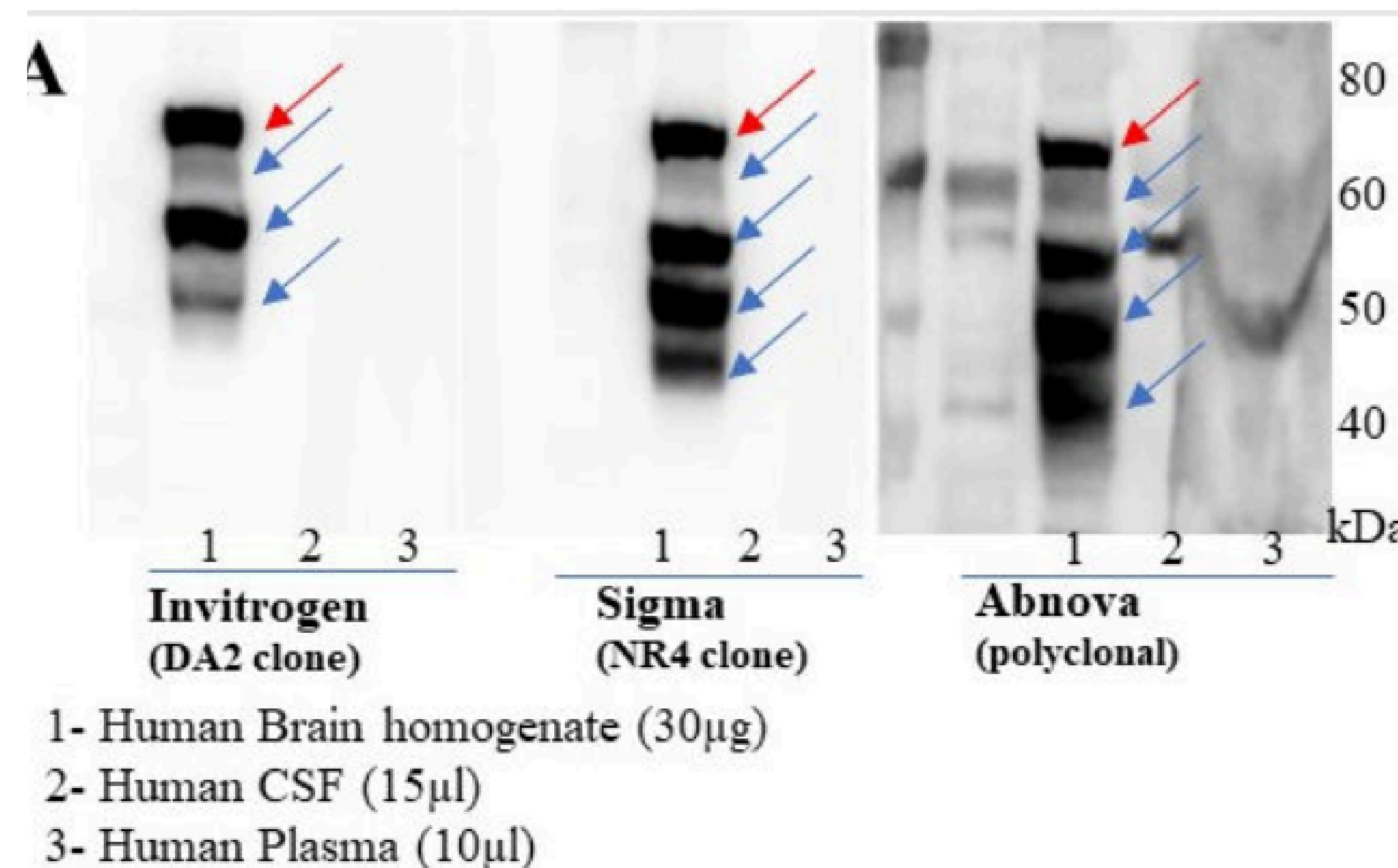
Aim: To investigate and characterise NfL variants in brain from AD patients, using optimised immunoprecipitation techniques, to improve diagnostic accuracy.

METHODS

Brain samples were collected and homogenized to extract proteins. Target proteins were enriched using immunoprecipitation, followed by analysis through Western blotting to assess protein expression and interactions.



RESULTS



Five isoforms of NFL including three major and two minor bands were detected in postmortem human brain homogenates using western blotting with 3 different antibodies, DA2, NR4 and Abnova (Figure A). A full length 68kda (red arrow) and four isoforms around 40-60kda (blue arrows) were observed. Blocking experiment below with pure NFL homogeneate further validated the band variant results in brain (Figure 2).

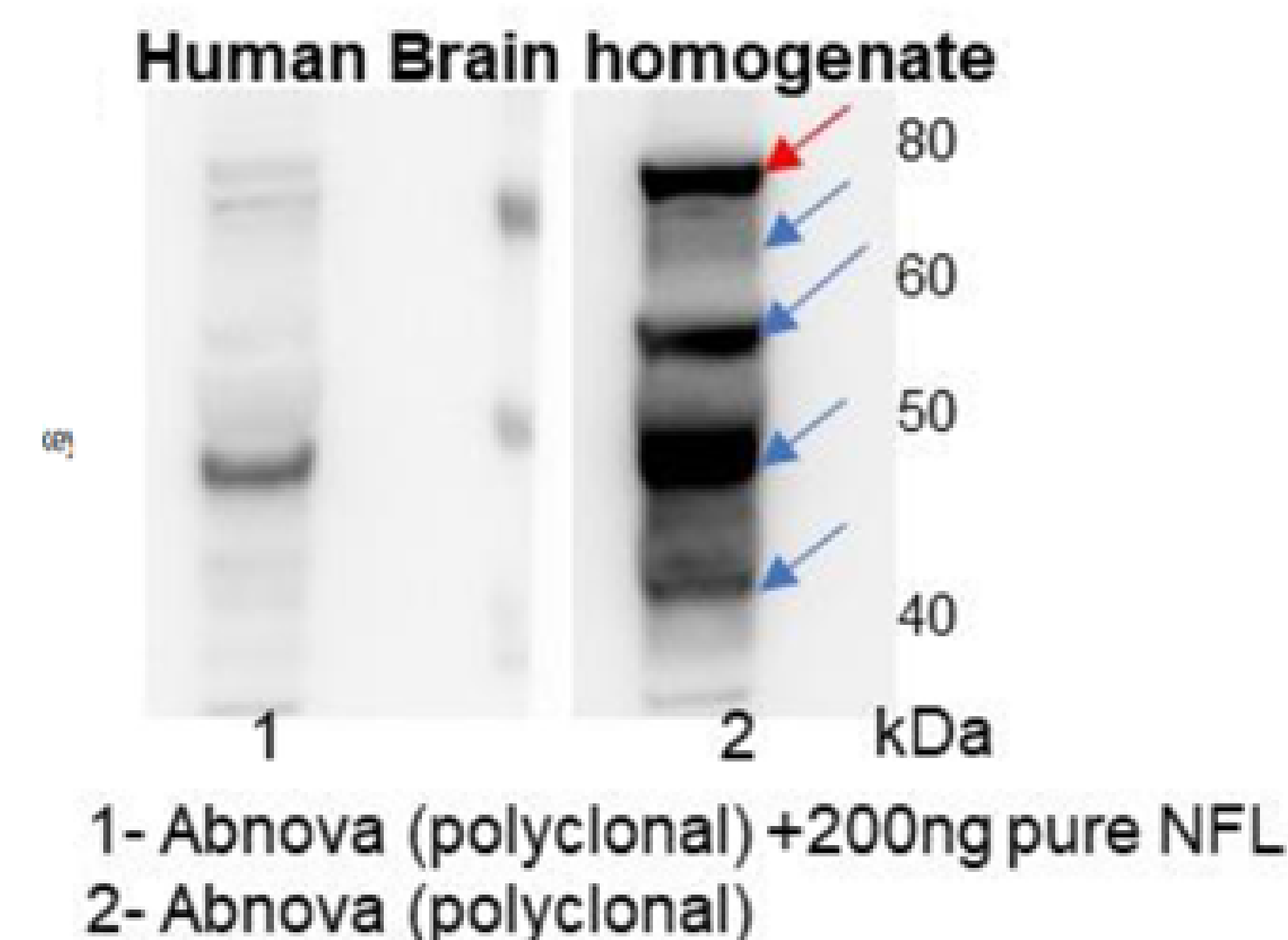
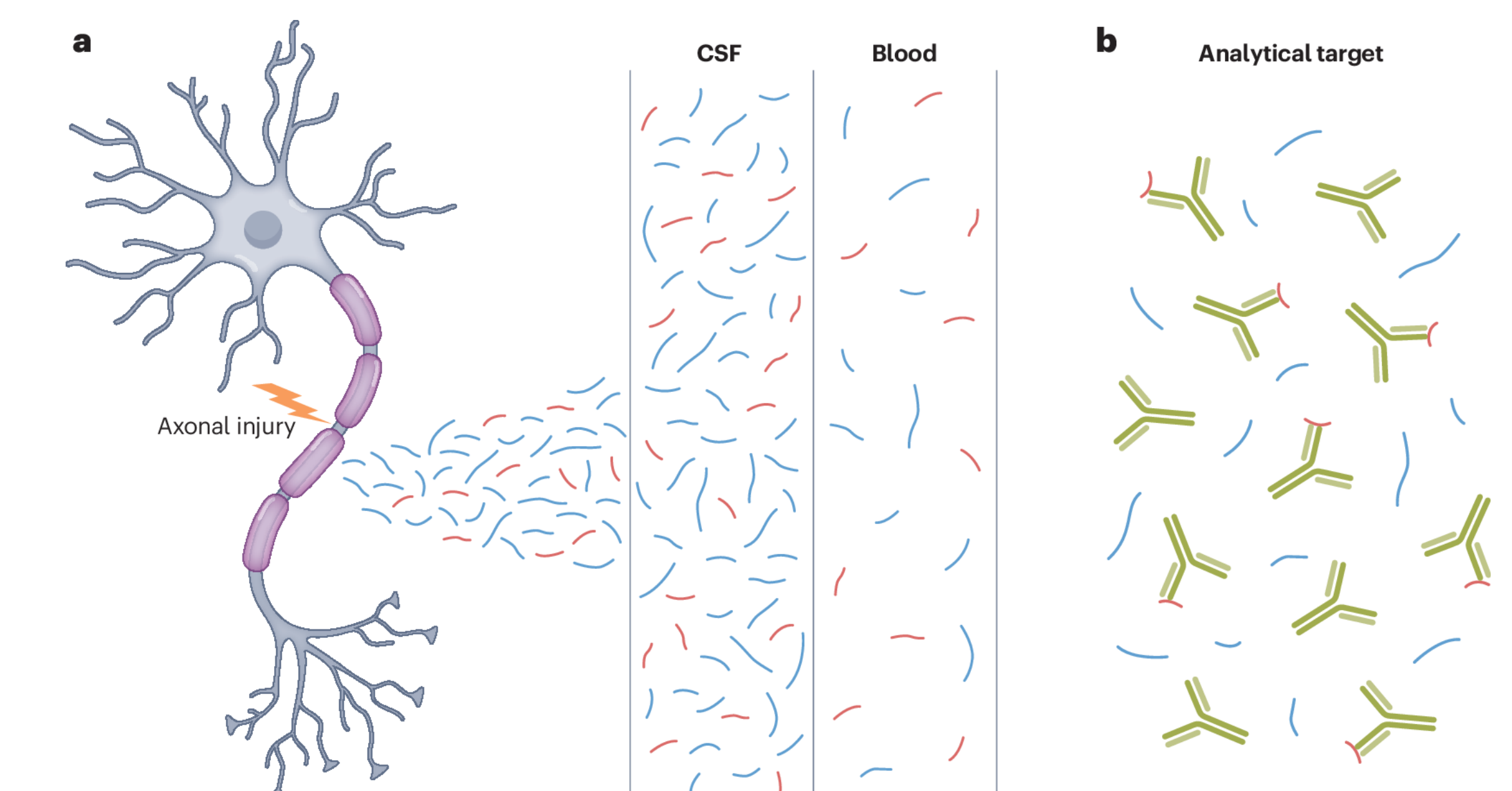


Figure 2: Validation of NFL protein isoforms in brain

CONCLUSION

Our findings indicate NFL exists as multiple distinct variants in Alzheimer's disease, extending beyond the traditionally measured ~68 kDa form. The presence of additional immunoreactive fragments, with preliminary evidence of disease-associated differences, suggests that NfL heterogeneity may carry clinically meaningful information. Further studies are being performed to further investigate and validate the studies, including mass spectrometry, proteomics analysis and advanced immunoassay techniques testing brain, CSF and blood. Variant-specific detection strategies may therefore improve diagnostic precision and support more refined biomarker-driven approaches in neurodegenerative disease.



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Sources:

- Khalil M et al., 2018. Neurofilaments as biomarkers in neurological disorders. *Nature Reviews Neurology*.
- Gaetani L et al., 2019. Neurofilament light chain as a biomarker in neurological disorders. *Nature Reviews Neurology*.
- Budelier MM et al., 2022. Fragment-specific quantification of neurofilament light chain by immunoprecipitation-mass spectrometry. *Science Advances*