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

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ABSTRACT

Epidemiological research has suggested that inhibition of tumor necrosis factor (TNF)-α in patients with rheumatoid arthritis (RA) reduces the overall risk of Alzheimer's disease (AD). TNF-α antagonists have been suggested as a potential treatment for AD. We used a two-sample Mendelian randomization design to examine the causal relationship between blood *TNF* expression, serum TNF-α levels, and RA on AD risk. Our results do not support a causal relationship between *TNF* expression, serum TNF-α levels, and RA on AD risk.

1. Introduction

Chronic systemic inflammation may be associated with an increased risk of developing dementia (Wyss-Coray, 2006), with higher levels of serum proinflammatory cytokines being reported in patients with Alzheimer's disease (AD) (Lai et al., 2017). Chronic systemic inflammation is characterized by the production of the proinflammatory cytokine tumor necrosis factor α (TNF-α) from macrophages. TNF-α is involved in the pathogenesis of chronic autoimmune disorders such as rheumatoid arthritis (RA) but also plays a role in activation of the innate immune response, including in microglial cells (Perry et al., 2007). Inflammation represents a potential means of modifying AD pathogenesis, with the link between peripheral inflammation, TNF-α, and neuroinflammation, suggesting that TNF-α inhibition may reduce the risk of AD. In this study, we use Mendelian randomization (MR) to test whether RA, *TNF* gene expression, and TNF-α levels are causally related to AD risk.

2. Methods

We obtained cis- expression quantitative trait loci (cis-eQTLs) data derived from whole blood for *TNF* expression from the eQTLGen project (n = 31,684) (Võsa et al., 2018), protein quantitative trait loci data derived from whole blood TNF-α levels (n = 13,577) (Sliz et al., 2019), and single nucleotide polymorphisms for RA from a previous genome wide association studies (GWAS) meta-analysis (14,361 cases, 43,923 controls) (Okada et al., 2014). Three independent ($r^2 > 0.001$, 1000-kb window) eQTLs for *TNF*, ten nominally significant ($p < 5e\text{-}6$) independent TNF-α protein quantitative trait loci, and 56 independent genome-wide significant ($p < 5e\text{-}8$) single nucleotide polymorphisms for RA were selected for analysis. The effect sizes of the eQTLs were estimated from z-statistics as previously described because they were not available in the summary data (Zhu et al., 2016). AD GWAS summary data were from a meta-analysis comprising 21,982 cases and 41,944 controls (Kunkle et al., 2019). Supplementary Table 1 presents the harmonized instruments.

We used two-sample Mendelian randomization to estimate causal effects using the Wald ratio for individual variants and an inverse-variance–weighted (IVW) fixed-effects meta-analysis for an overall estimate (Hemani et al., 2018). To account for potential violations of the assumptions underlying the IVW analysis, we conducted a sensitivity analysis using MR-Egger regression and the Weighted Median Estimator (Hemani et al., 2018). Heterogeneity was tested using Cochran's *Q* statistic (Hemani et al., 2018). The proportion of variance explained by each instrument and power were calculated as previously described (Brion et al., 2013; Shim et al., 2015). Code is available at https://github.com/marcoralab/MR_TNF.

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3. Results

The selected instruments for *TNF* expression, TNF-α levels, and RA risk explained 5.93% (F = 285), 1.74% (F = 24.1), and 19.2% (F = 247) of the variance, respectively. Given a sample size of 63,926 with the proportion of cases equal to 0.34, this study was adequately powered to detect an OR of any AD of 1.1 for *TNF* expression, 1.19 for TNF-α levels, and 1.055 for RA. There was no evidence of a causal association of *TNF* expression, TNF-α levels or RA on AD risk in the IVW, Weighted Median Estimator, or MR-Egger regression analyses (Supplementary Table 2). Similarly, there was no causal association for the individual *TNF* eQTLs. There was evidence of heterogeneity ($Q = 84.8$, $df = 54$, $p = 0.00472$) in RA analysis but not for the TNF ($Q = 3.46$, $df = 2$, $p = 0.177$) or TNF-α ($Q = 11.12$, $df = 9$, $p = 0.26$) analyses.

4. Discussion

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

Incidence of AD was reported to be lower in patients with RA in a meta-analysis of 10 studies; however, an MR analysis conducted using an earlier AD GWAS also found no causal effect of RA on AD (Policicchio et al., 2017). Although animal studies of AD models suggest that TNF-α inhibition ameliorates AD-related pathology, only a few human studies have been conducted (Ekert et al., 2018). An open-label clinical trial conducted in patients with mild to severe AD (n = 15) found that perispinal extrathecal administration of etanercept was associated with significant improvement in cognitive function (Tobinick et al., 2006). In contrast, a double-blind study of etanercept conducted in patients with mild to moderate AD (n = 41) over a 24-week period found that subcutaneous administration of etanercept showed no effect on cognitive, functional, or behavioral assessments (Butchart et al., 2015).

There are limitations to this study. First, this analysis was restricted to the expression of *TNF* mRNA in whole blood. Analysis in additional tissues may implicate *TNF* expression as a causal risk factor, however, the sample sizes available for other tissues are 30 x smaller than that of whole blood and thus have considerably reduced power (GTEx Consortium et al., 2017). Second, for the TNF-α analysis, we included nominally significant variants, which can bias results toward the null. Finally, these MR estimates represent the effect of lifelong exposure to increased *TNF* expression or TNF-α levels, whereas drugs generally have shorter periods of exposure and may not distinguish between critical periods of exposure (Walker et al., 2017).

Disclosure

SJA and AMG were supported by the JPB Foundation (http://www.jpbfoundation.org). SJA has no conflicts of interest to declare. AMG served on the scientific advisory board for Denali Therapeutics from 2015-2018. She has also served as a consultant for Biogen, Cognition Therapeutics, AbbVie, Pfizer, GSK, Eisai and Illumina.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.09.003.

References

Brion, M.-J.A., Shakhbazov, K., Visscher, P.M., 2013. Calculating statistical power in Mendelian randomization studies. Int. J. Epidemiol. 42, 1497–1501.

Butchart, J., Brook, L., Hopkins, V., Teeling, J., Püntener, U., Culliford, D., Sharples, R., Sharif, S., McFarlane, B., Raybould, R., Thomas, R., Passmore, P., Perry, V.H., Holmes, C., 2015. Etanercept in Alzheimer disease: a randomized, placebo-controlled, double-blind, phase 2 trial. Neurology 84, 2161–2168.

Ekert, J.O., Gould, R.L., Reynolds, G., Howard, R.J., 2018. TNF alpha inhibitors in Alzheimer's disease: a systematic review. Int. J. Geriatr. Psychiatry 33, 688–694.

GTEx Consortium, Laboratory, Data analysis &Coordinating Center (LDACC)—Analysis Working Group, Statistical Methods Groups—Analysis Working Group, Enhancing GTEx (eGTEx) Groups, NIH Common Fund, NIH/NCI, NIH/NHGRI, NIH/NIMH, NIH/NIDA, Biospecimen Collection Source Site—NDRI, Biospecimen Collection Source Site—RPCI, Biospecimen Core Resource—VARI, Brain Bank Repository—University of Miami Brain Endowment Bank, Leidos Biomedical—Project Management, ELSI Study, Genome Browser Data Integration &Visualization—EBI, Genome Browser Data Integration &Visualization—UCSC Genomics Institute, University of California Santa Cruz, Lead Analysts, Laboratory, Data Analysis &Coordinating Center (LDACC), NIH Program Management, Biospecimen Collection, Pathology, eQTL Manuscript Working Group, Battle, A., Brown, C.D., Engelhardt, B.E., Montgomery, S.B., 2017. Genetic effects on gene expression across human tissues. Nature 550, 204–213.

Hemani, G., Zheng, J., Elsworth, B., Wade, K.H., Haberland, V., Baird, D., Laurin, C., Burgess, S., Bowden, J., Langdon, R., Tan, V.Y., Yarmolinsky, J., Shihab, H.A., Timpson, N.J., Evans, D.M., Relton, C., Martin, R.M., Davey Smith, G., Gaunt, T.R., Haycock, P.C., 2018. The MR-base platform supports systematic causal inference across the human phenome. Elife 7, 1–29.

Kunkle, B.W., Grenier-Boley, B., Sims, R., Bis, J.C., Damotte, V., Naj, A.C., Boland, A., Vronskaya, M., van der Lee, S.J., Amlie-Wolf, A., Bellenguez, C., Frizatti, A., Chouraki, V., Martin, E.R., Sleegers, K., Badarinarayan, N., Jakobsdottir, J., Hamilton-Nelson, K.L., Moreno-Grau, S., Olaso, R., Raybould, R., Chen, Y., Kuzma, A.B., Hiltunen, M., Morgan, T., Ahmad, S., Vardarajan, B.N., Epelbaum, J., Hoffmann, P., Boada, M., Beecham, G.W., Garnier, J.-G., Harold, D., Fitzpatrick, A.L., Valladares, O., Moutet, M.-L., Gerrish, A., Smith, A.V., Qu, L., Bacq, D., Denning, N., Jian, X., Zhao, Y., Del Zompo, M., Fox, N.C., Choi, S.-H., Mateo, I., Hughes, J.T., Adams, H.H., Malamon, J., Sanchez-Garcia, F., Patel, Y., Brody, J.A., Dombroski, B.A., Naranjo, M.C.D., Daniilidou, M., Eiriksdottir, G., Mukherjee, S., Wallon, D., Uphill, J., Aspelund, T., Cantwell, L.B., Garzia, F., Galimberti, D., Hofer, E., Butkiewicz, M., Fin, B., Scarpini, E., Sarnowski, C., Bush, W.S., Meslage, S., Kornhuber, J., White, C.C., Song, Y., Barber, R.C., Engelborghs, S., Sordon, S., Voijnovic, D., Adams, P.M., Vandenberghe, R., Mayhaus, M., Cupples, L.A., Albert, M.S., De Deyn, P.P., Gu, W., Himali, J.J., Beekly, D., Squassina, A., Hartmann, A.M., Orellana, A., Blacker, D., Rodriguez-Rodriguez, E., Lovestone, S., Garcia, M.E., Doody, R.S., Munoz-Fernadez, C., Sussams, R., Lin, H., Fairchild, T.J., Benito, Y.A., Holmes, C., Karamujić-Čomić, H., Frosch, M.P., Thonberg, H., Maier, W., Roschupkin, G., Ghetti, B., Giedraitis, V., Kawalia, A., Li, S., Huebinger, R.M., Kilander, L., Moebus, S., Hernández, I., Kamboh, M.I., Brundin, R., Turton, J., Yang, Q., Katz, M.J., Concari, L., Lord, J., Beiser, A.S., Keene, C.D., Helisalmi, S., Kloszewska, I., Kukull, W.A., Koivisto, A.M., Lynch, A., Tarraga, L., Larson, E.B., Haapasalo, A., Lawlor, B., Mosley, T.H., Lipton, R.B., Solfrizzi, V., Gill, M., Longstreth, W.T., Montine, T.J., Frisardi, V., Diez-Fairen, M., Rivadeneira, F., Petersen, R.C., Deramecourt, V., Alvarez, I., Salani, F., Ciaramella, A., Boerwinkle, E., Reiman, E.M., Fievet, N., Rotter, J.I., Reisch, J.S., Hanon, O., Cupidi, C., Andre Uitterlinden, A.G., Royall, D.R., Dufouil, C., Maletta, R.G., de Rojas, I., Sano, M., Brice, A., Cecchetti, R., George-Hyslop, P.S., Ritchie, K., Tsolaki, M., Tsuang, D.W., Dubois, B., Craig, D., Wu, C.-K., Soininen, H., Avramidou, D., Albin, R.L., Fratiglioni, L., Germanou, A., Apostolova, L.G., Keller, L., Koutroumani, M., Arnold, S.E., Panza, F., Gkatzima, O., Asthana, S., Hannequin, D., Whitehead, P., Atwood, C.S., Caffarra, P., Hampel, H., Quintela, I., Carracedo, Á., Lannfelt, L., Rubinsztein, D.C., Barnes, L.L., Pasquier, F., Frölich, L., Barral, S., McGuinness, B., Beach, T.G., Johnston, J.A., Becker, J.T., Passmore, P., Bigio, E.H., Schott, J.M., Bird, T.D., Warren, J.D., Boeve, B.F., Lupton, M.K., Bowen, J.D., Proitsi, P., Boxer, A., Powell, J.F., Burke, J.R., Kauwe, J.S.K., Burns, J.M., Mancuso, M., Buxbaum, J., Bonuccelli, U., Cairns, N.J., McQuillin, A., Cao, C., Livingston, G., Carlson, C.S., Bass, N.J., Carlsson, C.M., Hardy, J., Carney, R.M., Bras, J., Carrasquillo, M.M., Guerreiro, R., Allen, M., Chui, H.C., Fisher, E., Masullo, C., Crocco, E.A., DeCarli, C., Bisceglio, G., Dick, M., Ma, L., Duara, R., Graff-Radford, N.R., Evans, D.A., Hodges, A., Faber, K.M., Scherer, M., Fallon, K.B., Riemenschneider, M., Fardo, D.W., Heun, R., Farlow, M.R., Kölsch, H., Ferris, S., Leber, M., Foroud, T.M., Heuser, I., Galasko, D.R., Giegling, I., Gearing, M., Hüll, M., Geschwind, D.H., Gilbert, J.R., Morris, J., Green, R.C., Mayo, K., Growdon, J.H., Feulner, T., Hamilton, R.L., Harrell, L.E., Drichel, D., Honig, L.S., Cushion, T.D., Huentelman, M.J., Hollingworth, P., Hulette, C.M., Hyman, B.T., Marshall, R., Jarvik, G.P., Meggy, A., Abner, E., Menzies, G.E., Jin, L.-W., Leonenko, G., Real, L.M., Jun, G.R., Baldwin, C.T., Grozeva, D., Karydas, A., Russo, G., Kaye, J.A., Kim, R., Jessen, F., Kowall, N.W., Vellas, B., Kramer, J.H., Vardy, E., LaFerla, F.M., Jöckel, K.-H., Lah, J.J., Dichgans, M., Leverenz, J.B., Mann, D., Levey, A.I., Pickering-Brown, S., Lieberman, A.P., 2019. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. Nat. Genet. 51, 414–430.

Lai, K.S.P., Liu, C.S., Rau, A., Lanctôt, K.L., Köhler, C.A., Pakosh, M., Carvalho, A.F., Herrmann, N., 2017. Peripheral inflammatory markers in Alzheimer's disease: a

systematic review and meta-analysis of 175 studies. J. Neurol. Neurosurg. Psychiatry 88, 876–882.

Okada, Y., Wu, D., Trynka, G., Raj, T., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Yoshida, S., Graham, R.R., Manoharan, A., Ortmann, W., Bhangale, T., Denny, J.C., Carroll, R.J., Eyler, A.E., Greenberg, J.D., Kremer, J.M., Pappas, D.A., Jiang, L., Yin, J., Ye, L., Su, D.-F., Yang, J., Xie, G., Keystone, E., Westra, H.-J., Esko, T., Metspalu, A., Zhou, X., Gupta, N., Mirel, D., Stahl, E.A., Diogo, D., Cui, J., Liao, K., Guo, M.H., Myouzen, K., Kawaguchi, T., Coenen, M.J.H., van Riel, P.L.C.M., van de Laar, M.A.F.J., Guchelaar, H.-J., Huizinga, T.W.J., Dieudé, P., Mariette, X., Bridges Jr., S.L., Zhernakova, A., Toes, R.E.M., Tak, P.P., Miceli-Richard, C., Bang, S.-Y., Lee, H.-S., Martin, J., Gonzalez-Gay, M.A., Rodriguez-Rodriguez, L., Rantapää-Dahlqvist, S., Arlestig, L., Choi, H.K., Kamatani, Y., Galan, P., Lathrop, M., RACI consortium, GARNET consortium, Eyre, S., Bowes, J., Barton, A., de Vries, N., Moreland, L.W., Criswell, L.A., Karlson, E.W., Taniguchi, A., Yamada, R., Kubo, M., Liu, J.S., Bae, S.-C., Worthington, J., Padyukov, L., Klareskog, L., Gregersen, P.K., Raychaudhuri, S., Stranger, B.E., De Jager, P.L., Franke, L., Visscher, P.M., Brown, M.A., Yamanaka, H., Mimori, T., Takahashi, A., Xu, H., Behrens, T.W., Siminovitch, K.A., Momohara, S., Matsuda, F., Yamamoto, K., Plenge, R.M., 2014. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 506, 376–381.

Perry, V.H., Cunningham, C., Holmes, C., 2007. Systemic infections and inflammation affect chronic neurodegeneration. Nat. Rev. Immunol. 7, 161–167.

Policicchio, S., Ahmad, A.N., Powell, J.F., Proitsi, P., 2017. Rheumatoid arthritis and risk for Alzheimer's disease: a systematic review and meta-analysis and a Mendelian randomization study. Sci. Rep. 7, 12861.

Shim, H., Chasman, D.I., Smith, J.D., Mora, S., Ridker, P.M., Nickerson, D.A., Krauss, R.M., Stephens, M., 2015. A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. PLoS One 10, e0120758.

Sliz, E., Kalaoja, M., Ahola-Olli, A., Raitakari, O., Perola, M., Salomaa, V., Lehtimäki, T., Karhu, T., Viinamäki, H., Salmi, M., Santalahti, K., Jalkanen, S., Jokelainen, J., Keinänen-Kiukaanniemi, S., Männikkö, M., Herzig, K.-H., Järvelin, M.-R., Sebert, S., Kettunen, J., 2019. Genome-wide association study identifies seven novel loci associating with circulating cytokines and cell adhesion molecules in Finns. J. Med. Genet. 56, 607–616.

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

Võsa, U., Claringbould, A., Westra, H.-J., Bonder, M.J., Deelen, P., Zeng, B., Kirsten, H., Saha, A., Kreuzhuber, R., Kasela, S., Pervjakova, N., Alvaes, I., Fave, M.-J., Agbessi, M., Christiansen, M., Jansen, R., Seppälä, I., Tong, L., Teumer, A., Schramm, K., Hemani, G., Verlouw, J., Yaghootkar, H., Sönmez, R., Brown, A., Kukushkina, V., Kalnapenkis, A., Rüeger, S., Porcu, E., Kronberg-Guzman, J., Kettunen, J., Powell, J., Lee, B., Zhang, F., Arindrarto, W., Beutner, F., BIOS Consortium, Brugge, H., i2QTL Consortium, Dmitreva, J., Elansary, M., Fairfax, B.P., Georges, M., Heijmans, B.T., Kähönen, M., Kim, Y., Knight, J.C., Kovacs, P., Krohn, K., Li, S., Loeffler, M., Marigorta, U.M., Mei, H., Momozawa, Y., Müller-Nurasyid, M., Nauck, M., Nivard, M., Penninx, B., Pritchard, J., Raitakari, O., Rotzchke, O., Slagboom, E.P., Stehouwer, C.D.A., Stumvoll, M., Sullivan, P., t Hoen, P.A.C., Thiery, J., Tönjes, A., van Dongen, J., van Iterson, M., Veldink, J., Völker, U., Wijmenga, C., Swertz, M., Andiappan, A., Montgomery, G.W., Ripatti, S., Perola, M., Kutalik, Z., Dermitzakis, E., Bergmann, S., Frayling, T., van Meurs, J., Prokisch, H., Ahsan, H., Pierce, B., Lehtimäki, T., Boomsma, D., Psaty, B.M., Gharib, S.A., Awadalla, P., Milani, L., Ouwehand, W., Downes, K., Stegle, O., Battle, A., Yang, J., Visscher, P.M., Scholz, M., Gibson, G., Esko, T., Franke, L., 2018. Unraveling the polygenic architecture of complex traits using blood eQTL metaanalysis. bioRxiv. https://doi.org/10.1101/447367.

Walker, V.M., Davey Smith, G., Davies, N.M., Martin, R.M., 2017. Mendelian randomization: a novel approach for the prediction of adverse drug events and drug repurposing opportunities. Int. J. Epidemiol. 46, 2078–2089.

Wyss-Coray, T., 2006. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat. Med. 12, 1005–1015.

Zhu, Z., Zhang, F., Hu, H., Bakshi, A., Robinson, M.R., Powell, J.E., Montgomery, G.W., Goddard, M.E., Wray, N.R., Visscher, P.M., Yang, J., 2016. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat. Genet. 48, 481–487.