nature portfolio

Peer Review File

An observational and genetic investigation into the association between psoriasis and risk of malignancy

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors used five different methods to study site-specific cancer and its relation to psoriasis using public datasets and data from the UK Biobank. Their data showed that psoriasis is causally associated with lung and breast cancer.

1. Several studies have looked at psoriasis and the risk of cancer, including two meta-analyses without consistent findings also for lung and breast cancer. Two two-sided Mendelian randomization studies on psoriasis and lung cancer have also shown controversial results. I therefore acknowledge the authors for using five different methods to study the association. However, the authors need to discuss the biological relevance of their findings. How significant is the increased risk of risk they find?

2. The authors write several times that their data support regular lung and breast cancer screening in patients with psoriasis. I disagree; there is no data on the risk compared to the risk of screening.

3. Why did the authors use data on psoriasis from a GWAS study from 2012 when a new larger is available from 2017 (Nat Commun. 2017 May 24:8:15382)?

4. In the PheWAS, are, breast cancer included in overall cancer risk, although only data on females are included.

5. In the abstract, the findings for the one-sample MR are shown as hazard ratio; however, in the result section, the results are shown as OR but with the exact same values. Please explain.

6. In the cis-eQTL analysis study, psoriasis-associated SNPs were studied, and an increased expression of ERAP1 is found in lung cancer; however, ERAP1 have already been shown to be associated with cancer and is not psoriasis-specific. Please explain.

7. In discussion lines 308+309, the authors state that their data confirmed that psoriasis is causally associated with breast and lung cancer; however, has it been shown before for both cancers?

8. One of the author's explanations for the inconsistency with previous findings is inconsistent diagnostic criteria for psoriasis in the included studies. However, many of the included studies use hospital-based diagnoses of psoriasis and may be much more correct than genetically identified patients with psoriasis; please explain.

Minor comments:

1. Figure 1 is a good overview; however, it is tricky to see which key strength fits to which analysis.

2. In the abstract, please write out the abbreviation for PRS.

3. Why is sup Table 17 referred to before sup Tables 4 to 16?

4. Why is Table 2 referred to before Table 1?

Reviewer #2 (Remarks to the Author):

This is an interesting paper. Prior research, including a large number of observational cohort studies and MR studies, have reported the relationship between PSO and cancer. This paper utilizes the raw data from the UKB to explore the relationship between PSO and cancers according to observational research and genetic analysis and finds that there is a certain causal association between PSO and lung/breast cancer. However, there are still some issues with this paper.

Major Concerns:

1. For observational studies

1.1 After carefully reviewing the authors' methodological section, I believe it is not the standard way to conduct a cohort study; when conducting a subgroup, did the authors exclude another cancer, or did some participants suffer from two or more cancers at the same time? Additionally, what is the overall risk of developing cancer?

1.2 Some confounders are inevitable, however, based on the UKB, many other factors that may contribute to cancer should be included, such as some medications. The authors have included more cancers, these confounders need to be further determined by the authors through a literature review. In addition, are there multiple collinear relationships between the variables included in the analysis? How do we deal with possible multiple collinear relationships?

1.3 The starting time of the study cohort is 2006-2010, and the ending time is 2019. This necessarily involves the issue of sample weighting, which needs to be paid attention to in statistical analysis, and it is recommended to conduct WQS analysis. In some analyses, the HR value is equal to 0 or 1, which may suggest mistakes in statistical methods or other possible interfering factors.

1.4 I am curious about the possible mediators through which PSO and cancers are related, therefore, mediation analysis is also worth trying.

2. Genetic analysis

2.1 One-sample MR seems unnecessary

2.2 In this article, two-sample MR is used to confirm the results of One-sample MR. Based on the limitations of single-sample MR, this seems redundant. Shouldn't the results of one-sample MR prove the results of two-sample MR? Or, why didn't the authors conduct a two-sample MR based on the original UKB data?

2.3 I am confused about the authors' choice of PSO data from Finn when conducting two-sample MR. Finn is a very special database; many other data may be more suitable.

2.4 Similar to observational studies, when conducting Phewas, confounders should be explained, and some cancers may have special risk factors.

2.5 About the authors' handling of gene pleiotropy. If possible, I would like to assess the relationship between PSO and the candidate cancers through various methods after two-sample MR analysis, such as CAUSE, LCV, and gene colocalization; even cross-trait methods should be tried. Of course, if doing so, it does not necessarily guarantee that the Eqtl's results will be consistent with those of these methods.

Minor Concerns

1. The methodological part needs to be more detailed.

2. The figures also need to be more beautiful; and the authors do not provide a detailed process of the study in Figure 1, for example, the inclusion and exclusion criteria are not clear.

3. In the Data availability section, the sources of public data need to be clarified.

4. Detailed information on public data should be compiled into a table attachment.

5. I am not sure if there is any other evidence needed for the original data from UKB.

6. It would be best if the authors could draw a possible mechanism diagram of the relationship between PSO and cancer, of course, the authors' eQTL results should be included in this figure.

Reviewer #1

The authors used five different methods to study site-specific cancer and its relation to psoriasis using public datasets and data from the UK Biobank. Their data showed that psoriasis is causally associated with lung and breast cancer.

Response：Thank you.

1. Several studies have looked at psoriasis and the risk of cancer, including two meta-analyses without consistent findings also for lung and breast cancer. Two two-sided Mendelian randomization studies on psoriasis and lung cancer have also shown controversial results. I therefore acknowledge the authors for using five different methods to study the association. However, the authors need to discuss the biological relevance of their findings. How significant is the increased risk of risk they find?

Response：Thanks for your comments. We speculate that genes derived from psoriasis susceptibility loci in cis-eQTL analysis may be related to the increased risk of cancers. Among these genes, ERAP1 is shown to be associated with both psoriasis and cancers in previous studies[1–3]. Since our study is not a mechanistic research, further study is needed to elucidate the exact mechanisms underlying the increased risk of cancers in psoriasis patients.

We have added the following discussion in the manuscript based on your suggestions (Page 18):

"By integrating public datasets of eQTL, TCGA and GTEx, our gene annotation revealed potential molecular association between psoriasis and lung or breast cancer. Among them, ERAP1, an endoplasmic reticulum aminopeptidase, may be one of the most promising candidates. On one hand, several genome-wide association studies and exome sequencing identified ERAP1 as an important susceptibility locus harbored gene for psoriasis[46-48]. On the other hand, patients with breast cancer or lung cancer often have abnormal expression level of ERAP1[49], which may lead to abnormal antigen processing, and subsequently facilitates tumor immune escape and malignant

progression[50]. However, the exact role of ERAP1 in the link between psoriasis and lung/breast cancer remains to be studied."

2. The authors write several times that their data support regular lung and breast cancer screening in patients with psoriasis. I disagree; there is no data on the risk compared to the risk of screening.

Response：Thanks for your comments. We agreed with your opinion, and removed these descriptions from the manuscript.

3. Why did the authors use data on psoriasis from a GWAS study from 2012 when a new larger is available from 2017 (Nat Commun. 2017 May 24:8:15382)?

Response：We appreciate your comments. We ultimately used the GWAS data from the 2012 study for the following reasons: First of all, although the study published in 2017 provides larger-scale GWAS data on psoriasis, we could not access the complete raw data through GWAS Catalog because it was not publicly shared. We have contacted the authors to request the data, but they didn't reply for several months. Secondly, the 2012 study had a substantial sample size of 10,588 psoriasis patients and 22,806 controls, with reliable data quality and relatively complete information. Considering these factors, we ultimately chose to use the 2012 dataset.

4. In the PheWAS, are, breast cancer included in overall cancer risk, although only data on females are included.

Response：Thank you for your comment. In our PheWAS results, the overall risk of breast cancer was increased in patients with psoriasis. However, when stratified by sex, it was only significant in females, which may be due to the relatively small number of male breast cancer cases (98 cases in 219645 male participants).

In our observational PheWAS, the HR for breast cancer in the male group was 0.92 (95% CI: 0.29, 2.92), with a p-value of 0.893.

In our PRS PheWAS, the OR for breast cancer in the male group was 0.98 (95% CI: 0.93, 1.03), with a p-value of 0.370.

We have added the results in Supplementary Table 14 and Supplementary Table 20.

5. In the abstract, the findings for the one-sample MR are shown as hazard ratio; however, in the result section, the results are shown as OR but with the exact same values. Please explain.

Response： We apologize for the mistake. Logistic regression was employed in our one-sample MR, and we should report these results as OR (95%CI). We thoroughly reviewed the abstract, tables, figures and main document to ensure the accuracy of the analyses and the consistency of the reporting.

6. In the cis-eQTL analysis study, psoriasis-associated SNPs were studied, and an increased expression of ERAP1 is found in lung cancer; however, ERAP1 have already been shown to be associated with cancer and is not psoriasis-specific. Please explain.

Response： Thank you. We used cis-eQTL analysis to screen harbored genes based on psoriasis susceptibility loci (namely psoriasis-associated SNPs). Then we investigate whether these harbored genes could explain the increased risk of cancer attributed to psoriasis. We employed four steps for the whole process.

Step 1: We obtained data of psoriasis susceptibility loci from public psoriasis GWAS datasets. After cis-eQTL analysis, we identified several harbored genes whose expression levels were strongly influenced by psoriasis susceptibility loci in normal lung and breast tissues.

Step 2: We compared the expression level of harbored genes between tumor tissues and normal tissues by using the TCGA dataset.

Step 3: We searched literature to examine whether these differentially expressed harbored genes were involved in both psoriasis and cancer.

Step 4: We identified ERAP1 as one of the possible harbored genes to link the psoriasis with cancer.

We have added the relevant discussion in the manuscript, as in our response to comment 1.

7. In discussion lines 308+309, the authors state that their data confirmed that psoriasis is causally associated with breast and lung cancer; however, has it been shown before for both cancers?

Response：We are not sure if your question refers to (1) whether previous studies have reported the causal relationship between psoriasis and breast/lung cancer, or (2) whether psoriasis preceded the development of breast/lung cancer. We will address both aspects in our response:

(1) If you mean the first interpretation:

By the time we completed our analyses, there were two articles analyzing the causal association between psoriasis and lung cancer with inconsistent results[6,7]. Both of them conducted Mendelian randomization (MR) analysis. Their conflicting evidence may be attributed to the insufficiencies in methodology in MR analysis according to guidelines of MR analysis[8]: Wang et al. used genetic variants with obvious heterogeneity[7], and Luo et al. selected genetic datasets for psoriasis and lung cancer both from the same population[6]. The causal association between psoriasis and increased risk of breast cancer was unreported. Beyond them, we conducted observational studies and genetic analysis to investigate the causal association between psoriasis and site-specific cancers. We also conducted two-sample MR analysis with a relatively low degree of genetic heterogeneity and using two non-overlapping populations. Our studies showed consistent results that psoriasis is causally associated with breast and lung cancer. This section was discussed in Page 17, paragraph 1-2.

(2) If you mean the second interpretation:

In our observational PheWAS and PRS PheWAS, we have ensured that the diagnosis of psoriasis preceded the diagnosis of cancers. We excluded cases whose cancer diagnosis preceded their psoriasis diagnosis and cases whose cancer disease was already diagnosed at baseline. We also used Cox regression models to control for time-related factors. This part has been stated in the methods section (Page 5, Study population) of our manuscript.

In our original draft, we did not perform similar exclusions in the one-sample MR

analysis. To further validate our findings, we re-conducted the one-sample MR analysis after excluding baseline cancer cases and cases whose cancer diagnosis preceded the psoriasis diagnosis (the same exclusion criteria as our PRS PheWAS analysis). Our updated results support a causal relationship between psoriasis and breast and lung cancer. These one-sample MR results are attached below. The statistical analysis methods are the same in our manuscript page 10:

Table 1: one-sample MR analysis excluding baseline cancer and psoriasis participants diagnosed with any type of cancer before their diagnosis of psoriasis

Cancer	**Sex**	**Case/Total**	**OR (95%CI)**	***p* value**
C34 Malignant neoplasm of bronchus and lung	Male and Female	3950/387475	1.11 (1.02,1.21)	0.015
	Male	2076/179871	1.16 (1.02,1.31)	0.021
	Female	1874/207604	1.07 (0.95,1.20)	0.269
C50 Malignant neoplasm of breast	Male and Female	8338/382505	1.07 (1.01,1.14)	0.027
	Male	79/179981	0.74 (0.39,1.42)	0.373
	Female	8259/202524	1.07 (1.01,1.14)	0.021

Abbreviation: MR, Mendelian randomization. OR, Odds Ratio. CI, Confidence Interval. MR-Egger, Mendelian Randomisation-Egger. MR-PRESSO, MR-pleiotropy residual sum and outlier.

Finally, we also performed a reverse two-sample MR analysis, using lung and breast cancer as the exposure factor and psoriasis as the outcome. The results suggest that lung and breast cancer were not causally associated with the risk of developing psoriasis. This supports our conclusion that the observed association is unidirectional and that psoriasis increases the risk of cancers. Our two-sample MR results are attached below.

GWAS data used:

Lung cancer database: GWAS Catalog (inquiry code: GCST90011812)

Breast cancer database: (IEU-OpenGWAS project inquiry code: ebi-a-GCST004988)

Psoriasis database: (IEU-OpenGWAS project inquiry code: finn-b-L12_PSORIASIS)

Table 2: Reverse causal association between cancers and psoriasis.

Exposure	Methods	OR (95%CI)	*p* value
Lung cancer	MR Egger	1.01 (0.92,1.10)	0.908
	Weighted median	1.03 (0.96,1.11)	0.432
	Inverse variance weighted	1.01 (0.96,1.06)	0.830
	Weighted mode	1.04 (0.96,1.12)	0.395
Breast cancer	MR Egger	1.04 (0.92,1.18)	0.518
	Weighted median	1.04 (0.94,1.15)	0.429
	Inverse variance weighted	1.04 (0.97,1.10)	0.258
	Weighted mode	1.04 (0.93,1.17)	0.467

Abbreviation: MR, Mendelian randomization. OR, Odds Ratio. CI, Confidence Interval. MR-Egger, Mendelian Randomisation-Egger. MR-PRESSO, MR-pleiotropy residual sum and outlier.

8. One of the author's explanations for the inconsistency with previous findings is inconsistent diagnostic criteria for psoriasis in the included studies. However, many of the included studies use hospital-based diagnoses of psoriasis and may be much more correct than genetically identified patients with psoriasis; please explain.

Response：We appreciate your comments.

In previously reported large cohort studies, the diagnosis of psoriasis was originated from various sources, including national medical record systems[9,10], self-reports[11], and health insurance system[12]. However, their detailed descriptions of the diagnostic criteria were lacking. The diagnosis of psoriasis mainly depends on the subjective judgment of the doctor[13]. A skin biopsy can be the gold standard but it is not routinely conducted[14]. Given the various methods for diagnosing psoriasis, such as characteristic appearance of skin lesions, histopathology or dermoscopy, we cannot confirm which methods were used. Therefore, the heterogeneity and subjectivity of current hospital diagnoses might also lead to inconsistency across different studies.

We used both hospital-based diagnoses and genetically identified psoriasis in our study. In observational PheWAS, we investigated the association between clinically

diagnosed psoriasis and incident cancers, while in PRS PheWAS and MR analysis, we investigated the association between genetically predicted psoriasis and cancers. Both studies showed consistent results on the relationship between psoriasis and lung or breast cancer.

Minor comments:

1. Figure 1 is a good overview; however, it is tricky to see which key strength fits to which analysis.

Response：Thank you for your suggestions. Our Table 2 summarize the strengths and limitations of the five analyses in our study. The names of analyses 1-5 in Figure 1 and Table 2 correspond to each other. To facilitate cross-reading with Table 2, we have modified Figure 1 to add color to the names of analyses.

2. In the abstract, please write out the abbreviation for PRS.

Response：Thank you. We have added the abbreviation for PRS.

3. Why is sup Table 17 referred to before sup Tables 4 to 16?

Response：Thank you. We have adjusted the order of the tables.

4. Why is Table 2 referred to before Table 1?

Response：Thank you. We have adjusted the order of the tables.

Reviewer #2

This is an interesting paper. Prior research, including a large number of observational cohort studies and MR studies, have reported the relationship between PSO and cancer. This paper utilizes the raw data from the UK Biobank to explore the relationship between PSO and cancers according to observational research and genetic analysis and finds that there is a certain causal association between PSO and lung/breast cancer. However, there are still some issues with this paper.

Response：Thank you.

Major Concerns:

1. For observational studies

1.1 After carefully reviewing the authors' methodological section, I believe it is not the standard way to conduct a cohort study; when conducting a subgroup, did the authors exclude another cancer, or did some participants suffer from two or more cancers at the same time? Additionally, what is the overall risk of developing cancer?

Response：We appreciate your comments.

(1) In our initial analysis, we did not specifically exclude individuals with more than one cancer diagnosis and some participants have multiple types of cancer. In the revised manuscript, we re-analyzed the data, requiring that each participant has only one type of cancer diagnosis. The statistical analysis methods are the same in our manuscript page 10. We found that the results were consistent with our original findings, showing an association between psoriasis and lung or breast cancer in the observational PheWAS. We have included these results in the supplementary Table 16-18. We also added these data in result section in page 13:

"After excluding patients who had multiple cancer diagnoses, psoriasis is still significantly associated with a higher risk of lung cancer (C34, HR 1.56, 95% CI 1.14 to 2.12) and breast cancer (C50, HR 1.28, 95% CI 1.09 to 1.49) (**Supplementary Tables 16-18**)."

(2) We also investigated the overall risk of developing cancer among individuals with psoriasis in our manuscript. Please see results below: when we gradually added confounding factors into the model, the association between psoriasis and overall cancer risk gradually weakened, while psoriasis is still significantly associated with lung cancer or breast cancer.

Table 3 Multi-variable Cox regression analysis for overall risk of cancers associated with psoriasis

	Whole population			Male			Female		
	Case/Total	HR (95%CI)	*p* value	Case/Total	HR (95%CI)	*p* value	Case/Total	HR (95%CI)	*p* value
Model 1	65158/455169	1.13(1.08,1.17)	< 0.001	34288/210944	1.11(1.05,1.17)	< 0.001	30870/244225	1.11(1.04,1.19)	0.001
Model 2	65158/455169	1.07(1.02,1.11)	0.003	34288/210944	1.04(0.99,1.11)	0.132	30870/244225	1.09(1.02,1.16)	0.010
Model 3	65158/455169	1.04(1.00,1.09)	0.066	34288/210944	1.03(0.97,1.09)	0.373	30870/244225	1.06(0.99,1.13)	0.106
Model 4	65158/455169	1.03(0.99,1.08)	0.121	34288/210944	1.01(0.96,1.07)	0.632	30870/244225	1.05(0.99,1.13)	0.120

Model 1: Crude

Model 2: Model 1 further adjusted for age and sex

Model 3: Model 2 further adjusted for race, smoking status, alcohol frequency and physical activity

Model 4: Model 3 further adjusted for BMI, glucocorticoids, methotrexate and cyclosporin

1.2 Some confounders are inevitable, however, based on the UK Biobank, many other factors that may contribute to cancer should be included, such as some medications. The authors have included more cancers, these confounders need to be further determined by the authors through a literature review. In addition, are there multiple collinear relationships between the variables included in the analysis? How do we deal with possible multiple collinear relationships?

Response：We appreciate your comments.

In the PheWAS analysis, we have now included additional covariates to adjust for potential confounding factors. These include:

(1) Medications for psoriasis treatment, including use of immunosuppressants such as glucocorticoids, methotrexate, and cyclosporine.

(2) Cancer-specific confounders based on the literature review: for skin cancer, we

further adjusted for sun exposure levels (UK Biobank data: Time spend outdoors in summer, Time spent outdoors in winter); for breast cancer and female reproductive system malignancies, we further adjusted for age at menarche, number of offspring, and menopausal status (UK Biobank data: Age when periods started [menarche], Number of live births, Had menopause); for colorectal cancer, we further adjusted for red meat and fiber intake levels (UK Biobank data: median data of Beef intake, Lamb/mutton intake and Pork intake, median data of Cooked vegetable intake and Salad/raw vegetable intake).

After adjusting for the aforementioned confounding variables, our results still support the association between psoriasis and the risk of breast and lung cancer. We revised manuscript in page 6, Covariates adjusted for in multivariable regression analysis section:

"Covariates were selected based on their potential associations with both psoriasis and cancer risk. Sex from UK Biobank data was primarily based on self-reporting. Smoking status was categorized into three levels of never, previous, and current smoking. Alcohol intake frequency was categorized into six levels, including daily or almost daily, three or four times a week, once or twice a week, one to three times a month, special occasions only, and never. Physical activity was categorized into three levels, specifically low intensity, moderate intensity, and high intensity. Medication use (including glucocorticoids, methotrexate, cyclosporine) was defined as whether the corresponding medication was used or not.

We further included additional covariates for selected cancer outcomes. For skin cancer (C43-C44), we additionally adjusted for sun exposure[16]. Sun exposure was defined as the time spend outdoors in summer and in winter. For female reproductive system malignancies and breast cancer(C50-C58), we further adjusted for age at menarche, number of live births, and menopausal status[17–20]. For colorectal cancer(C18-C20), we further adjusted for red meat and fiber intake levels[21,22]. Red meat intake levels were defined as the median data of beef, lamb/mutton and pork intake. Fiber intake levels were defined as the median data of cooked vegetable and salad/raw vegetable intake."

(3) To address the issue of potential multicollinearity among the variables included in our observational study, we performed a multicollinearity analysis. Variance inflation factor (VIF) is less than 4, which means there was no significant multicollinearity in our PheWAS analysis.

Table 4: Multicollinearity test

Variable	VIF
Age	1.050578786
Sex	1.067241094
Ethnic	1.050517826
Smoking status	1.081748532
Alcohol frequency	1.138206560
Physical activity	1.026519181
BMI	1.061912669
Glucocorticoids	1.007071499
Methotrexate	1.021860043
Cyclosporin	1.004452129
Red meat intake	1.054064310
Fiber intake	1.028300560

Abbreviation: VIF (Variance Inflation Factor).

VIF less than 4 means there was no significant multicollinearity.

1.3 The starting time of the study cohort is 2006-2010, and the ending time is 2019. This necessarily involves the issue of sample weighting, which needs to be paid attention to in statistical analysis, and it is recommended to conduct WQS analysis. In some analyses, the HR value is equal to 0 or 1, which may suggest mistakes in statistical methods or other possible interfering factors.

Response：We appreciate your comments.

(1) The issue of sample weighting

We conducted a literature review on the Weighted Quantile Sum (WQS) regression method. We found that WQS is commonly used to investigate the combined effect of multiple continuous variables on one outcome[15–17]. However, in our model, our exposure variable psoriasis is a binary variable, and we have multiple cancer outcomes. We also consulted with professors in statistics, and they suggested that WQS may not

be suitable for our model. Nevertheless, we do need to consider the factor of sample weighting, as the UK Biobank cohort enrollment period varies from 2006 to 2010. Instead of WQS, we reanalyzed using the following two alternative methods:

Method 1: Weighted Cox regression

To address the issue of sample weighting, we calculated sample weights from baseline date of participants by using exponential decay function. And the idea was derived from this site (https://mathcracker .com/exponential-decay-formula). Then we used "coxph" function in R package "survival" to conduct weighted Cox regression by assign parameter "weights". We also adjusted for age, sex, BMI, smoking status, alcohol intake frequency, physical activity, and medication use. For breast cancer we further adjusted for age at menarche, number of live births, and menopausal status. These results remain consistent with our previous findings.

Table 5: Weighted Cox regression results

Cancers	**Group**	**Case/Total**	**HR (95%CI)**	***p* value**
C34 Malignant neoplasm of bronchus and lung	All	4828/476296	1.14(0.99,1.32)	0.074
	Male	2502/219504	1.21(1.00,1.46)	0.049
	Female	2326/256792	1.05(0.84,1.31)	0.651
C50 Malignant neoplasm of breast	All	10173/470084	1.23(1.10,1.37)	< 0.001
	Male	98/219645	0.90(0.29.2.82)	0.857
	Female	10075/250439	1.23(0.11,1.37)	< 0.001

Method 2: Cox regression adjusting starting time as the confounder

Since our analysis might be biased by different starting time of the cohort, we considered the cohort effect by extracting the years of entry date and adjusting this covariate "years" in Cox regression. We also adjusted for age, sex, BMI, smoking status, alcohol intake frequency, physical activity, and medication use. For breast cancer we further adjusted for age at menarche, number of live births, and menopausal status. The results obtained were consistent with our previous findings.

Table 6: Multi-variable Cox regression analysis adjusted for cohort effect

Cancers	Group	Case/Total	HR (95%CI)	*p* value
C34 Malignant neoplasm of bronchus and lung	All	4828/476296	1.15(0.99,1.33)	0.057
	Male	2502/219504	1.23(1.02,1.48)	0.034
	Female	2326/256792	1.06(0.846,1.32)	0.630
C50 Malignant neoplasm of breast	All	10173/470084	1.24(1.11,1.38)	< 0.001
	Male	98/219645	0.92(0.29,2.91)	0.888
	Female	10075/250439	1.24(1.11,1.38)	< 0.001

(2) For the issue of HR value equal to 0 or 1

We examined the cancer types the reviewer mentioned with HR values equal to 0 or 1 in our initial results. For some cancers, such as C18 in Supplementary Table 14, the HR value of 1.00 is the result of rounding to two decimal places, indicating that the risk of psoriasis for this type of cancer approaches null. We consider these results to be reliable. The abnormal HR values for certain cancers (C01, C05, C13, C30, C52, C65, C75, C84, C93, C95), are due to the small sample size. We have listed all the cancers with abnormal HR values and the cancer cases of psoriasis patients (as shown below).

It can be observed that for all these cancer types in whole population, the number of cases among psoriasis patients is less than 6, or even 0. After being divided into male and female subgroups, the number of cases of these cancers at least in one of the sexes was 0, which led to the inability to estimate HR values and resulted in abnormal results. We apologize for the confusion caused by these results. We have revised the description of our methodology section. We added "We also excluded the types of cancers with cases less than 6 in the psoriasis patients." in Page 6, Study population section.

Table 7: Cancer cases of psoriasis patients in certain cancers：

Cancers	Non-PSO Non-CA (N)	PSO Non-CA (N)	Non-PSO CA (N)	PSO CA(N)
C01 Malignant neoplasm of base of tongue	462890	13457	198	6
C05 Malignant neoplasm of palate	463041	13459	64	4
C13 Malignant neoplasm of hypopharynx	463066	13461	61	1
C30 Malignant neoplasm of nasal cavity and middle ear	463065	13462	58	1
C52 Malignant neoplasm of vagina	463068	13463	52	0
C65 Malignant neoplasm of renal pelvis	462985	13457	132	6

C75 Malignant neoplasm of other endocrine glands and related structures	463043	13463	69	0
C84 Peripheral and cutaneous T-cell lymphomas	462933	13453	170	5
C93 Monocytic leukaemia	463034	13462	99	1
C95 Leukaemia of unspecified cell type	463003	13461	108	2

Abbreviation: PSO, psoriasis.CA, cancer.

1.4 I am curious about the possible mediators through which PSO and cancers are related, therefore, mediation analysis is also worth trying.

Response：Thanks for your suggestions. UK Biobank provides metabolomic results for a subset of patients. We examined potential mediating effects of the top differentially expressed molecules on the association between psoriasis and cancers. The findings (Table 8 and Table 9) revealed that the mediation effects were weak.

Table 8: Mediation analysis of differential metabolites linking psoriasis to lung cancer:

	ACME		Proportion
	beta	*p* value	mediated
Cholesteryl Esters to Total Lipids in Very Large VLDL percentage	3.13E-05	0.16	1.25%
Triglycerides to Total Lipids in Very Large HDL percentage	2.63E-06	0.78	0.13%
Cholesterol to Total Lipids in Very Large VLDL percentage	2.94E-05	0.26	0.85%
Triglycerides to Total Lipids in Very Large VLDL percentage	3.14E-05	0.14	1.22%
Triglycerides to Total Lipids in Large HDL percentage	4.77E-06	0.48	0.11%
Free Cholesterol to Total Lipids in Very Large HDL percentage	1.43E-05	0.16	0.35%
Triglycerides to Total Lipids in Medium HDL percentage	9.04E-06	0.42	0.27%
Cholesteryl Esters to Total Lipids in Large VLDL percentage	2.87E-05	0.16	0.99%
Triglycerides to Total Lipids in Small HDL percentage	2.35E-05	0.18	0.75%
Phospholipids to Total Lipids in Large VLDL percentage	9.23E-06	0.54	0.22%
Phospholipids to Total Lipids in Chylomicrons and Extremely Large VLDL percentage	2.54E-05	0.14	0.90%
Total Triglycerides	-2.67E-06	0.78	-
Docosahexaenoic Acid to Total Fatty Acids percentage	7.31E-05	0.02	2.43%
Triglycerides in VLDL	-1.61E-06	1.00	-
Glucose	-8.02E-06	0.52	-

Abbreviation: ACME, Average Causal Mediation Effect.

Table 9: Mediation analysis of differential metabolites linking psoriasis to breast cancer:

	ACME		Proportion
	beta	***p* value**	**mediated**
Triglycerides to Total Lipids in Large HDL percentage	3.17E-05	0.40	-
Free Cholesterol to Total Lipids in Very Large HDL percentage	-2.00E-05	0.40	0.24%
Triglycerides to Total Lipids in Medium HDL percentage	7.35E-05	0.06	-
Phospholipids to Total Lipids in Large VLDL percentage	1.52E-05	0.46	-
Phospholipids to Total Lipids in Chylomicrons and Extremely Large VLDL percentage	4.22E-05	0.10	-
Omega-6 Fatty Acids to Omega-3 Fatty Acids ratio	-6.13E-05	0.06	0.88%
Omega-3 Fatty Acids to Total Fatty Acids percentage	1.94E-06	1.00	0.04%
Total Triglycerides	2.54E-05	0.48	-
Docosahexaenoic Acid to Total Fatty Acids percentage	2.65E-05	0.22	-
Triglycerides in VLDL	1.96E-05	0.56	-
Glucose	3.47E-05	0.06	-
Monounsaturated Fatty Acids	4.98E-05	0.26	-
Total Lipids in Large HDL	-1.85E-05	0.22	0.26%
Total Lipids in Chylomicrons and Extremely Large VLDL	3.47E-05	0.28	-
Total Lipids in VLDL	-8.64E-06	0.66	0.09%

Abbreviation: ACME, Average Causal Mediation Effect.

2. Genetic analysis

2.1 One-sample MR seems unnecessary

Response：Thank you for your comments. One-sample MR integrates clinical diagnoses and genetic information from a single study population, allowing us to explore causal relationships between exposures and outcome variables based on different subgroups. As this approach differs from the two-sample MR [18–20], similar results from one-sample and two-sample MR may indicate robust relation between psoriasis and cancer.

2.2 In this article, two-sample MR is used to confirm the results of One-sample MR. Based on the limitations of single-sample MR, this seems redundant. Shouldn't the results of one-sample MR prove the results of two-sample MR? Or, why didn't the authors conduct a two-sample MR based on the original UK Biobank data?

Response： Thank you for your comments.

(1) One-sample MR and two-sample MR have their own advantages and limitations. One-sample MR is less affected by population heterogeneity, but may have a higher false positive rate due to weak instrument variables[18]. In two-sample MR without sample overlap, bias caused by weak instrument variables is towards the null, which does not lead to false positive results [17]. Moreover, two-sample MR can use summary data from larger sample sizes, which means an increased power to detect causal relationships. However, only having access to summary data limit the flexibility in selecting research topics and conducting subgroup analysis (e.g., inability to conduct sex-specific analyses). Therefore, in order to strengthen our confidence and to analyze by sex in the causal estimates, both methods are necessary.

We have revised some of the descriptions in the results in page 14, Two-sample MR analysis section：

"We conducted two-sample MR analysis as it is less prone to false-positive bias than one-sample MR analysis."

(2) In two-sample MR analysis, the exposure and outcome GWAS data should be

originated from independent, non-overlapping populations to avoid bias introduced by sample overlap. Partially overlapping samples may inflate the correlation between genetic instrument variables and the exposure and outcome, leading to MR estimates deviating from the true causal effect [8].

The original text states: "*However, as several large consortia have overlapping studies, participants may overlap between the datasets used to estimate the genetic associations with the exposure and outcome. In this case, the direction and size of the bias varies linearly depending on the degree of overlap (formally, depending on the degree of correlation between the genetic association estimates).*"

The overlap issue mentioned in the text refers to the overlap of the same participants, which is inappropriate according to the principles. Therefore, we cannot simultaneously include exposure GWAS data and outcome GWAS data from the UK Biobank in our two-sample MR analysis.

2.3 I am confused about the authors' choice of PSO data from Finn when conducting two-sample MR. Finn is a very special database; many other data may be more suitable.

Response: Currently, the two largest publicly available GWAS databases for psoriasis in Europeans are the UK Biobank and FinnGen. Based on the aforementioned principle of non-overlapping samples in two-sample MR analysis[21], the GWAS data for psoriasis should not be sourced from the UK Biobank since the GWAS data for some outcomes originate from the UK Biobank. So, we used the Finnish psoriasis database.

2.4 Similar to observational studies, when conducting Phewas, confounders should be explained, and some cancers may have special risk factors.

Response: We appreciate your comments. We have revised the methodology section regarding the adjustment for confounding factors. We have included additional adjustments in the PheWAS analysis, as mentioned in our previous response 1.2.

2.5 About the authors' handling of gene pleiotropy. If possible, I would like to

assess the relationship between PSO and the candidate cancers through various methods after two-sample MR analysis, such as CAUSE, LCV, and gene colocalization; even cross-trait methods should be tried. Of course, if doing so, it does not necessarily guarantee that the Eqtl's results will be consistent with those of these methods.

Response: Thank you for your comments. To address the potential pleiotropy in the two-sample MR analysis, we have conducted the following additional analyses:

(1) For your recommended methods

We have performed additional CAUSE (Causal Analysis Using Summary Effect Estimates) to assess whether there is potential pleiotropy in the two-sample MR analysis. And we also performed cross-trait methods by using LDSC (Linkage Disequilibrium Score Regression). In table 10, the MR-Egger regression and MR-PRESSO results did not show any correlated horizontal pleiotropy ($p > 0.05$). The CAUSE results indicate that there is no uncorrelated horizontal pleiotropy with the effect size interval crossing zero (eta value represents effect size). LDSC also suggests that there is no genetic correlation between psoriasis and cancer, indicating that they do not share a similar genetic basis ($p > 0.05$).

Table 10: Pleiotropy test and LDSC:

	MR–Egger	MR-PRESSO	CAUSE	LDSC
Outcomes	***p* value**	***p* value**	**eta**	***p* value**
Lung cancer	0.815	0.160	0.00 (-0.35,0.29)	0.084
Breast cancer	0.512	0.111	0.00 (-0.09, 0.09)	0.754

(2) For other methods：

To investigate whether the SNPs used in the two-sample MR analysis are associated with the outcome cancers, we also performed logistic regression analysis, adjusting for potential confounders such as age, sex, and the top 10 principal components. The results showed that these instrumental variables were not significantly associated with cancer outcomes, suggesting that the SNPs are valid instrumental variables for psoriasis and do not directly influence the cancer outcomes. These results have been included in

supplementary Table 24.

We further conducted cross-trait analyses by examining the association between psoriasis-related SNPs and known risk factors for lung cancer and breast cancer, including smoking, hormone levels (oestradiol, SHBG and testosterone), and obesity (BMI). We performed logistic regression analysis using the instrumental SNPs from the two-sample MR analysis and these risk factors, adjusting for potential confounders such as age, sex, and the top 10 principal components. The results indicated that these instrumental variables were not significantly associated with cancer risk factors, reducing the possibility that the instrumental variables directly influence cancer development by affecting these risk factors. These results have been included in supplementary Table 25.

We added the description in page 15, Two-sample MR analysis section:

"Most of psoriasis susceptibility loci included as instrument variables in two-sample MR analysis were not significantly associated with breast or lung cancer outcomes **(Supplementary table 24)**. These instrumental variables were also not associated with common risk factors for breast cancer or lung cancer, including BMI, oestradiol, SHBG, testosterone and smoking **(Supplementary table 25)**."

Minor Concerns

1. The methodological part needs to be more detailed.

Response：We have revised the Methods section to provide more detailed information. Please refer to the red text.

2. The figures also need to be more beautiful; and the authors do not provide a detailed process of the study in Figure 1, for example, the inclusion and exclusion criteria are not clear.

Response：Thank you for your comments. We have added marks to make the inclusion and exclusion process clearer in Figure 1. Additionally, we have improved the

presentation of the other figures.

3. In the Data availability section, the sources of public data need to be clarified.

Response： The data availability statement is modified. We added the following statement in page 20, the data availability section：

Summary-level data from publicly available GWAS can be obtained through the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/) and the GWAS Catalog (https://www.ebi.ac.uk/gwas/). Gene expression RNA-seq data and clinical data from TCGA and GTEx RNA datasets can be accessed via the UCSC XENA database (http://xena.ucsc.edu/).

4. Detailed information on public data should be compiled into a table attachment.

Response： We have added the detailed information on the public data used in our study into a table, which is now included as Supplementary Table 3.

5. I am not sure if there is any other evidence needed for the original data from UK Biobank.

Response: We appreciate your comments. According to previous studies, researchers usually provided the UK Biobank Application ID and UK Biobank website in the Data availability section[22,23]. We added the following statement in page 20, the data availability section：

The current analysis was approved by UK Biobank in August 2020 with the ID 66536. The individual participant data collected for the current study cannot be shared without UK Biobank's explicit written approval. The UK Biobank data are available through a standard application protocol (https://www.ukbiobank.ac.uk/register-apply/).

6. It would be best if the authors could draw a possible mechanism diagram of the relationship between PSO and cancer, of course, the authors' eQTL results should be included in this figure.

Response: Thank you for your suggestions. We tried to draw a picture of the possible

mechanism as follow. However, this study is more about correlation and causality rather than detailing a comprehensive mechanistic pathway. Most of our analysis focuses on the association between two diseases, and our hypothesized mechanisms are indirect as we do not have concrete evidence regarding the precise mechanisms involved. We have added this picture in Supplementary Figure 5.

We added "The possible mechanisms of cancer development in psoriasis patients are summarized in Supplementary Figure 5." In page 19.

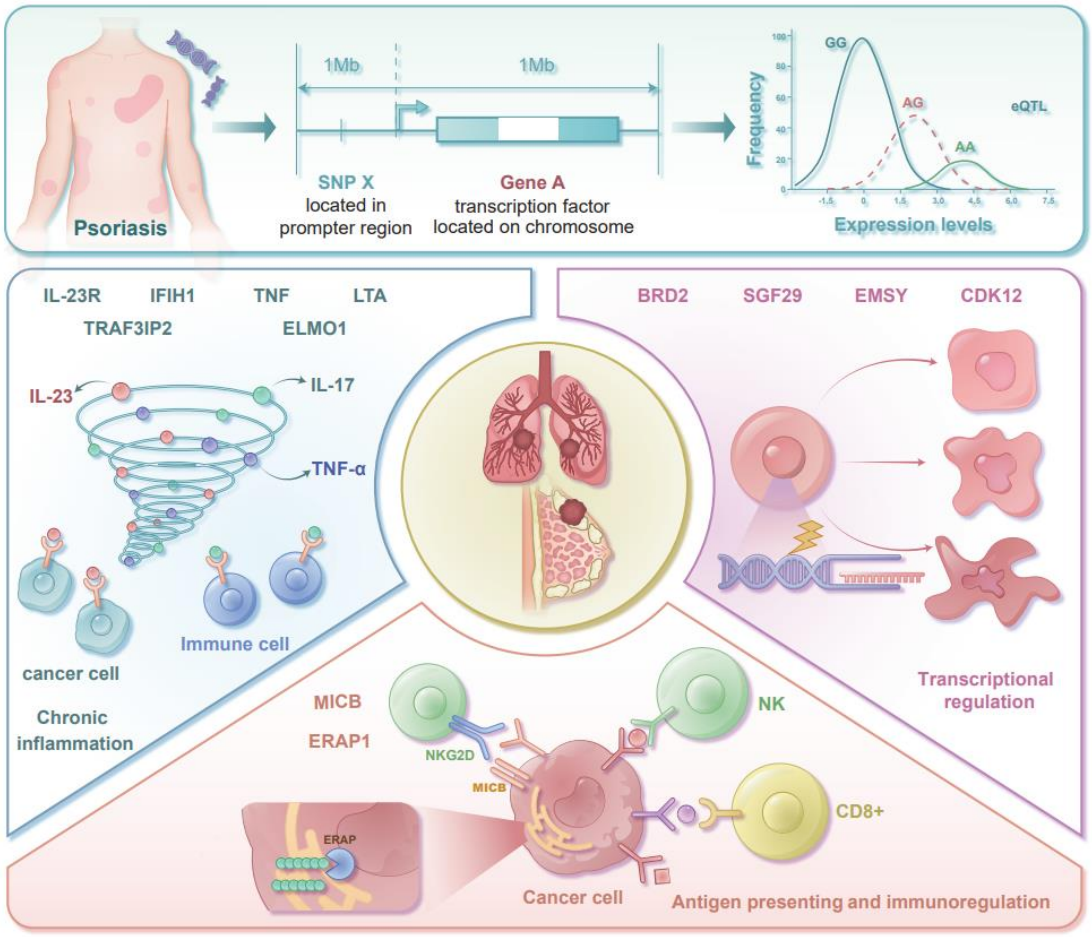

Figure 1：Possible mechanisms of cancer development in psoriasis patients

We propose that the mechanism between psoriasis and cancer may be due to the potential effects of psoriasis susceptibility loci on the expression of certain genes in breast and lung tissues. (1) The altered expression of genes related to transcriptional regulation and cell cycle regulation, such as BRD2, SGF29, EMSY and CDK12, may lead to uncontrolled cell transformation into tumor cells. (2) Chronic inflammation

caused by upregulated cytokines and activated NF-κB pathway and the IL-23/Th17 axis related to genes including IL-23R, IFIH1, TNF, LTA, TRAF3IP2 and ELMO1 may play a role in tumor development and progression. (3) Genes related to MHC I molecules, such as MICB and ERAP1, play a role in antigen processing and presentation. Changes in ERAP1 may lead to altered peptide antigen modification in the endoplasmic reticulum[1]. MICB, as a ligand for NKG2D, promotes NK cell cytotoxicity upon binding to the NKG2D receptor on the surface of NK cells[24]. Alterations in these genes may lead to changes in antigen presentation and immune regulation disorders, which could potentially promote tumor immune escape.

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REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The manuscript is significantly improved, and the authors have answered most of my questions.

A few comments:

1. Page 3, lines 45-46, it would be correct to write that cancer is one of the leading causes of death.

2. In the discussion, the authors must comment on the different findings in the one-sample and two-sample MR and explain why they performed both.

3. In the discussion, the authors should elaborate on the clinical relevance of their findings, particularly the implications of the limited increased risk.

4. I believe the authors need to clarify that the connection with ERAP1 may be a fortuitous finding, as ERAP1 is already known to be upregulated in both lung and breast cancer.

Reviewer #2 (Remarks to the Author):

The author's response is very detailed, and I suggest that the author elaborate on the confounding factors of each cancer in the appendix. Other than that, I have no other questions.

Reviewer #1 (Remarks to the Author):

The manuscript is significantly improved, and the authors have answered most of my questions.

A few comments:

1. Page 3, lines 45-46, it would be correct to write that cancer is one of the leading causes of death.

Response：Thanks for your comments. We have revised the manuscript according to your suggestion. The sentence on page 3, lines 45-46 has been modified to " Cancer has been reported to be one of the leading causes in patients with psoriasis[2],".

2. In the discussion, the authors must comment on the different findings in the one-sample and two-sample MR and explain why they performed both.

Response：We appreciate your comments. We have added the following discussion in the manuscript, Page 9:

Using unselected PheWAS analysis, our observational data not only confirmed previous reports that psoriasis is associated with higher risks of site-specific cancers in lung, kidney, liver, bladder, nonmelanoma skin, oral cavity, lymph nodes and non-Hodgkin's lymphoma[3,4,7] but also revealed some unreported associations between psoriasis and cancers of the breast, penis, anal canal and mesothelioma. Furthermore, our genetic analysis (including PRS and MR analyses) confirmed the causal relationship between psoriasis and lung cancer/breast cancer. Previous meta-analyses have several shortcomings that may lead to biased results, including significant heterogeneity across the included studies, inconsistent diagnostic criteria for psoriasis, and insufficient adjustment for potential confounders linked to cancer. Hence, previous reports on the multiple sites of malignancy related to psoriasis[3,4] should be interpreted cautiously. It should be noted that not all results from one-sample MR and two-sample MR were consistent. In one-sample MR analyses, we found causal associations between psoriasis and several cancers, including anal canal cancer, lung cancer, breast cancer, kidney cancer, SOS and follicular non-Hodgkin's lymphoma. In two-sample MR analyses, we only observed causal associations between psoriasis and lung cancer and breast cancer. One-sample MR may generate a false positive result due to its weak instrument variables[14]. For two-

sample MR without sample overlap, bias caused by weak instrument variables is towards the null, which rarely lead to false positive result[14]. Moreover, two-sample MR often refers to summary data from larger sample size, which enhances a power to detect potential causal relationships. However, only having access to summary data limits the flexibility in conducting subgroup analysis. To cautiously and flexibly interpret the causal association between psoriasis and cancers, we used both one-sample MR and two-sample MR analyses. The consistent results from both methods strengthen the robustness of the findings on the association between psoriasis and lung cancer and breast cancer.

3. In the discussion, the authors should elaborate on the clinical relevance of their findings, particularly the implications of the limited increased risk.

Response：Thanks for your comments. We have added the discussion on Page 10:

In our study, measurement errors, confounding factors, and false-positive bias were gradually controlled from observational PheWAS, PRS PheWAS, and one-sample analysis to two-sample MR analysis[14,16,17]. Although the genetically increased risks are not as high as observational results, our data warrant a clinical attention to lung cancer and breast cancer among patients with psoriasis.

4. I believe the authors need to clarify that the connection with ERAP1 may be a fortuitous finding, as ERAP1 is already known to be upregulated in both lung and breast cancer.

Response：Thanks for your comments. We have revised the description in the discussion section, Page 11:

By integrating public datasets of eQTL, TCGA and GTEx, our gene annotation revealed potential molecular association between psoriasis and lung or breast cancer. Among them, we fortuitously found that ERAP1, an endoplasmic reticulum aminopeptidase, may be one of the promising candidates. On one hand, several genome-wide association studies and exome sequencing identified ERAP1 as an important susceptibility locus harbored gene for psoriasis[26–28].

Reviewer #2 (Remarks to the Author):

The author's response is very detailed, and I suggest that the author elaborate on the confounding factors of each cancer in the appendix. Other than that, I have no other questions.

Response：We appreciate your comments. We have added these data into Supplementary table 17.