Peer Review File

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Reviewer comments

Peng et al. performed a mendelian randomization analysis to understand a causal relationship between bipolar and breast cancer. MR analyses demonstrated that genetically predicted BD was causally associated with an increased risk of breast cancer (OR = 1.058; 95% CI 1.023-1.093, p < 0.001). When results were examined by immunohistochemical type, a strong association was observed between genetically predicted BD and estrogen receptor (ER) -positive breast cancer rather than ER-negative breast cancer. The manuscript has a huge space for improvement, and I have several major comments.

1. The bipolar GWAS is not the most updated GWAS, check out with the psychiatric genomic consortium. If I didn't remember wrong, there are 31 IVs associated with BD according to the most recently published GWAS. Please update the manuscript using new IVs. Check Eli A. Stahl 2019 Nature Genetics paper, PMID 31043756

Reply 1: Thank you very much for your comment. After updating our manuscript using new IVs, the results demonstrated that genetically predicted BD was causally associated with an increased risk of breast cancer (OR = 1.059; 95% CI 1.008-1.112, p = 0.0229). When results were examined by immunohistochemical type, no causal effects between genetically predicted BD and estrogen receptor (ER) -positive breast cancer (OR = 1.049, 95%CI 0.999-1.102 p = 0.0556) and ER-negative breast cancer (OR = 1.032, 95%CI 0.953-1.116 p = 0.4407) were observed.
Changes in the text: Page 5, line 129 to Page 6, line 148; Page 8, line 209 to Page 9, line 237.

2. As the author stated in the method section, genetic predisposition towards BD could be associated with the potential confounders and mediators underlying the mechanisms from BD to breast cancer. Instead of performing additional MR, check out a method named MVMR – multivariable MR, in which one can adjust for the confounders in a MR framework.

Reply 2: Thank you very much for your comment. Multivariable Mendelian randomization (MVMR) is a novel enlargement of MR that uses genetic variants associated with multiple, potentially related exposures to estimate the effect of each exposure on a single outcome, which allows for equivalent analysis to mediation within the MR framework and therefore can also be used to estimate mediation effects. Thus, it is a suitable method for ambiguous SNP-related phenotypes. For example, intelligence and education level are two independent phenotypes, yet common genetic variables are shared between both, and potential mechanisms may interfere each other (1). In similar cases, MVMR is required to elucidate the respective direct effect of each exposure to the outcome phenotype, conditional on the other exposures included in the model.
However, in regard to our study, MVMR may not be applicable due to several reasons. First, we retrieved and identified SNPs from several GWASs of bipolar disorder (BD) with large sample sizes and tight correlation ($P < 5*10^{-8}$). Second, our study depicted a robust association of our chosen SNPs with BD (F > 1000), and a strong statistical power (>80%) further enhanced the stability of our results. Third, by performing literature search on each SNP of instrument variables, neither of them was associated with phenotypes other than BD. In conclusion, the SNPs of our study were solely related to an exposure phenotype, and it was unnecessary and inapplicable to perform MVMR. Thus, additional MR analyses were performed in our study to identify whether BD-breast cancer

association was influenced by potential confounders and mediators based on existing literature, which is also an effective method used by predecessors (2). Our study indicated that genetically predicted BD was not causally associated with obesity (OR 0.982, 95%CI 0.900-1.071, P = 0.6756 for obesity class 1; OR 1.004, 95%CI 0.888-1.136, P = 0.9446 for obesity class 2; OR 0.931, 95%CI 0.756-1.146, P = 0.4979 for obesity class 3), alcohol consumption (OR 0.999, 95%CI 0.987-1.012, P = 0.9170), smoking (OR 0.998, 95%CI 0.991-1.005, P = 0.5092 for previous smoker; OR 1.000, 95%CI 0.995-1.005, P = 0.8926 for current smoker) and use of lithium product (OR 1.005, 95%CI 0.977-1.034, P = 0.710), revealing relatively great credibility of the outcomes.

3. Is it likely that cancer status would reversely cause mental health conditions? perform a bi-directional MR.

Reply 3: Thank you very much for your comment. It is quite reasonable that cancer patients, especially women and young adults, are likely to experience persisting negative mood, like depression and cancer-related fears, which may potentially lead to mental health illness (3). Therefore, to further explore the causal relationship, we performed a bi-directional MR to seek whether breast cancer status would reversely cause BD. Using breast cancer as an exposure phenotype and BD as an outcome, our bi-directional MR result demonstrated an absence of casual relationship between breast cancer and BD (OR 1.001, 95% CI 0.913-1.098, P = 0.9799).
Changes in the text: Page 7, line 180 to line 183; Page 8, line 221 to line 223.

4. Cut the introduction, try to write succinctly. Do not repeat introduction in discussion

Reply 4: Thank you very much for your comment. We have polished our manuscript to make it more succinctly.
Changes in the text: Page 4, line 96 to line 102.

Reference

1. Davies N, Hill W, Anderson E, Sanderson E, Deary I, Davey Smith G. Multivariable two-sample Mendelian randomization estimates of the effects of intelligence and education on health. eLife. 2019;8.
2. Zhou H, Zhang Y, Liu J, Yang Y, Fang W, Hong S, et al. Education and lung cancer: a Mendelian randomization study. Int J Epidemiol. 2019;48(3):743-50.
3. Yi JC, Syrjala KL. Anxiety and Depression in Cancer Survivors. Med Clin North Am. 2017;101(6):1099-113.