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1 **Plasma levels of soluble TREM2 and neurofilament light chain in *TREM2* rare variant**
2 **carriers**

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6

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46 **Abstract**

47 Results from recent clinical studies suggest that cerebrospinal fluid (CSF) biomarkers that are
48 indicative of Alzheimer's disease (AD) can be replicated in blood, *e.g.*, amyloid-beta peptides
49 ($A\beta_{42}$ & $A\beta_{40}$) and neurofilament light chain (NFL). Such data proposes that blood is a rich
50 source of potential biomarkers reflecting central nervous system pathophysiology and should
51 be fully explored for biomarkers that show promise in CSF. Recently, soluble fragments of the
52 triggering receptor expressed on myeloid cells 2 (sTREM2) protein in CSF have been reported
53 to be increased in prodromal AD and also in individuals with *TREM2* rare genetic variants that
54 increase the likelihood of developing dementia. In this study, we measured the levels of plasma
55 sTREM2 and plasma NFL in 48 confirmed *TREM2* rare variants carriers and 49 non-carriers.
56 Our results indicate that there are no changes in plasma sTREM2 and NFL between *TREM2*
57 rare variants carriers and non-carriers. Furthermore, plasma sTREM2 is not different between
58 healthy controls, mild cognitive impairment (MCI) or AD. In conclusion, plasma sTREM2 does
59 not mimic the recent changes found in CSF sTREM2.

60

61 **Key words**

62 Alzheimer's disease, sTREM2, blood, biomarkers, neurofilament light chain

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68 **Introduction**

69 There has been considerable progress in the search for blood-based biomarkers able to capture
70 the clinical course and underlying pathophysiology of Alzheimer's disease (AD), for review
71 see (1). Reduced plasma $A\beta_{42}/A\beta_{40}$ ratio (2-4) and increased neurofilament light chain (NFL)
72 (5-7) are becoming consistently reported in AD and, encouragingly, these findings mimic the
73 more established observations seen in cerebrospinal fluid (CSF) (8). NFL is not a specific
74 biomarker for AD (9). Increases are observed in a number of neurodegenerative disorders (10-
75 12), owing to its global reflection of axonal injury or degeneration. Yet, translating other co-
76 pathology markers of neurodegeneration from CSF to blood has been less successful. For
77 example, plasma total tau (T-tau) increases in AD (13) but it seems to have limited clinical
78 utility and phosphorylated form of tau (P-tau) has proven difficult to establish as a reliable
79 measure in blood despite recent promise (14). Likewise, TDP-43 (15), alpha-synuclein (16) and
80 the post-synaptic dendritic biomarker, neurogranin (17), all have substantial and specific
81 peripheral expression but levels appear to be unrelated to changes in the central nervous system.

82 The triggering receptor expressed on myeloid cells 2 (TREM2) is an innate immune receptor
83 that guides essential functions of microglia. Rare variants in *TREM2* strongly increase the
84 likelihood of developing AD, frontotemporal dementia (FTD), Parkinson's disease (PD) and
85 amyotrophic lateral sclerosis (ALS) (18-23). TREM2 is a type-1 transmembrane protein and its
86 ectodomain is shed at the plasma membrane by ADAM family proteases C-terminal at histidine
87 157 position (24-27). The resulting soluble fragment (sTREM2) is released into the
88 extracellular space and can be found in CSF and plasma (28, 29). Recently, the concentrations
89 of CSF sTREM2 have been shown to be increased in early symptomatic stages of sporadic (30-
90 34) and autosomal dominant AD patients (35). Interestingly, $A\beta$ pathology and tau-related
91 neurodegeneration may impact levels of CSF sTREM2 differently (33). Moreover, it has been

92 shown that the concentrations of CSF sTREM2 vary between different disease-associated
93 *TREM2* genetic variant carriers (32, 33).

94 Unlike CSF sTREM2, levels of sTREM2 in blood have been poorly investigated. In this study,
95 we investigate plasma concentrations of sTREM2 in patients with AD and mild cognitive
96 impairment (MCI) compared with aged-matched healthy controls. Furthermore, in a novel
97 approach, we also report on blood concentrations of sTREM2 and NFL in *TREM2* rare variants
98 carriers.

99

100 **Methods**

101 *Participants*

102 Samples from a total of 97 participants were used for these analyses (Table 1). The majority of
103 samples (n=82) were from the longitudinal AD cohorts managed at King's College London
104 (KCL; AddNeuroMed (36), Kings Health Partners-Dementia Case Register (KHP-DCR) a UK
105 clinic and population based study (37) and the MRC AD Biomarker study (38)). Further
106 samples were obtained from DEGESCO (n=11, Dementia Genetics Spanish Consortium (39,
107 40)) and EDAR (n=4, Beta amyloid oligomers in the early diagnosis of AD and as marker for
108 treatment response (41)). Informed consent for all participants was obtained according to the
109 Declaration of Helsinki (1991) and protocols and procedures were approved by the relevant
110 local ethical committee at each site. The cohorts as mentioned above were genetically analysed
111 to identify known or novel non-synonymous variants in exon 2 of the *TREM2* gene, previously
112 linked to pathogenic risk or predicted to be detrimental. Of the 48 participants identified with a
113 *TREM2* pathogenic variant ($TREM2^{var}$, Table 1), 10 were control, 10 had MCI and 28 had a
114 dementia diagnosis (AD). Similar age-matched non-carrier control (n=10), MCI (n=8) and AD
115 (n=31) samples were also included.

116

117 *Plasma measures of sTREM2 and NFL*

118 Plasma sTREM2 was measured using an in-house electrochemoluminescent assay on the
119 MesoScale Discovery SECTOR imager 6000 (MesoScale Discovery (MSD) Maryland, US)
120 using a method adapted from Kleinberger et al (29). The capture antibody was the biotinylated
121 polyclonal goat anti-human TREM2 (0.25 μ g/mL R&D Systems, Minneapolis, US) and the
122 detector antibody was monoclonal mouse anti-human TREM2 (1 μ g/MI Santa Cruz
123 Biotechnology, Texas, US). A standard curve for calculations of unknowns was constructed
124 using recombinant human TREM2 (4000 – 62.5 pg/mL, Sino Biological, Beijing, China) and
125 plasma samples were diluted 1:4 before being assayed. For a more comprehensive description
126 of the method please see (29). For NFL, the commercially available NF-light assay on an HD-
127 1 Simoa instrument (Quanterix, Lexington, MA, USA) was utilized. All biochemical analyses
128 were performed at the Institute of Neurology at University College London (UCL).

129

130 *Statistical analysis*

131 Data normality was determined by the D'Agostino-Pearson test and statistical evaluation was
132 performed on log₁₀-transformed data. After transformation, the data followed a normal
133 distribution. All data analysis reported has been performed on log₁₀-transformed sTREM2 and
134 NFL but the untransformed values are shown in descriptive tables and figures. To study the
135 association of plasma measures with demographic data a Pearson product-moment correlation
136 was utilized for age and MMSE whereas *t*-test (sTREM2) or a one-way analysis of covariance
137 (ANCOVA, NFL) for gender and APOE status. Only age was a significant predictor of plasma
138 NFL, the subsequent analyses were therefore conducted including age as a confounder. A *t*-test
139 or ANCOVA were conducted to determine clinical group differences between blood

140 biomarkers. ANCOVA analyses were followed by a Bonferroni corrected *post hoc* pairwise
141 comparison where appropriate. A partial correlation, adjusted by age, tested the association
142 between plasma sTREM2 and plasma NFL. Statistical analysis was performed using IBM SPSS
143 Statistics, version 25 (Armonk, NY, USA).

144

145 **Results**

146 Forty-eight confirmed *TREM2* rare variant carriers and 49 non-carriers were included in the
147 study. The *TREM2* rare variant carrier group comprised of 10 different variants: p.Q33X,
148 p.D39N, p.R47H, p.P59L, p.R62H, p.D87N, p.T96K, p.Q691H, p.H703Y and p.L868R. The
149 demographical characteristics of the cohort are described in Table 1. There were no differences
150 in age between *TREM2* rare variant carriers (M = 75.2, SD = 7.3) and non-carriers (M = 76.1,
151 SD = 6.7). The inclusion of gender was very similar across groups (carriers, 26/48 female
152 [54.2%]; non-carriers, 27/49 female [55.1%]). Finally, there was no statistically significant
153 differences in MMSE (carriers, M = 23.1, SD = 6.4; non-carriers, M = 23.3, SD = 5.0) or clinical
154 diagnosis between the two groups (Table 1).

155 In the whole cohort, plasma sTREM2 was not associated with age ($r = 0.060$; $P = 0.562$), gender
156 ($P = 0.083$), *APOE* $\epsilon 4$ status ($P = 0.237$) or MMSE ($r = -0.018$; $P = 0.858$). There were no
157 differences in the levels of plasma sTREM2 between *TREM2* rare variant carriers (M = 7,346
158 ng/L, SD = 5,526 ng/L) or non-carriers (M = 8,750 ng/L, SD = 5,265 ng/L), $t(95) = 1.696$, $P =$
159 0.093 (Fig. 1A). There were no significant differences in plasma sTREM2 between carriers of
160 different *TREM2* rare variants ($F(3, 84) = 1.68$, $P = 0.177$, Fig 1B). Note, we only included
161 *TREM2* rare variants with >2 individuals per group in this analysis. Adding clinical diagnosis
162 as covariate did not change the result ($P = 0.171$). Next, we tested whether plasma sTREM2
163 levels differ between clinical diagnoses, regardless of the *TREM2* rare variant status, between

164 AD (M = 8,859 ng/L, SD = 5,951 ng/L), MCI (M = 6,204 ng/L, SD = 2,572 ng/L) and controls
165 (M = 7,352 ng/L, SD = 5,318 ng/L). Plasma sTREM2 levels were not different between these
166 groups ($F(2, 94) = 1.84$, $P = 0.164$, Fig 1C). Adjusting for the effect of age and gender did not
167 change the result of sTREM2 in plasma.

168 As expected, plasma NFL concentrations were significantly associated with age ($r = 0.202$, $P =$
169 0.047). After accounting for the effect of age, plasma NFL levels were found to be associated
170 with MMSE ($r = -0.353$, $P = 0.0004$), and tended to be higher in females (M = 25.6, SD = 13.1
171 ng/L) compared to males (M = 25.1, SD = 22.8 ng/L; $P = 0.079$). Levels were not affected by
172 *APOE* $\epsilon 4$ status ($P = 0.899$). There were no differences in plasma NFL between *TREM2* rare
173 variant carriers (M = 24.6 ng/L, SD = 19.1 ng/L) and non-carriers (M = 26.1 ng/L, SD = 17.1
174 ng/L) ($F(1, 94) = 0.505$, $P = 0.479$, Fig 2A), after adjusting for the effect of age. Similar to
175 plasma sTREM2, there were no differences in plasma NFL when comparing the different
176 *TREM2* rare variants ($F(3, 83) = 0.113$, $P = 0.952$, Fig 2B). This remained true when correcting
177 for the effect of the clinical diagnosis ($P = 0.633$). As expected, differences in plasma NFL were
178 observed between the three clinical groups ($F(2, 93) = 4.89$, $P = 0.010$). Bonferroni corrected
179 *post hoc* pairwise comparison demonstrated that the AD group (M = 29.0 ng/L, SD = 21.6 ng/L)
180 had significantly higher levels of NFL compared to controls (M = 18.8 ng/L, SD = 7.2 ng/L; P
181 = 0.025) but not to MCI (M = 20.5 ng/L, SD = 8.1 ng/L; $P = 0.096$, Fig 2C). A further
182 adjustment for the effect of gender did not change these NFL findings.

183 In the whole cohort, when correcting for age, a significant positive correlation between plasma
184 sTREM2 and plasma NFL was observed ($r = 0.245$, $P = 0.016$). However, this correlation is
185 driven by the symptomatic individuals (MCI and AD) as no significant association of plasma
186 sTREM2 and plasma NFL was observed in the control group ($r = 0.250$, $P = 0.302$) but a
187 tendency in the symptomatic group ($r = 0.223$, $P = 0.053$).

188

189 **Discussion**

190 This is the first study to have comprehensively investigated if the levels of plasma sTREM2
191 and an neurodegenerative marker, plasma NFL, differ between *TREM2* rare variant carriers and
192 non-carriers. Our main finding demonstrates that there are no significant differences in plasma
193 sTREM2 and NFL between these groups. Furthermore, we also show that plasma sTREM2
194 does not differ between controls, MCI and AD.

195 While sTREM2 has been extensively studied in CSF, only a few studies have reported sTREM2
196 in blood. Piccio and co-workers (28) demonstrated that serum sTREM2 levels did not differ
197 between multiple sclerosis, other inflammatory neurologic diseases and non-inflammatory
198 controls. Kleinberger et al. (29) found no difference in plasma sTREM2 between healthy
199 controls, AD and FTD. The lack of separation between AD and controls was then independently
200 replicated (32). In a different approach, Ohara et al. showed that increased serum sTREM2 is
201 associated with increased risk to develop dementia in Japanese population (42). Herein, we also
202 demonstrate that plasma sTREM2 is not different between *TREM2* rare variant carriers and
203 non-carriers, nor with respect to clinical diagnosis. Therefore, although readily detectable,
204 sTREM2 in blood is not useful to discriminate those people with a clinical diagnostic group, or
205 those with a *TREM2* variant associated with AD. It should be noted that sTREM2 in blood most
206 likely has a peripheral rather than a central nervous system (CNS) origin from microglia. This
207 is in agreement with the ubiquitous expression pattern of *TREM2* at both mRNA and protein
208 levels in Human Protein Atlas ([https://www.proteinatlas.org/ENSG00000095970-
209 TREM2/tissue](https://www.proteinatlas.org/ENSG00000095970-TREM2/tissue)). *TREM2* is highly expressed in cells from a myeloid lineage, such as
210 monocytes, macrophages, Kupffer cells or osteoclasts (43-46).

211 Previously, it was shown that both plasma and CSF sTREM2 were useful to detect those with
212 homozygous *TREM2* mutations (e.g. p.T66M, p.W198X, p.Q33X and p.Y38C) that lead to
213 Nasu-Hakola disease or an FTD-like syndrome (47, 48). In these diseases, sTREM2 in blood

214 or CSF is almost absent, which is in line with the impaired cell surface transport and shedding
215 that occur with these mutations (29, 49). In contrast, heterozygous *TREM2* rare variant carriers
216 have a less obvious and inconsistent pattern of CSF sTREM2. While the *TREM2* p.R47H rare
217 variant is associated with increased CSF sTREM2, other *TREM2* variants (e.g. p.L211P;
218 T96K/L211P/W191X) have been reported to be associated with decreased or unchanged levels
219 of CSF sTREM2 (e.g. R62H) (32, 33) (Deming et al. Sci Trans Med 2019 *in press*). Our
220 findings could be explained by how these rare variants differently affect the processing and
221 shedding of TREM2. For example, individual variants are believed to impact TREM2
222 production (Q33X), expression or turnover at the cell surface (R47H) (29, 50), α -secretase
223 cleavage of the extracellular soluble ectodomain (H157Y) (27, 51) and/or ligand binding
224 (R47H, R62H, T96K, H157Y) (52-54). Surprisingly, we did not find such differences in
225 plasma, suggesting a different regulation of TREM2 in the periphery and the CNS. We also
226 investigated plasma NFL, but here we also found no differences between *TREM2* rare variant
227 carriers and non-carriers, even when introducing clinical diagnosis as a co-variable, suggesting
228 that the neuronal injury is no different in AD irrespective of whether someone has a *TREM2*
229 rare variant. These findings are agreement with clinical data that find AD cases with a *TREM2*
230 p.R47H rare variant are clinically indistinguishable from other AD, albeit those with a *TREM2*
231 rare variant generally have an earlier age of onset (55). Finally, the finding that sTREM2 is
232 associated with NFL in AD is consistent with the association of CSF sTREM2 with T-tau,
233 another marker of neurodegeneration, and suggests that inflammatory response might be
234 coupled to neurodegeneration (56).

235 This study has some limitations. Despite the remarkable number of individuals with rare
236 variants that were included, some individual variants were represented by only very small
237 numbers, which precluded a comparison between them. Additionally, these samples did not
238 have the core AD CSF biomarkers to confirm diagnosis, disease stage or link analyses

239 individually to Tau or amyloid burden, and hence we were limited to using clinical diagnosis.
240 The main strengths are the fact that we used very reliable and well-established assays for both
241 sTREM2 and NFL. The study was designed in such a way that an important total number of
242 *TREM2* rare variants were carefully matched to a control group in terms of age, gender and
243 clinical diagnosis.

244 In conclusion, this study, for the first time, demonstrates that the levels of sTREM2 and NFL
245 in plasma do not differ between *TREM2* rare variants carriers and non-carriers. Furthermore,
246 we confirm previous reports that sTREM2 is not changed in AD or MCI compared with aged-
247 matched controls. Therefore, we conclude that, although plasma sTREM2 may be useful to
248 detect *TREM2* homozygous mutations, plasma sTREM2 is not a reliable biomarker to detect
249 *TREM2* rare variant status nor suspected AD.

250

251 **Figure legends**

252

253 **Fig 1. The concentrations of plasma sTREM2.** No change in the levels of sTREM2 between
254 *TREM2* rare variant carriers and non-carriers (A, B). A non-significant increase in sTREM2
255 was observed in AD patients compared to MCI and aged-matched controls (C).

256

257 **Fig 2. The concentrations of plasma NFL.** No change in the concentrations of NFL between
258 *TREM2* rare variants and non-carriers (A, B). A significant increase in NFL was observed in
259 AD patients compared to MCI and aged-matched controls which was not influenced by *TREM2*
260 mutation status (C).

261

262

263 **Abbreviations**

264 AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ANCOVA, analysis of
265 covariance; CNS, central nervous system; CSF, cerebrospinal fluid; FTD, frontotemporal
266 dementia; MCI, mild cognitive impairment; MMSE, mini mental state examination; MSD,
267 MesoScale Discovery; NFL, neurofilament light chain; P-tau, phosphorylated tau; PD,
268 Parkinson's disease; Simoa, Single molecule array; TREM2, triggering receptor expressed on
269 myeloid cells 2; T-tau, total tau

270

271 **Declarations**

272 *Ethics approval and consent to participate*

273 Not applicable.

274 *Consent for publication*

275 Not applicable.

276 *Availability of data and material*

277 The datasets used and/or analysed during the current study are available from the corresponding
278 author on reasonable request.

279 *Competing interests*

280 KB has served as a consultant or at advisory boards for Alector, Alzheon, CogRx, Biogen, Lilly,
281 Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in
282 Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all
283 unrelated to the work presented in this paper. HZ has served at scientific advisory boards for
284 Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by

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298 *Authors' contributions*

299 N.J.A, A.K.H and H.Z. provided the initial concept and study design. A.H performed all data
300 acquisition. N.J.A and M.S.A performed data analysis, interpreted the data and co-wrote the
301 manuscript. All authors contributed to the content of the publication, critically reviewed and
302 edited the manuscript.

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504

505 **Table 1.** Demographic and clinical characteristics of *TREM2* rare variant carriers and non-
 506 carriers

	<i>TREM2</i> non-carriers (n=49)	<i>TREM2</i> rare variant carriers (n=48)	<i>TREM2</i> rare variant carriers			
			p.R47H (n=26)	p.T96K (n=8)	p.D87N (n=5)	Others (n=9)
Age, years (SD)	76.1 (6.7)	75.2 (7.3)	73.9 (8.4)	76.6 (4.8)	72.4 (6.8)	76.9 (4.1)
Female, n (%)	27/49 (55.1)	26/48 (54.2)	12 (46.2)	5 (55.5)	3 (60)	6 (75)
<i>APOE</i> ε4 carriers, n (%)	24 (68.6) ^a	21 (59.7) ^b	11	3	1	6
MMSE, n (SD)	23.3 (5.0)	23.1 (6.4)	21 (7.5)	25 (5.0)	28.8 (4.5)	24.7 (2.3)
Diagnosis, n (%)	AD, 31/49 (63.3) MCI, 8/49 (16.3) Ctrl, 10/49 (20.4)	AD, 28/48 (58.4) MCI, 10/48 (20.8) Ctrl, 10/48 (20.8)	18/26 (69) 4/26 (15.5) 4/26 (15.5)	2/9 (25.0) 3/9 (37.5) 3/9 (37.5)	1/5 (16.7) 2/5 (33.3) 2/5 (50.0)	7/9 (87.5) 1/9 (12.5) 0/9
sTREM2, ng/L (SD)	8,750 (5,265)	7,346 (5,526)	7,294 (6791)	8,761.8 (4840)	6,431 (4107)	7,009 (1889)
NFL, ng/L (SD)	26.1 (17.1)	24.6 (19.1)	25.7 (23.8)	25.2 (17.8)	23.1 (7.7)	21.3 (7.6)