

Mendelian randomization indicates that TNF is not causally associated with Alzheimer's disease

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1 **Abstract**

2 INTRODUCTION: Epidemiological research has suggested that inhibition of tumor necrosis
3 factor (TNF)- α in patients with rheumatoid arthritis (RA) reduces the overall risk of Alzheimer's
4 disease (AD). TNF- α antagonists have been suggested as a potential treatment for AD.

5 METHODS: We used a two-sample Mendelian randomization design to examine the causal
6 relationship between blood *TNF* expression, serum TNF- α levels, and RA on AD risk.

7 RESULTS: Our results do not support a causal relationship between *TNF* expression, serum
8 TNF- α levels or RA on AD risk.

9 DISCUSSION: These results suggest that TNF- α antagonists are unlikely to reduce the risk of
10 AD.

11

12 **Keywords:** Alzheimer's disease; Tumor necrosis factor; TNF; rheumatoid arthritis; Mendelian
13 Randomization

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16 **1. Introduction**

17 Alzheimer's disease (AD) is a debilitating neurological condition that is characterized by a
18 progressive deterioration in cognitive function and concomitant functional decline. While the
19 classical neuropathological hallmarks of AD are the deposition of neuritic plaques and
20 neurofibrillary tangles, increasing evidence has highlighted the role of neuroinflammation and
21 microglial innate immune response in AD pathogenesis [1].

22

23 Chronic systemic inflammation may also be associated with an increased risk of developing
24 dementia [2], with higher levels of serum proinflammatory cytokines being reported in patients
25 with AD [3]. Chronic systemic inflammation is characterized by the production of the
26 proinflammatory cytokine tumor necrosis factor α (TNF- α) from macrophages. TNF- α is involved
27 in the pathogenesis of chronic autoimmune disorders such as rheumatoid arthritis (RA), but also
28 plays a role in activation of the central innate immune response, including in microglial cells [4].
29 Inflammation represents a potential means of modifying AD pathogenesis, with the link between
30 peripheral inflammation, TNF- α and neuroinflammation suggesting that TNF- α inhibition may
31 reduce the risk of AD.

32

33 AD was found to be more prevalent in RA patients in a nested case-control study of 8.5 million
34 commercially insured adults. Additionally, it was observed that the risk of AD was lower among
35 RA patients who had been exposed to the TNF- α inhibitor etanercept, suggesting that anti-TNF
36 therapy with etanercept could be a potential treatment for AD [5]. The potential role of
37 etanercept as a treatment for AD has gained further media exposure from a recent Washington
38 Post report that analysis conducted by Pfizer, which holds the patent for etanercept outside of
39 the USA, observed a similar decrease in risk of AD in RA patients exposed to etanercept based
40 on insurance claim data [6]. Pfizer, however, elected not to proceed with a clinical trial which

41 was estimated to cost \$80 million, according to critics, because etanercept was reaching the
42 end of its patent life, though Pfizer denies this was a factor [6].

43
44 While randomized control trials (RCTs) can be reliably used for estimating causal effects, they
45 are expensive to conduct and not all drug targets can be tested in an RCT framework due to
46 ethical considerations or the time-scale involved may be prohibitive. A novel method for
47 estimating the causal effect of drug targets on a disease outcome is Mendelian randomization
48 (MR). MR is a method that estimates the causal effect of an exposure on an outcome by using
49 genetic variants as a proxy for the exposure administered as an intervention in an RCT [7]. MR
50 is analogous to an RCT due to the random allocation of genotypes from parents to offspring at
51 conception (randomization in an RCT) and is thus not affected by reverse causation or
52 confounding variables. In the context of drug development, genetic variants are selected that
53 mimic the action of a drug target, with one allele associated with an altered gene or protein
54 expression (the drug in an RCT) to that of a neutral allele that serves as a reference (the
55 placebo in an RCT) [7]. If the altered genetic allele is associated with a pathway that is
56 causative of the disease, the MR study will detect a change in the clinical outcome. A drug
57 target that has a causative effect on disease is a potential target for drug development, whereas
58 the reverse is true if it is not causative.

59
60 In this study, we use Mendelian randomization to evaluate if RA, *TNF* gene expression and
61 $TNF-\alpha$ levels are causally related to AD risk.

62 **2. Methods**

63 **2.1 Instrument Selection**

64 We obtained cis-eQTL data derived from whole blood for *TNF* expression from the eQTLGen
65 project (n = 31,684) [8], pQTL data derived from whole blood $TNF-\alpha$ levels (n = 8,293) [9], and

66 genome-wide significant SNPs for RA from a previous GWAS meta-analysis (14,361 RA cases
67 and 43,923 controls) [10]. To obtain independent SNPs, linkage disequilibrium clumping was
68 performed by excluding SNPs that had an $r^2 > 0.001$ with another variant with a smaller p-value
69 association within 1000kb use a reference panel of European individuals from 1000 Genomes
70 Project (phase 3). Three independent eQTLs for *TNF*, six nominally significant ($p < 5e-6$)
71 independent $TNF-\alpha$ pQTLs, 56 independent SNPs for RA were selected for analysis. As the
72 effect sizes of the eQTLs were not available in the summary data, the effect sizes were
73 estimated from z-statistics as previously described [11].

74

75 The GWAS summary data for AD were from the most recent meta-analysis conducted by the
76 International Genomics of Alzheimer's Project comprised of 21,982 cases and 41,944
77 cognitively normal controls (Stage 1 discovery) [12]. The SNPs corresponding to the *TNF*
78 eQTLs, $TNF-\alpha$ pQTLs, and RA SNPs were extracted from the AD GWAS and were harmonized.

79

80 **2.2 Mendelian Randomization Analysis**

81 We used two-sample MR to estimate the causal effect of *TNF* expression, $TNF-\alpha$ levels, and RA
82 on AD. For each variant, we calculated an instrumental variable ratio estimate by dividing the
83 SNP-exposure by SNP-outcome and coefficients. An overall estimate of the causal effect was
84 calculated by combining the individual SNP estimates in a fixed-effects meta-analysis using an
85 inverse-variance weighted (IVW) approach [13]. In order to account for potential violations of the
86 assumptions underlying the IVW analysis, we conducted a sensitivity analysis using MR-Egger
87 regression, which allows all variants to be subject to direct effects [13] and the Weighted
88 Median Estimator (WME), which takes the median effect of all available variants, allowing 50%
89 of variants to exhibit horizontal pleiotropy [13]. Heterogeneity was tested using Cochran's Q
90 statistic [13].

91 The proportion of variance in the exposure explained by each instrument were calculated as
92 previously described [14]. Power calculations were conducted using the mRnd power
93 calculation tool [15]. All statistical analyses were conducted using R version 3.5.2, with
94 Mendelian randomization analysis was performed using the 'TwoSampleMR' package [13].

95

96 **Results**

97 The selected instruments for *TNF* expression, TNF- α levels, and RA risk explained 5.93% ($F =$
98 285), 1.68% ($F = 23.6$), and 19.2% ($F = 247$) of the variance respectively. Given a sample size
99 of 63,926 with the proportion of cases equal to 0.34, this study was adequately powered to
100 detect an OR of any AD of 1.1 for *TNF* expression, 1.19 for TNF- α levels and 1.055 for RA.

101

102 There was no evidence of a causal association of *TNF* expression, TNF- α levels or RA on AD
103 risk in the IVW, WME, or MR-Egger regression analyses (Table 2). Similarly, there was no
104 causal association for the individual *TNF* eQTLs. There was evidence of heterogeneity ($Q =$
105 84.8, $df = 54$, $p = 0.00472$) in RA analysis, but not for the TNF ($Q = 3.46$, $df = 2$, $p = 0.177$) or
106 TNF- α ($Q = 2.78$, $df = 5$, $p = 0.733$) analysis.

107

108 **Discussion**

109 This study examined the causal association of blood *TNF* expression, serum TNF- α levels and
110 RA with AD risk using Mendelian randomization. Despite adequate statistical power to detect an
111 effect, we do not find any evidence that increased *TNF* expression, TNF- α levels or RA risk are
112 causally associated with increased AD risk. These results suggest that TNF- α antagonists, such
113 as etanercept, are unlikely to reduce the risk of AD.

114

115 Incidence of AD was reported to be lower in RA patients in a meta-analysis of 10 studies,
116 however, an MR analysis conducted using an earlier AD GWAS also found no causal effect of
117 RA on AD [16]. While animal studies of AD models suggest that TNF- α inhibition ameliorates
118 AD-related pathology, only a few human studies have been conducted [17]. An open-label
119 clinical trial conducted in mild-severe AD patients (n = 15) found that perispinal extrathecal
120 administration of etanercept was associated with significant improvement in cognitive function
121 [18]. In contrast, a double-blind study of etanercept conducted in patients with mild-moderate
122 AD (n = 41) over a 24-week period, found that subcutaneous administration of etanercept
123 showed no effect on cognitive, functional or behavioral assessments [19].

124
125 The results of this study should be interpreted in conjunction with its limitations. First, the
126 analysis conducted here was restricted to the expression of *TNF* mRNA in whole blood, the
127 tissue in which the largest eQTL studies have been conducted to date. Analysis in additional
128 tissues may implicate *TNF* expression as a causal risk factor, however, the sample sizes
129 available for other tissues are 30x smaller than that of whole blood and thus have considerably
130 reduced power [20]. Second, the TNF- α GWAS did not contain any genome-wide significant
131 hits, as such, we used nominally significant hits which can result in the inclusion of weak
132 instruments and bias results towards the null. Finally, these MR estimates represent the effect
133 of lifelong exposure to increased *TNF* expression or TNF- α levels, while drugs generally have
134 shorter periods of exposure, and may not distinguish between critical periods of exposure [7].

135
136 In conclusion, this Mendelian randomization analysis does not support a causal effect of
137 increased blood *TNF* expression, serum TNF- α levels or RA risk on the risk of AD. These
138 results suggest that, in contrast to recent reports, TNF- α antagonists are unlikely to result in
139 decreased risk of AD. Furthermore, this study highlights how incorporating genetic data into the

140 drug discovery process using Mendelian randomization can improve the drug discovery
141 process.

142

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145

146 **Conflicts of Interest**

147 SJA has no conflicts of interest to declare.

148 AMG served on the scientific advisory board for Denali Therapeutics from 2015-2018. She has
149 also served as a consultant for Biogen, Cognition Therapeutics, AbbVie, Pfizer, GSK, Eisai and
150 Illumina.

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208

209 **Tables**

210

211 **Table 1: Causal effect of *TNF* expression, $TNF-\alpha$ levels and rheumatoid arthritis risk on**

212 **AD**

	β (se)	OR (95% CI)	p
TNF - LOAD			
rs1121800	0.06 (0.05)	1.07 (0.97, 1.17)	0.20
rs72855945	0.17 (0.19)	1.19 (0.83, 1.72)	0.35
rs9469017	-0.24 (0.17)	0.79 (0.57, 1.1)	0.16
IVW	0.05 (0.05)	1.05 (0.96, 1.15)	0.30
WME	0.06 (0.05)	1.06 (0.97, 1.17)	0.21
MR Egger	0.1 (0.26)	1.11 (0.67, 1.83)	0.76
TNF-α - LOAD			
IVW	-0.03 (0.04)	0.97 (0.89, 1.06)	0.52
WME	-0.03 (0.06)	0.97 (0.87, 1.08)	0.58
MR Egger	-0.07 (0.07)	0.93 (0.81, 1.06)	0.34
RA - LOAD			
IVW	-0.01 (0.01)	0.99 (0.97, 1.01)	0.37
WME	0.01 (0.02)	1.01 (0.97, 1.06)	0.48
MR Egger	-0.02 (0.03)	0.98 (0.93, 1.03)	0.51

213

214 *TNF*: Tumor necrosis factor mRNA expression; $TNF-\alpha$: Serum tumor necrosis factor- α levels;

215 RA: rheumatoid arthritis

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