

Neurofilament-light as biomarker for AD in DS

1 **Neurofilament light as a blood biomarker for neurodegeneration in Down syndrome**

2

3 Andre Strydom MBChB MRCPsych PhD ^{a,b,c} *

4 Amanda Heslegrave PhD ^d

5 Carla M Startin PhD ^{a,b,c}

6 Kin Y Mok FRCP(Edin) PhD ^{c,d,e}

7 John Hardy PhD ^{c,d,f}

8 Jurgen Groet PhD ^{c,g}

9 Dean Nizetic MD PhD ^{c,g,h}

10 Henrik Zetterberg MD PhD ^{d,i,j}

11 And The LonDownS Consortium

12

13 ^a Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, Psychology and
14 Neuroscience, Kings College London, London, UK

15 ^b Division of Psychiatry, University College London, London, UK

16 ^c The LonDownS Consortium

17 ^d Department of Molecular Neuroscience, Institute of Neurology, University College London, London,
18 UK

19 ^e Division of Life Science, Hong Kong University of Science and Technology, Hong Kong SAR, People's
20 Republic of China

21 ^f Reta Lila Weston Institute, Institute of Neurology, University College London, London, UK

22 ^g Blizard Institute, Barts and the London School of Medicine, Queen Mary University of London,
23 London, UK

Neurofilament-light as biomarker for AD in DS

24 ^h Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore

25 ⁱ Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The

26 Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

27 ^j UK Dementia Research Institute at UCL, London, UK

28

29 * Corresponding author: andre.strydom@kcl.ac.uk

30 Address: Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry,

31 Psychology and Neuroscience, Kings College London, 16 De Crespigny Park, London, SE5 8AF, UK

32

33 **Author contributions**

34 Conception and design of the study: AS, HZ; acquisition of data: AH, KM, JH, JG, DN, HZ; analysis of

35 data: AS, CS; writing the manuscript: AS, CS; revising the manuscript for important intellectual

36 content: all co-authors.

37

38 **Conflicts of interest**

39 AS has consulted for Ono pharmaceuticals, is an adviser to the UK Down Syndrome Association, and

40 is an advisory board member of the LuMind Foundation (USA). HZ has served at advisory boards of

41 Roche Diagnostics, Eli Lilly, and Teva, and is a co-founder of Brain Biomarker Solutions in Gothenburg

42 AB, a GU Ventures-based platform company at the University of Gothenburg.

43 No other authors have any competing interests to declare.

44

45 Title characters: 79

46 Abstract word count: 150

47 Body word count: 1261

48 Number of figures / tables: 2

Neurofilament-light as biomarker for AD in DS

49 Abstract

50 INTRODUCTION: Down syndrome (DS) may be considered a genetic form of Alzheimer's disease (AD)
51 due to universal development of AD neuropathology, but diagnosis and treatment trials are
52 hampered by a lack of reliable blood biomarkers. A potential biomarker is neurofilament light (NF-L),
53 due to its association with axonal damage in neurodegenerative conditions.

54 METHODS: We measured blood NF-L concentration in 100 adults with DS using Simoa NF-light®
55 assays, and examined relationships with age, and cross-sectional and longitudinal dementia
56 diagnosis.

57 RESULTS: NF-L levels increased with age (Spearman's rho = 0.789, p<0.001), with a steep increase
58 after age 40, and were predictive of dementia status (p=0.022 adjusting for age, sex, and APOE4) but
59 showed no relationship with longstanding epilepsy or premorbid ability. Baseline NF-L levels were
60 associated with longitudinal dementia status.

61 DISCUSSION: NF-L is a biomarker for neurodegeneration in DS, with potential for use in future
62 clinical trials to prevent or delay dementia.

63

64 **Keywords:** Down syndrome, Alzheimer's disease, dementia, neurofilament light, biomarker

65

66 Research in context

67 **Systematic review:** The authors reviewed the literature using PubMed searches supplemented with
68 our knowledge of pending papers in this research area. While blood NF-L has been associated with
69 clinical features of progression in a number of neurodegenerative conditions, we have not identified
70 any reports of NF-L associated with cognitive decline in DS, a genetic form of AD.

71 **Interpretation:** Our findings demonstrate the potential utility of NF-L as a blood biomarker of
72 neurodegeneration in DS, a population that may not be able to tolerate more invasive procedures
73 such as neuroimaging and lumbar punctures to track progression.

Neurofilament-light as biomarker for AD in DS

74 **Future directions:** The association between NF-L and other markers of longitudinal AD progression

75 should be explored further in future work.

Neurofilament-light as biomarker for AD in DS

76 1. Introduction

77 Down syndrome (DS), caused by the trisomy, translocation, or partial trisomy of chromosome 21, is
78 the most common genetic cause of intellectual disability (ID) with an estimated population of 6
79 million worldwide. Dementia is a common feature of the ageing process in DS due to the triplication
80 of the amyloid precursor protein (APP) on chromosome 21 leading to brain pathology indicative of
81 Alzheimer's disease (AD) [1], a cumulative incidence for dementia in excess of 90% by the age of 65
82 [2], and a mean age at dementia diagnosis of 55 [3]. DS is therefore a genetic form of AD alongside
83 autosomal dominant causes of AD [4].

84 Neurofilament light (NF-L) is one of the scaffolding cytoskeleton proteins of myelinated subcortical
85 axons [5] and can now be reliably measured in blood using ultrasensitive single molecule array
86 technology. Blood concentration of NF-L correlates well with corresponding CSF measures [6] and
87 reflects axonal damage in neurological disorders, including frontotemporal dementia [7], multiple
88 sclerosis [8], and familial and sporadic AD [9, 10]. NF-L correlates with other measures of disease
89 stage and severity [10] but the utility of NF-L in populations with other genetic forms of AD is yet to
90 be fully explored.

91 We aimed to explore the relationship between plasma NF-L levels, age, and dementia status in
92 individuals with DS, as well as its independence from sex effects, premorbid intellectual ability levels,
93 and longstanding epilepsy.

94 2. Methods

95 The North West Wales National Health Service (NHS) Research Ethics Committee provided ethical
96 approval for a longitudinal study of cognitive ability and dementia in DS (13/WA/0194). For those
97 with decision-making capacity written consent was obtained, while for those who did not have
98 decision-making capacity a consultee indicated their agreement to participation.

99 Participants aged 16 and older were recruited across England via care homes, support groups, and
100 local NHS sites. Participants with an acute physical or mental health condition were excluded until

Neurofilament-light as biomarker for AD in DS

101 they had recovered; other details of the cohort have been previously described [11]. DS status was
102 confirmed using DNA from saliva or blood and genotyped using Illumina OmniExpressExome arrays
103 (San Diego, CA, USA); trisomy status was visually confirmed in GenomeStudio (see Table 1). APOE
104 was determined using Thermo Fisher Scientific Taqman assays for rs7412 and rs429358 (Waltham,
105 MA, USA).

106 Assessment included a detailed interview with carers using the Cambridge Examination of Mental
107 Disorders of Older People with Down's syndrome (CAMDEX-DS) [12] to determine decline in several
108 domains including memory. Premorbid ID level was defined according to the ICD10 diagnostic
109 system's descriptions of mild, moderate, and severe ID, based on carers' reports of the individuals'
110 best ever level of functioning [13]. At baseline, dementia was defined as a confirmed clinical
111 diagnosis. At follow-up, participants were classified according to whether they had retained or been
112 given a diagnosis of dementia, or were being investigated for dementia.

113 Blood samples from 100 individuals were collected in lithium heparin tubes (Fisher, Loughborough,
114 UK) and sent overnight for processing. Blood was layered over a similar amount of ficoll (GE
115 Healthcare, Little Chalfont, UK), then centrifuged in a swing-out rotor for 40 minutes at 400g without
116 brake. Plasma samples were stored at -80°C. Plasma NF-L concentration was measured by the same
117 laboratory technician with reagents from a single lot using the Simoa NF-light® assay (a digital
118 sandwich immunoassay employing antibodies directed against the rod domain of NF-L) on an HD-1
119 Simoa analyzer, according to the protocol issued by the manufacturer (Quanterix, Lexington, MA,
120 USA). Samples were run in duplicate and coefficients of variance (CV) for duplicates were set to be
121 below 12%. All samples measured within the range spanned by the limits of quantification and inter-
122 assay CV for the high and low concentration quality controls were 6.6 and 8.1% respectively.

123 All statistical tests were 2-sided and statistical significance was set at p<0.05. We tested associations
124 between plasma NF-L samples and demographic or clinical factors using Mann-Whiney U, Kruskal-
125 Wallis, and Spearman rank correlation tests as appropriate. Associations between plasma NF-L and

Neurofilament-light as biomarker for AD in DS

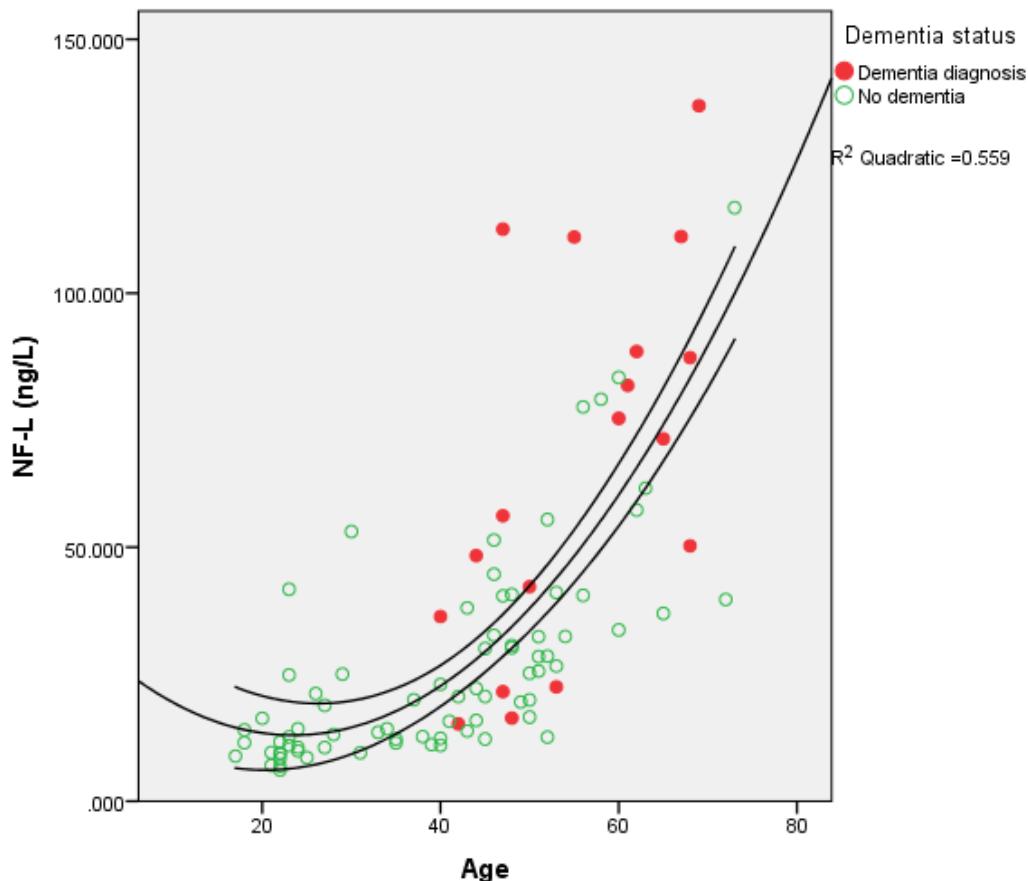
126 dementia diagnosis were tested using logistic regression and log-transformed NF-L values, with
127 adjustment for age and sex; we also adjusted for APOE4 status cross-sectionally.

128 **3. Results**

129 NF-L levels were obtained from 100 participants (age range 17-73); 5 results were excluded after
130 failing to meet CV thresholds meaning 95 adults were included in subsequent analyses. Of adults
131 aged 36 and older who are being targeted for longitudinal follow-up, 29/63 (46%) had completed a
132 follow-up assessment at the time of this report (mean number of months between assessments
133 23.4, SD 3.9). One individual had suffered an occlusive cerebrovascular event 4-6 months prior to
134 donating the blood sample and converted to dementia status at follow-up but was an outlier with an
135 NF-L level of 481.97 ng/L, thus was excluded from cross-sectional analysis. For the remaining 94
136 individuals NF-L concentration had a median value of 22.74 ng/L, range 6.11-136.91 ng/L. At
137 baseline, 18 of 94 participants had a clinical diagnosis of dementia (Table 1).

138 NF-L levels did not differ by premorbid ID level (Kruskal-Wallis test, $p=0.195$), sex (Mann-Whitney U
139 test, $p=0.837$) or longstanding epilepsy (Mann-Whitney U test, $p=0.858$). NF-L level and age of
140 participants were significantly correlated (Spearman's rho = 0.789, $p<0.001$) (Figure 1), such that
141 those aged 35 and older had significantly higher levels of NF-L compared to younger individuals
142 (median 11.52 vs. 32.42, Mann-Whitney U test $p<0.001$). Those with dementia had significantly
143 higher levels of NF-L (median 63.76 ng/L vs. 19.96 ng/L; Mann-Whitney U test $p<0.001$), and a
144 logistic regression model adjusting for age, sex, and APOE4 status revealed that NF-L levels remained
145 predictive of dementia status ($p=0.022$).

Neurofilament-light as biomarker for AD in DS



146
147 Figure 1. NF-L concentration by age of individuals with DS.

148

149 Seven (24.1%) of 29 individuals with follow-up cognitive data had a clinical diagnosis of dementia at
150 baseline, all with the dementia diagnosis retained at follow-up, with a further two (6.9%) individuals
151 converting to dementia status by follow-up, while three (10.3%) participants were under
152 investigation for dementia at follow-up. Predictive validity of NF-L levels at baseline was explored by
153 combining individuals with confirmed or suspected dementia at follow-up (n=12, median NF-L 77.38
154 ng/L), and comparing them against those who remained dementia-free (median NF-L 19.94 ng/L).
155 Higher levels of NF-L at baseline predicted the likelihood of dementia at follow-up, even when
156 adjusted for age and sex ($p=0.036$).

157

158

Neurofilament-light as biomarker for AD in DS

159 4. Discussion

160 We demonstrated that NF-L measured in blood using an ultra-sensitive assay is strongly associated
161 with age and dementia status in individuals with DS, and baseline levels were predictive of dementia
162 diagnosis over time. Furthermore, NF-L levels did not differ according to severity of premorbid ID or
163 by longstanding epilepsy diagnosis (a common neurological comorbidity in DS), suggesting that it is a
164 stable and feasible biomarker that can be used in clinical populations.

165 Our results indicate that this marker could pinpoint onset of neurodegeneration in DS. NF-L showed
166 an age relationship in keeping with post-mortem data and amyloid positron emission tomography
167 (PET) studies of AD pathology in adults with DS [1, 14]. Although NF-L is a marker of axonal damage,
168 and thus not specific to AD [7-9, 15], in a genetically predisposed population such as DS where AD is
169 almost always the cause of dementia, the lack of specificity is arguably less of an issue and NF-L
170 could potentially be used as a biomarker for treatment response. Normalization of serum/plasma
171 NF-L in response to treatment has already been demonstrated in patients with multiple sclerosis [8,
172 16].

173 Although further work is required to establish long-term predictive and concurrent validity of NF-L,
174 our data suggests that this biomarker could be instrumental in allowing an experimental medicine
175 approach in DS and other high-risk populations to test treatments that might prevent or delay
176 dementia onset.

177

178 Acknowledgements

179 The authors would like to thank all the participants in this study for their time.
180 This work was funded by a Wellcome Trust Strategic Award (grant number: 098330/Z/12/Z)
181 conferred upon The London Down Syndrome (LonDownS) Consortium. The funder had no role in

Neurofilament-light as biomarker for AD in DS

182 study design; the collection, analysis, or interpretation of the data; the writing of the report; or the
183 decision to submit the report for publication.

184 HZ is a Wallenberg Academy Fellow and receives support from the European Research Council, the
185 Swedish Research Council, and Frimurarestiftelsen. HZ is further supported by the Wolfson
186 Foundation, the Wellcome Trust, and the UK Dementia Research Institute. DN is also supported by
187 the National Medical Research Council Singapore (NMRC/CIRG/1438/2015) and Singapore Ministry
188 of Education AcRF-Tier2 (2015-T2-1-023). This research was further supported by the National
189 Institute for Health Research networks (mental health, dementias and neurology) and participating
190 NHS trusts. We would like to thank our NHS network of sites that helped to identify participants.

191 The LonDownS Consortium principal investigators are Andre Strydom (chief investigator),
192 Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, Psychology and
193 Neuroscience, King's College London, London, UK, and Division of Psychiatry, University College
194 London, London, UK; Elizabeth Fisher, Department of Neurodegenerative Disease, UCL Institute of
195 Neurology, London, UK; Dean Nizetic, Blizzard Institute, Barts and the London School of Medicine,
196 Queen Mary University of London, London, UK, and Lee Kong Chian School of Medicine, Nanyang
197 Technological University, Singapore, Singapore; John Hardy, Reta Lila Weston Institute, Institute of
198 Neurology, University College London, London, UK, and UK Dementia Research Institute at UCL,
199 London, UK; Victor Tybulewicz, Francis Crick Institute, London, UK, and Department of Medicine,
200 Imperial College, London, UK; and Annette Karmiloff-Smith (Birkbeck University of London, London,
201 UK, deceased). Other members of The LonDownS Consortium who contributed to data collection are
202 Sarah Hamburg and Rosalyn Hithersay (both Department of Forensic and Neurodevelopmental
203 Sciences, Institute of Psychiatry, Psychology and Neuroscience, Kings College London, London, UK,
204 and Division of Psychiatry, University College London, London, UK).

Neurofilament-light as biomarker for AD in DS

205 References

206 [1] Wiseman FK, Al-Janabi T, Hardy J, Karmiloff-Smith A, Nizetic D, Tybulewicz VL, et al. A genetic
207 cause of Alzheimer disease: mechanistic insights from Down syndrome. *Nat Rev Neurosci*.
208 2015;16:564-74.

209 [2] McCarron M, McCallion P, Reilly E, Dunne P, Carroll R, Mulryan N. A prospective 20-year
210 longitudinal follow-up of dementia in persons with Down syndrome. *J Intellect Disabil Res*.
211 2017;61:843-52.

212 [3] Sinai A, Mokrysz C, Bernal J, Bohnen I, Bonell S, Courtenay K, et al. Predictors of Age of Diagnosis
213 and Survival of Alzheimer's Disease in Down Syndrome. *J Alzheimers Dis*. 2018;61:717-28.

214 [4] Zis P, Strydom A. Clinical aspects and biomarkers of Alzheimer's disease in Down syndrome. *Free
215 Radic Biol Med*. 2017.

216 [5] Zetterberg H. Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for
217 Neurodegeneration. *Neuron*. 2016;91:1-3.

218 [6] Gisslen M, Price RW, Andreasson U, Norgren N, Nilsson S, Hagberg L, et al. Plasma Concentration
219 of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-
220 Sectional Study. *EBioMedicine*. 2016;3:135-40.

221 [7] Rohrer JD, Woollacott IO, Dick KM, Brotherhood E, Gordon E, Fellows A, et al. Serum
222 neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia.
223 *Neurology*. 2016;87:1329-36.

224 [8] Disanto G, Barro C, Benkert P, Naegelin Y, Schadelin S, Giardiello A, et al. Serum Neurofilament
225 light: A biomarker of neuronal damage in multiple sclerosis. *Ann Neurol*. 2017;81:857-70.

226 [9] Mattsson N, Andreasson U, Zetterberg H, Blennow K, Alzheimer's Disease Neuroimaging I.
227 Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer
228 Disease. *JAMA Neurol*. 2017;74:557-66.

229 [10] Weston PSJ, Poole T, Ryan NS, Nair A, Liang Y, Macpherson K, et al. Serum neurofilament light in
230 familial Alzheimer disease: A marker of early neurodegeneration. *Neurology*. 2017;89:2167-75.

Neurofilament-light as biomarker for AD in DS

231 [11] Startin CM, Hamburg S, Hithersay R, Davies A, Rodger E, Aggarwal N, et al. The LonDownS adult
232 cognitive assessment to study cognitive abilities and decline in Down syndrome. *Wellcome Open*
233 Res. 2016;1:11.

234 [12] Ball SL, Holland AJ, Huppert FA, Treppner P, Watson P, Hon J. The modified CAMDEX informant
235 interview is a valid and reliable tool for use in the diagnosis of dementia in adults with Down's
236 syndrome. *J Intellect Disabil Res.* 2004;48:611-20.

237 [13] Strydom A, Hassiotis A, King M, Livingston G. The relationship of dementia prevalence in older
238 adults with intellectual disability (ID) to age and severity of ID. *Psychol Med.* 2009;39:13-21.

239 [14] Annus T, Wilson LR, Hong YT, Acosta-Cabronero J, Fryer TD, Cardenas-Blanco A, et al. The
240 pattern of amyloid accumulation in the brains of adults with Down syndrome. *Alzheimers Dement.*
241 2016;12:538-45.

242 [15] Lu CH, Macdonald-Wallis C, Gray E, Pearce N, Petzold A, Norgren N, et al. Neurofilament light
243 chain: A prognostic biomarker in amyotrophic lateral sclerosis. *Neurology.* 2015;84:2247-57.

244 [16] Piehl F, Kockum I, Khademi M, Blennow K, Lycke J, Zetterberg H, et al. Plasma neurofilament
245 light chain levels in patients with MS switching from injectable therapies to fingolimod. *Mult Scler.*
246 2017;1352458517715132.

247

Neurofilament-light as biomarker for AD in DS

	All participants	Dementia (baseline)	No dementia (baseline)	Participants with follow up data
Number	94	18	76	29
Age at baseline (mean ± standard deviation (range))	42.68±14.87 (17- 73)	55.17±9.92 (40- 69)	39.72±14.34 (17- 73)	52.63±8.88 (40- 72)
DS type	89 (94.7%) trisomy, 2 (2.1%) translocation, 3 (3.2%) unknown	18 (100.0%) trisomy translocation, 3 (3.9%) unknown	71 (93.4%) trisomy, 2 (2.6%) translocation, 3 (3.9%) unknown	28 (96.6%) trisomy, 1 (3.4%) unknown
Sex	41 (43.6%) female, 53 (56.4%) male	6 (33.3%) female, 12 (66.7%) male	35 (46.1%) female, 41 (55.9%) male	10 (34.5%) female, 19 (65.5%) male
Ethnicity	85 (90.4%) white, 9 (9.6%) other	17 (94.4.0%) white, 1 (5.6%) other	68 (89.5%) white, 8 (10.5%) other	27 (93.1%) white, 2 (6.8%) other
Pre-dementia ID level	37 (39.4%) mild, 47 (50%) moderate, 9 (9.6%) severe, 1 (1.2%) unknown	6 (33.3%) mild, 9 (50.0%) moderate, 3 (16.7%) severe	31 (40.8%) mild, 38 (50%) moderate, 6 (7.9%) severe, 1 (1.3%) unknown	13 (44.8%) mild, 12 (41.4%) moderate, 3 (10.3%) severe, 1 (3.4%) unknown

Neurofilament-light as biomarker for AD in DS

APOE status	68 (72.3%) non-APOE4 carrier, 23 (24.5%) APOE4 carrier, 3 (3.2%) unknown	12 (66.7%) non-APOE4 carrier, 5 (27.8%) APOE4 carrier, 1 (5.5%) unknown	56 (73.7%) non-APOE4 carrier, 18 (23.7%) APOE4 carrier, 2 (2.6%) unknown	22 (75.9%) non-APOE4 carriers, 6 (20.7%) APOE4 carrier, 1 (3.4%) unknown
NFL level (median (range)) ng/L	22.74 (6.11-136.91)	63.76 (15.21-136.91)	19.96 (6.11-116.84)	32.67 (12.23 – 481.97)

248 Table 1. Participant demographics of all participants included in group and subgroup analyses