**Pleiotropy Analysis**

In Mendelian randomization study, one important issue is the potential violation of assumption 2 and 3 through pleiotropy occurring when a genetic instrument is associated with a study outcome through biological pathways outside the exposure of interest. Here, we performed an assessment for pleiotropy to assure that the selected genetic variants do not exert effects on AD risk through biological pathways independent of serum calcium levels. A number of steps were taken to reduce the risk of pleiotropy.

In 2017, a major review by The Lancet has identified nine potentially modifiable risk factors linked to dementia1. This review suggests that around 35% of dementia is attributable to a combination of these nine risk factors1. In stage 1, we referred the major review and evaluated the potential pleiotropy using eight known confounders including years of educational attainment from Social Science Genetic Association Consortium (SSGAC)2, type 2 diabetes from DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium3, cigarettes smoked per day from the Tobacco and Genetics Consortium (TGC) 4, major depressive disorder from Psychiatric Genomics Consortium (PGC)5, blood pressure including systolic blood pressure (SBP) and diastolic blood pressure (DBP) from the International Consortium of Blood Pressure (ICBP)6, hearing loss from UK biobank7.

In stage 2, we evaluated the potential pleiotropic associations of these 43 variants with mineral supplements (including calcium, fish oil (including cod liver oil), glucosamine, iron, selenium, zinc and others), and vitamin supplements (including folic acid or folate (Vit B9), vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, multivitamins and others)7. We then particularly investigated continuous alcohol and dichotomous alcohol as potential confounder8.

In stage 4, we obtained the summary data of Aβ42, tau, and ptau levels in cerebrospinal fluid (CSF) from an Alzheimer's endophenotypes and disease modifiers GWA study containing 3146 particpants9. In stage 1-4, the significance threshold for the association of these 43 variants with these known and potential confounders is P < 1.16E-3 (a Bonferroni correction, P < 0.05/43).

In addition to the known confounders above, there may also be some unknown confounders. In stage 5, we selected a statistical method to evaluate the potential pleiotropic associations of these 39 genetic variants with known and unknown confounders. The method is MR-Egger intercept test, which could provide an assessment of the validity of the instrumental variable assumptions, and provide a statistical test the presence of potential pleiotropy10. Here, we used the method to iteratively prune the corresponding variant list until there is pleiotropy, which allows for an agnostic assessment of pleiotropy caused by both known and unknown confounders11.

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