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Mendelian Randomization Analysis shows that Elevated Eosinophil Cell Count Increases the Risk of Autoimmune disorders but Protects against Skin Cancer

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ABSTRACT

Background: Eosinophils are immune cells which are critical to the pathophysiology of autoimmune disorders and skin cancer. We performed an integrated causal inference analysis with Mendelian randomization to demonstrate that although individuals with reduced eosinophil cell count are protected against autoimmune disorders such as asthma, they are at increased risk of skin cancer.
Methods: Epidemiology and public health has historically relied on observational studies to identify risk factors for disease; however, these methods are limited by reverse causation and confounding effects. In this study, we utilize genetic epidemiology and Mendelian randomization, a methodology that removes the risk for reverse causation, reduces pathways for confounding variables, and is an effective tool in identifying causal effects between risk factors and outcomes. Our analysis combines results from 12 genetic analyses from 5 different studies to explore the differential effect of eosinophil cell count on autoimmune and skin cancer disease risk.
Results: Raised eosinophil count resulted in increased risk of multiple autoimmune disorders including psoriasis (OR 0.0029 (95% CI: 0.0013-0.0046), P-value = 5.0×10^{-4}), ankylosing spondylitis (OR 1.397×10^{-3} (95% CI: 0.0006-0.002), P-value = 4.0×10^{-4}), and rheumatoid arthritis (OR 0.0011 (95% CI: 0.0004-0.0019), P-value = 2.1×10^{-3}). In contrast, increased eosinophil cell count was protective against malignant melanoma (OR -0.001 (95% CI: -0.0017-(-0.0003)), P-value = 0.0074) and basal cell carcinoma (OR -0.0012 (95% CI: -0.0024-(-0.00007)), P-value = 3.7×10^{-2}).
Conclusions: Results indicated that the causal effect of increased eosinophil count differentially increases the risk of immune related disorders and decreases the risk of oncology related skin diseases.

Introduction

Eosinophils are white blood cells that are critical for immune action and response. In order to fight against invading pathogens that threaten the immune system, eosinophils detect pathogens and promote immune responses through the expression of complement and Fc receptors (Travers & Rothenberg, 2015). Moreover, evidence suggests that eosinophils have complex structures, containing both pro-inflammatory and anti-inflammatory properties, which lead to varied behaviors in autoimmune and oncology related diseases (Biton et al., 2016). It has been shown that eosinophils can both cause disease such as autoimmune disorders and protect against disease such as melanoma (Varricchi et al., 2018). With MR analysis, we investigate the causal effects of eosinophil count (EC) on different disease outcomes and show that individuals with high EC can either be protected against or at higher risk for different disease outcomes, such as generalized rheumatoid arthritis (RA), multiple sclerosis, and several oncology related outcomes, including basal cell carcinoma and malignant melanoma. In this study we utilize a statistical approach which leverages genome

wide association studies to perform a causal analysis to determine the role of EC in autoimmune diseases and skin cancers.

Methods

The Mendelian randomization (MR) technique relies on a series of instrumental variables which are genetic variations known to be associated with the exposure (EC) and with association statistics with the outcome measure (autoimmune disease or oncology related disorder).

Dataset

Results of 12 genetic analyses were integrated from 5 different studies in our analysis. Genetic data was brought together from the sources and repositories shown in Table 1. The resulting data was compiled using the R programming language in RStudio to prepare for MR analyses. The rsID, chromosome and base-pair position, reference and alternative alleles of the genetic variation, estimated effect of the genetic variation on the outcome, and p-value for the estimated effect of the genetic variation on the outcome was obtained for each genetic variation. The TwoSampleMR package containing data from genome wide association studies was used in the MR analyses.

Table 1. Genome wide association study data utilized in MR experiment

Trait	Author	Sample Size	Disease Cases	Source
Ankylosing spondylitis	Ben Elsworth	462,933	1,296	DOI: 10.1101/2020.08.10.244293
Malignant melanoma	B. Neale	361,194	1,672	Neal lab repository
Eosinophil cell count	D. Vuckovic	563,946		PMID: 32888494
Knee osteoarthritis	Tachmazidou I.	403,124	24,955	PMID: 30664745
Multiple sclerosis	Ben Elsworth	462,933	1,679	DOI: 10.1101/2020.08.10.244293
Psoriasis	Ben Elsworth	462,933	5,314	DOI: 10.1101/2020.08.10.244293
Rheumatoid arthritis	Ben Elsworth	463,010	1,523	DOI: 10.1101/2020.08.10.244293
Basal cell carcinoma	Ben Elsworth	462,933	4,290	DOI: 10.1101/2020.08.10.244293
Spine arthritis/spondylitis	Ben Elsworth	462,933	4,033	DOI: 10.1101/2020.08.10.244293
Osteoarthritis of the hip or knee	Tachmazidou I.	417,596	39,427	PMID: 30664745
Hip osteoarthritis	Zeggini	14,275	3,266	PMID: 22763110
Gout	Ben Elsworth	463,010	1,042	DOI: 10.1101/2020.08.10.244293

Genetic data for the 12 human phenotypes studied in our MR experiment were collected and integrated across multiple sources.

Mendelian randomization sensitivity analyses

MR can result in pleiotropy, a condition where one single gene affects several unrelated phenotypic traits, since it utilizes a large number of genetic variants, leading to the creation of biased causal results (Lin et al., 2021). MR Egger and Inverse Variance Weighted are two major MR methods that can be used to fight against pleiotropy; MR Egger assists in providing a more unbiased estimate but lacks statistical power, while Inverse Variance Weighted method maintains significant statistical power while inferring the strength of the causal effect between the exposure and outcome (Lin et al., 2021).

Software pipeline

Overall, this process is known as an MR experiment, which uses a series of instrumental variables as genetic variations to infer the causal effects of multiple risk factors on a single outcome. It is more efficient and methodical than a regular MR experiment that infers the causal effect of a single risk factor on a single outcome.

The experiment utilized R programming in RStudio to statistically compute the examined exposure and outcomes to retrieve p-values and odds ratios, produce forest plots between the exposures and outcomes to inspect and visualize potential relationships, and create MR programs from the TwoSampleMR package for each disease outcome, in which the package was employed to extract the effects of the genetic variations aligned with the exposure on the outcome to evaluate the causal relation. After the extraction that utilizes the TwoSampleMR package, using R programming, a loop was run on each exposure to receive its instrument data and their effects on the disease outcome, and inside the same loop, each exposure and disease outcome data was harmonized for them to situate on the exact same reference allele. Each outcome in the study went through this process.

Regarding data visualization, specifically, datasets were created for each exposure to provide data on the nSNP and p-values of the exposure on disease outcome for both MR methods performed, Inverse Variance Weighted and MR Egger. Since the Inverse Variance Weighted method maintains high statistical power, the values retrieved from it were selected over those from the MR Egger method. The analysis of the Inverse Variance Weighted method's p-values of each exposure on outcome included analyzing whether p-values were less than or equal to 0.05 at a 95% confidence interval, indicating statistically significant results that reject the null hypothesis and deduce that the exposure is a risk factor of the disease outcome. Scatterplots were also created for each exposure, plotting the SNP effect on the exposure against the SNP effect on the disease outcome. Finally, forest plots were then created to visualize the significant exposures and outcomes after the analysis of all the retrieved p-values.

Results

Most traditional applications of MR study singular risk factors with outcomes to determine causal effect. However, this approach is limited because in many cases, a risk factor may be protective for some categories of disease and causative for others. In this analysis we study the effect of EC on major autoimmune and oncology disease outcomes to assess the differential causal effect of eosinophils on disease outcomes.

Higher eosinophil cell count causally associated greater risk of autoimmune disorders such as psoriasis and lower risk of skin cancer

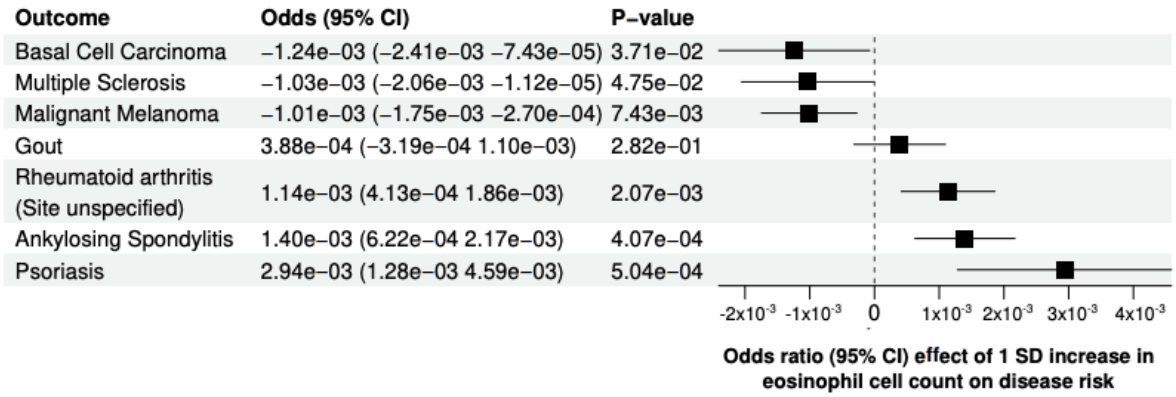

Figure 1. Forest plot representing effect of eosinophil count on autoimmune and oncology related disease outcomes. Eosinophil count increased risks of the autoimmune diseases but protected against oncology disorders.

Eosinophil count increased the risks of the autoimmune diseases, such as rheumatoid arthritis (OR 0.0011 (95% CI: 0.0004-0.0019), P-value = $2.1x10^{-3}$), ankylosing spondylitis (OR $1.4x10^{-3}$ (95% CI: 0.0006-0.002), P-value = $4.0x10^{-4}$), and psoriasis (OR 0.0029 (95% CI: 0.0013-0.0046), P-value = $5.0x10^{-4}$) but protected against oncology disorders such as basal cell carcinoma (OR -0.0012 (95% CI: -0.0024-(-0.00007)), P-value = $3.7x10^{-2}$) and malignant melanoma (OR -0.001 (95% CI: -0.0017-(-0.0003)), P-value = $7.4x10^{-3}$). Intriguingly, multiple sclerosis (OR $-1.034x10^{-3}$ (95% CI: -0.0021-(-0.00001)), P-value = $4.8x10^{-2}$) is an autoimmune disease that did not follow this trend due to no causal association with increased eosinophil count. Gout is a non-autoimmune condition and has no association with raised eosinophil count (OR 0.0004 (95% CI: 0.0003-0.0012), P-value = 0.28).

Higher eosinophil cell count shows an association with reduced hip osteoarthritis but not knee or spine osteoarthritis

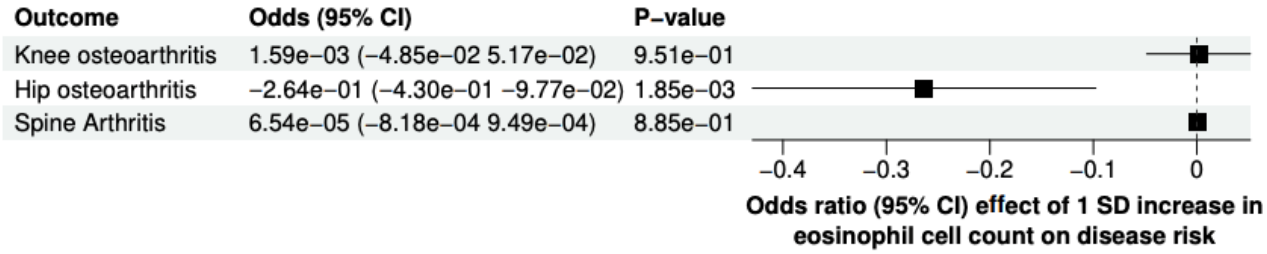

Figure 2. Forest plot representing effect of eosinophil count on three arthritis related diseases. Eosinophil count protected against hip osteoarthritis (OR -0.2636 (95% CI: -0.4296-(-0.0977)), P-value = $1.8x10^{-3}$) but had no effect on the risks of spine arthritis (OR $6.538x10^{-5}$ (95% CI: -0.0008-0.0009), P-value = 0.88) and knee osteoarthritis (OR 0.0016 (95% CI: -0.0485-0.0517), P-value = 0.95).

Our MR analysis identifies increased risk of multiple autoimmune disorders resulting from higher EC, including psoriasis (OR 0.0029 (95% CI: 0.0013-0.0046), P-value = $5.0x10^{-4}$), ankylosing spondylitis (OR $1.4x10^{-3}$ (95% CI: 0.0006-0.002), P-value = $4.0x10^{-4}$), and RA (OR 0.0011 (95% CI: 0.0004-0.0019), P-value = $2.1x10^{-3}$) (Figure 2). These results reflect the known etiology of these disease outcomes which are driven by autoimmune activity ("Eosinophilia"). Our analysis shows that arthritis driven by autoimmune activity shows an increased risk with higher EC (RA, OR 0.0011 (95% CI: 0.0004-0.0019), P-value = $2.1x10^{-3}$). However, osteoarthritis which is driven by wear and tear at the joint shows no causal association, such as spine and knee osteoarthritis (OR $6.5x10^{-5}$ (95% CI: -0.0008-0.0009), P-value = 0.88) and (OR 0.0016 (95% CI: -0.0485-0.0517), P-value = 0.95). Additionally, gout, a non-autoimmune condition, had no significant association with raised EC (OR 0.0004 (95% CI: 0.0003-0.0012), P-value = 0.28). Intriguingly, in the case of hip osteoarthritis, we identify a protective causal association between increased EC and odds ratio of disease (OR -0.2636 (95% CI: -0.4296-(-0.0977)), P-value = $1.8x10^{-3}$). However, multiple sclerosis (OR $-1.034x10^{-3}$ (95% CI: -0.0021-(-0.00001)), P-value = $4.8x10^{-2}$) was another autoimmune disease that did not follow this trend due to no causal association with increased EC. In contrast, eosinophil cells are also known to perform critical anti-tumorigenic roles, therefore, patients at higher risk of autoimmune disorders as a result of higher EC may be conversely protected against skin cancer risk. Our MR analysis shows a causal relationship between increased EC and malignant melanoma (OR -0.001 (95% CI: -0.0017-(-0.0003)), P-value = $7.4x10^{-3}$) and basal cell carcinoma (OR -0.0012 (95% CI: -0.0024-(-0.00007)), P-value = $3.7x10^{-2}$) (Figure 1). Analysis of further cancer types are limited by availability of well powered genome-wide association studies. This difference in eosinophil behavior can be explained by the fact that they contain anti-tumorigenic (counteract the formation of tumors) and protumorigenic molecules (promote formation of tumors) (Varricchi et al.).

Discussion

Eosinophils are white blood cells that can regulate inflammation in the immune system and destroy foreign substances ("Eosinophilia"). To isolate and control disease sites in the immune system, eosinophils can build up to cause inflammation ("Eosinophilia"). An increase in EC can be in response to certain autoimmune diseases. In several autoimmune diseases (psoriasis, ankylosing spondylitis, RA, and gout), high eosinophil blood counts are present as a result of the inflammation present ("Eosinophilia"). In contrast, increased cell counts of eosinophils had no association with knee or spine osteoarthritis, reflecting the known mechanism of the 'wear and tear' disease. Additionally, the protective causal effect of raised eosinophils on hip osteoarthritis requires further investigation. Even though evidence suggests that eosinophils increase to cause inflammation against autoimmune diseases, multiple studies propose that eosinophils instead protect against certain arthritis-related diseases, such as RA (Qin et al., 2021). Interleukin 33, a strong activator of eosinophils, has been perceived to have pro-inflammatory and anti-inflammatory properties in autoimmune diseases (Biton et al.). Eosinophils can either promote inflammation and increase in count to fight against autoimmune diseases or restrain from inflammatory response and protect against autoimmune disease (Biton et al.). This discovery can explain the varied results obtained in Figure 2, as well as clarify why multiple sclerosis is an autoimmune disease but is protected by EC. Moreover, based on the results in Figure 1, ECs were observed to protect against certain oncology related disorders: malignant melanoma and basal cell carcinoma. This difference in eosinophil behavior can be explained by the fact that they contain anti-tumorigenic (counteract the formation of tumors) and protumorigenic molecules (promote formation of tumors) (Varricchi et al.). Specifically, eosinophils play an anti-tumoral role in most cancer types– skin melanoma cancer, colorectal, breast, and gastric cancer– and a pro-tumoral action in blood, lung, and ovarian (Varricchi et al.). One particular study found that the activation of eosinophils could be a therapeutic strategy for tumors like melanoma, which supports the idea that eosinophils can protect against cancers (Varricchi et al.). Additionally, another study conducted to determine the role of eosinophils as on-treatment biomarkers concluded that melanoma patients who developed eosinophilia at any point in their course of disease had a significantly longer survival (Moreira et al., 2017). Specifically, out of 173 melanoma patients, those with more than 20% eosinophils had a prolonged survival with a median of 35 months, while those who did not have additional

amounts of eosinophils only had a median of 16 months (Moreira et al., 2017). These findings supported the results of the oncology related diseases in Figure 1, further strengthening the effectiveness of MR.

Conclusion

MR allows for causal inferences in observational studies that are vulnerable to confounding variables. Results indicated that the causal effect of increased EC differentially increases the risk of immune related disorders and decreases the risk of oncology related diseases. This difference in eosinophil behavior between the two types of diseases can be elucidated by its specific composition, which contains antitumorigenic and protumorigenic molecules as well as pro-inflammatory and anti-inflammatory properties. Further study on the roles of eosinophils in different types of arthritis is required.

Abbreviations:

MR- Mendelian randomization
RA- Rheumatoid arthritis
EC- Eosinophil count

Limitations

MR analysis relies on instrumental variables derived from genome-wide association analysis which is reliant on study sample sizes, in particular disease case counts. Therefore, our analysis is limited to disease outcomes which are well studied and at higher incidence. Additionally, the results may not be generalizable to all ethnic groups as the data primarily involves participants of European ancestry.

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