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:** Alzheiemr'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-α 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-α 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 r2 > 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-α 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-α 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-α 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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References

[1] Mhatre SD, Tsai CA, Rubin AJ, James ML, Andreasson KI. Microglial malfunction: the third rail in the development of Alzheimer's disease. Trends Neurosci 2015;38:621–36.

[2] Wyss-Coray T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 2006;12:1005–15.

[3] Lai KSP, Liu CS, Rau A, Lanctôt KL, Köhler CA, Pakosh M, et al. Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. J Neurol Neurosurg Psychiatry 2017;88:876–82.

[4] Perry VH, Cunningham C, Holmes C. Systemic infections and inflammation affect chronic neurodegeneration. Nat Rev Immunol 2007;7:161–7.

[5] Chou RC, Kane M, Ghimire S, Gautam S, Gui J. Treatment for Rheumatoid Arthritis and Risk of Alzheimer's Disease: A Nested Case-Control Analysis. CNS Drugs 2016;30:1111–20.

[6] Rowland C. Pfizer had clues its blockbuster drug could prevent Alzheimer's. Why didn't it tell the world? The Washington Post 2019.

[7] Walker VM, Davey Smith G, Davies NM, Martin RM. Mendelian randomization: a novel approach for the prediction of adverse drug events and drug repurposing opportunities. Int J Epidemiol 2017;46:2078–89.

[8] Võsa U, Claringbould A, Westra H-J, Bonder MJ, Deelen P, Zeng B, et al. Unraveling the polygenic architecture of complex traits using blood eQTL metaanalysis. bioRxiv 2018:447367. doi:10.1101/447367.

[9] Ahola-Olli AV, Würtz P, Havulinna AS, Aalto K, Pitkänen N, Lehtimäki T, et al. Genome-wide Association Study Identifies 27 Loci Influencing Concentrations of Circulating Cytokines and Growth Factors. Am J Hum Genet 2017;100:40–50.

[10] Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 2014;506:376–81.

[11] Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet 2016;48:481–7.

[12] Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. Nat Genet 2019. doi:10.1038/s41588-019-0358-2.

[13] Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife 2018;7. doi:10.7554/eLife.34408.

[14] Shim H, Chasman DI, Smith JD, Mora S, Ridker PM, Nickerson DA, et al. A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. PLoS One 2015;10:e0120758.

[15] Brion M-JA, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. Int J Epidemiol 2013;42:1497–501.

[16] Policicchio S, Ahmad AN, Powell JF, Proitsi P. Rheumatoid arthritis and risk for Alzheimer's disease: a systematic review and meta-analysis and a Mendelian Randomization study. Sci Rep 2017;7:12861.

[17] Ekert JO, Gould RL, Reynolds G, Howard RJ. TNF alpha inhibitors in Alzheimer's disease: A systematic review. Int J Geriatr Psychiatry 2018;33:688–94.

[18] Tobinick E, Gross H, Weinberger A, Cohen H. TNF-alpha modulation for treatment of Alzheimer's disease: a 6-month pilot study. MedGenMed 2006;8:25.

[19] Butchart J, Brook L, Hopkins V, Teeling J, Püntener U, Culliford D, et al. Etanercept in Alzheimer disease: A randomized, placebo-controlled, double-blind, phase 2 trial.

203   Neurology 2015;84:2161–8.
204 [20] GTEx Consortium, Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis
205   Working Group, Statistical Methods groups—Analysis Working Group, Enhancing GTEx
206   (eGTEx) groups, NIH Common Fund, NIH/NCI, et al. Genetic effects on gene expression
207   across human tissues. Nature 2017;550:204–13.

208

209 **Tables**

210

211 **Table 1: Causal effect of *TNF* expression, TNF-α 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-α: Serum tumor necrosis factor-α levels;

215 RA: rheumatoid arthritis

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