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Elevated platelet count appears to be causally associated with increased risk of lung cancer: A Mendelian randomization analysis

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Competing interests

The authors below are claiming a Conflict of Interest.
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Abbreviations: SNPs, single nucleotide polymorphisms; NSCLC, non-small cell lung cancer; OR, odds ratio; PLT, platelet count; MR, Mendelian Randomization; IV, instrumental variable; GWAS, genome-wide association studies; SMR, summary data-based Mendelian randomization; AC, adenocarcinoma; SqCC, squamous cell carcinoma; SCLC, small cell lung cancer; IVW, inverse-variance weighted.

Abstract

Background: Platelets are a critical element in coagulation and inflammation, and activated platelets are linked to cancer risk through diverse mechanisms. However, a causal relationship between platelets and risk of lung cancer remains unclear.

Methods: We performed single and combined multiple instrumental variable Mendelian Randomization (MR) analysis by an inverse-weighted (IVW) method, in addition to a series of sensitivity analyses. Summary data for associations between single nucleotide polymorphisms (SNPs) and platelet count is from a recent publication including 48,666 Caucasian Europeans and International Lung Cancer Consortium and Transdisciplinary Research in Cancer of the Lung data consisting of 29,266 cases and 56,450 controls analyze associations between candidate single nucleotide polymorphisms and lung cancer risk.

Results: Multiple instrumental variable analysis incorporating six SNPs showed a 62% increased risk of overall NSCLC (OR, 1.62; 95%CI, 1.15–2.27; P = 0.005) and 200% increased risk for small cell lung cancer (OR, 3.00; 95%CI, 1.27–7.06; P = 0.01), respectively. Results showed only a trending association with NSCLC histological subtypes, which may be due to insufficient sample size and/or weak effect size. A series of sensitivity analysis retained these findings.

Conclusion: Our findings suggest a causal relationship between elevated platelet count and increased risk of lung cancer and provide evidence of possible anti-platelet interventions for lung cancer prevention.

Impact: Our findings suggest a causal relationship of increased platelet count and risk of lung cancer, which also provide a better understanding of lung cancer etiology and potential evidence for anti-platelet interventions for lung cancer prevention.

Introduction

Lung cancer, a highly invasive, rapidly metastasizing cancer, has been the leading cause of cancer deaths worldwide for decades, accounting for more than one million deaths each year [1] . Smoking is a major risk factor for lung cancer and accounts for about 80% of male and 50% of female lung cancer cases [2]. In addition, environmental–occupational exposures [3, 4] , lifestyle, and genetic variants [5] have been broadly explored as risks/predisposing factors for lung cancer. However, aspects of lung cancer risk remain largely unexplained and thus warrant further study.

The lung was recently noted to play a major role in platelet biogenesis and act as an ideal bioreactor for production of mature platelets from megakaryocytes, which account for ~50% of total platelet production [6] . Platelets are an important element in coagulation and inflammation, and diverse mechanisms link activated platelets to cancer progression [7, 8]. It has been identified that several variants in those chromosomal regions associated with platelet count (PLT) have associations with myocardial infraction, autoimmune and hematologic disorders. Tumor-educated blood platelets (TEPs) have emerged as promising biomarker sources for non-invasive detection of cancer, and it was demonstrated to discriminates patients with NSCLC from healthy individuals and patients with various non-cancerous inflammatory conditions [9, 10].Indeed, high platelet count (PLT) is associated with increased mortality in a variety of cancers, including malignant mesothelioma [11] , gynecological malignancies [12], and breast cancer [13]. In addition, platelet-to-lymphocyte ratio and mean platelet volume also add value in early diagnosis of lung cancer [14] and prognosis prediction [15, 16]. These findings, taken together, indicate that disordered platelet production may be connected to lung carcinogenesis. However, due to potential unmeasured confounders in observational studies, the association between PLT and lung cancer risk remains unclear.

Mendelian Randomization (MR) is based on the principle that an individual's genotype is randomized at conception[17] and utilizes genetic variants as instrumental variables (IV) for the association between phenotypic exposures and outcomes to eliminate bias due to unmeasured confounders. Genetic variants used as instrumental variables should meet the following assumptions: (1) genetic variants are associated with exposure, (2) genetic variants affect outcome only via the

exposure, and (3) genetic variants are not associated with any confounders of the exposure–outcome association.[18] By finding a genetic marker that satisfies instrumental variable assumptions, Mendelian randomization analysis has been broadly used to estimate unconfounded associations between exposure and outcome [19], such as the effect of higher adult height on escalated cancer risk [20-24].

In this study, we performed summary data-based Mendelian randomization (SMR) [25] analysis which is the extension of two sample Mendelian randomization, using curated platelet count-related SNPs as instrumental variables to evaluate the association between platelet count and lung cancer risk by using summary statistics from recent large scale genome-wide association studies (GWAS).

Materials and Methods

Data source and study population

Mendelian Randomization analysis was conducted to estimate the effect of platelet count (X) on risk of lung cancer (Y) using genetic variants (G) as instrumental variables.[26] According to the MR analysis diagram described in Figure 1, we used coefficients of genetic variants on platelet count (b_{XG}) and their standard errors (SE_{XG}) from the recently published study of Gieger et al., which pooled 23 studies and included approximately 48,666 individuals of European descent [27].

The 54 genetic variants were identified that were associated with PLT (Table S1). One of the key assumptions underlying Mendelian Randomization is that the genetic variants (SNPs) used as instrumental variables are only related to the outcome of interest through the exposure variable under study. No pleiotropic pathways should exist from platelet-related SNPs to lung cancers through intermediates other than platelet count. Thus, six genetic variants (rs17030845, rs6141, rs3792366, rs210134, rs708382, and rs6065) where further selected as qualified instrumental variables that have prior functional knowledge supporting their association with platelets and no apparent link to cancer through intermediates other than platelets. By the way, the SNP rs6141 in THPO narrowly misses the level required for nominal significance ($P<5\times10^{-8}$) with $P=6.18\times10^{-8}$ in Europeans, but shows genome-wide significance in Japanese [28] . Therefore, it is still included serving as instrument variable for platelet count.

Coefficients (β_{YG}) and corresponding standard errors (SE_{YG}) of the association

between genetic variants and lung cancer risk were obtained from meta-analysis of existing Oncoarray and TRICL GWAS studies, which were detailed previously [29]. Briefly, overall non-small cell lung cancer (NSCLC) samples were composed from Oncoarray and TRICL GWASs, including 29,266 cases and 56,450 controls, and subgroup analyses were performed for 11,273 adenocarcinoma (AC), 7,426 squamous cell carcinoma (SqCC), and 2,664 small cell lung cancer (SCLC) cases (Table S2).

Mendelian Randomization (MR) analysis

Mendelian Randomization analysis with multiple instrumental variables was performed using an inverse-variance weighted (IVW) method combining the effect of genetic variants by weighted score. This score was used as an instrumental variable to estimate the effect of PLT on lung cancer risk [26]:

$$\hat{b}_{YX\_IVW} = \frac{\sum_{i=1}^{N}\left(\frac{b_{XG_i} b_{YG_i}}{SE_{YG_i}^2}\right)}{\sum_{i=1}^{N}\left(\frac{b_{XG_i}}{SE_{YG_i}}\right)^2}, SE_{YX\_IVW} = \sqrt{\frac{1}{\sum_{i=1}^{N}\left(\frac{b_{XG_i}}{SE_{YG_i}}\right)^2}} \quad (1)$$

In which $N = 6$ represents the number of instrumental variables included, and b_{YX_IVW} and SE_{YX_IVW} represent the effect of platelet count on lung cancer risk in log(OR) scale and its corresponding standard error. Associations of platelet count on risk of overall NSCLC and individual subtypes were analyzed. Results are presented as OR for lung cancer risk per 100×10^9/L increment of platelet count.

Additionally, penalized IVW, robust IVW, MR-Egger, penalized MR-Egger, and robust MR-Egger methods were used for sensitivity analyses to evaluate robustness of the findings [30]. Step forward modeling was used to add an optimal instrumental variable each time from the left 48 SNPs, adding to the 6 curated SNPs for multiple instrumental variable analysis, until there was no improvement of statistical significance (*P*-value) for the test of causal effect. The modeling process was terminated when no added SNP increased -log10 (*P*-value) by 20% or 10%. Besides, MR analysis with a single IV (one SNP at a time) was performed as supplementary. Effect of PLT on Lung cancer risk [b_{YX} in log odds ratio (OR) scale] and its standard error (SE_{YX}) were estimated as follows [31]:

$$\hat{b}_{YX} = \frac{b_{YG}}{b_{XG}}, SE_{YX} = \frac{SE_{YG}}{b_{XG}} \quad (2)$$

All analyses were performed using R Software Version 3.3.1 (The R Foundation). All tests were two-sided, and $P \leq 0.05$ was considered statistically significant unless stated otherwise.

Results

Among 48,666 Europeans, 54 SNPs were quantitatively associated with platelet count with $P \leq 5\times10^{-8}$ (Table S1) [27]. Associations of those 54 SNPs with risk of lung cancer were analyzed among 29,266 cases and 56,450 controls from OncoArray and previous GWAS studies. Demographics and study descriptions were detailed previously [29] and are briefly listed in Table S2 as well. Summarized association results of SNPs and lung cancer risk are listed in Table S3. According to instrumental variable assumptions that had evidence only related to platelets, six SNPs which are relatively independent and situated in different chromosomes were selected for MR analysis (Table 1), and 48 SNPs were excluded (Table S4).

In multiple IV analysis combining all six relatively independent SNPs situated in different chromosomes, a significant association between PLT and overall NSCLC risk is revealed, showing that each 100×10^{9}/L increment of PLT was associated with a 62% increase in NSCLC risk (95%CI, 1.15–2.27; $P = 0.005$) (Figure 2A and Figure 3A). In addition, five different methods of sensitivity analysis, including penalized IVW, robust IVW, MR-Egger, penalized MR-Egger, and robust MR-Egger, retained this association (Table 2). In NSCLC subtype analysis, it failed to detected significant associations between PLT and the risk of lung Adenocarcinoma (AC) (OR, 1.51; 95%CI, 0.92–2.48; $P = 0.11$) (Figure 2B and Figure 3B) and squamous cells carcinomas (SqCC) (OR, 1.59; 95%CI, 0.86–2.92; $P = 0.14$) (Figure 2C and Figure 3C). On the other hand, it is suggested that PLT is significantly associated with the risk of small cell lung cancer (SCLC) (OR, 3.00; 95%CI, 1.27–7.06; $P = 0.01$) (Figure 2D and Figure 3D). The results of single IV are presented in supplementary (Table S5). No correction was conducted for them because a single weak instrument will have lower power to reject the null hypothesis [32].

We also performed a step forward modeling strategy to include more instrumental SNPs in the multiple instrumental variable model. Including more SNPs as instrumental variables yielded similar, yet more significant, causal estimates (Table S6 and Figure S1).

Discussion

This Mendelian randomization study suggests that each 100×10^9/L increment in platelets results in a 62% increased risk of non-small cell lung cancer and, notably, a 200% increased risk of small cell lung cancer. However, this study failed to show evidence of a relationship between PLT and risk of AC and SqCC, probably resulting from insufficient sample size. As comparing with SCLC, the effect size of PLT on AC and SqCC are weaker, larger sample size is needed [33].

Platelets have been studied for decades as an important regulator of inflammation and thrombosis [34], which are broadly interrelated with human carcinogenesis [13]. Platelets are also recognized as a stimulator of proangiogenic factors [13] and a major source of vascular endothelial growth factor (VEGF) [35], platelet-derived growth factor (PDGF) [36, 37], and basic fibroblast growth factor (bFGF) [37], which act as promoters of tumor growth in lung [38-44]. New evidence suggests that platelets are relevant to defensive, physiological immune responses of the lungs and to inflammatory lung diseases [45]. Thus, higher platelet count has a potential biological connection to increased risk of lung cancer. Interestingly, p-selectin, an important adhesion molecule expressed on the surface of activated platelets, is more highly expressed in lung adenocarcinomas and squamous cell carcinomas than in healthy populations [46]. These results indicate a considerable role of platelets in lung carcinogenesis.

Intriguingly, a recent study indicates that cancer cells depend on platelets to avoid anoikis and succeed in metastasis [47]. Platelets induce resistance to anoikis in vitro and are critical for metastasis in vivo by activating RhoA-MYPT1-PP1-mediated YAP1 dephosphorylation and promoting its nuclear translocation to inhibit apoptosis. However, the unknown underlying mechanism warrants future well-designed functional experiments to clarify the role of platelets in these cellular processes.

In addition, anti-platelet agents, such as purinergic antagonists, are used clinically because they affect inflammatory pathways [48]. Recent publications demonstrate that platelets suppress T-cell responses against tumors through production and activation of immunosuppressive factors. These results suggest the use of a combination of immunotherapy and platelet inhibitors, such as aspirin [49, 50] and clopidogrel, as a therapeutic strategy against cancer [51, 52]. Therefore, it is possible that anti-platelet therapy could reduce lung cancer risk.

However, we acknowledge some limitations in our study. First, some associations between genetic instrumental variables and phenotype (platelet count) were insufficient and thus may result in a "weak instrument" phenomenon [53]. Second, in some scenarios, inconsistent results were observed between inverse-variance weighted and MR-Egger (or regular and penalized/robust) models. This phenomenon indicates that genetic variants probably have horizontal pleiotropy, and thus MR assumptions are likely violated [54]. Moreover, there is heterogeneity across results incorporating different SNP sets as instrumental variables, which indicates that the instrumental variable should be curated carefully before Mendelian randomization analysis. In this study, all platelet count-related SNPs were curated, and six were retained to better satisfy MR assumptions. Third, a linear association was assumed between PLT and lung cancer risk. However, the shape could be non-linear and thus warrants further study incorporating individual-level data. Fourth, we only evaluated platelet count as a potential causal factor, whereas platelet function plays a comparable causal role in this pathway. More detailed platelet information should be measured in future studies, including immature platelet fractions and function. In addition, we assumed that study populations used for the genetic instrument for platelet count and for risk of lung cancer were representative of the same general Caucasian population, which may not be true. Therefore, additional functional studies are needed to further evaluate the mechanisms that underlie associations between platelets and lung cancer risk.

Nonetheless, our findings do suggest a role of platelet count in risk of lung cancer. The results provide a better understanding of lung cancer etiology and evidence for a possible role of anti-platelet interventions in lung cancer prevention.

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Table 1. SNPs of specific platelet-related genes

SNP	Chr:Position (hg19)	Gene	Reference allele	Effect allele	EAF (%)	Function	b (95% CI)	P
rs17030845	2: 43687879	THADA	C	T	09.65	intron	–3.58 (–4.67, –2.49)	1.27×10^{-10}
rs6141	3: 184090266	THPO	T	C	47.39	3’ UTR	–2.47 (–3.36, –1.57)	6.18×10^{-8}
rs3792366	3: 122839876	PDIA5	A	G	38.68	intron	2.153 (1.44, 2.87)	3.60×10^{-9}
rs210134	6: 33540209	BAK1	G	A	29.29	500bp downstream	–4.96 (–5.73, –4.18)	7.11×10^{-36}
rs708382	17: 42442344	FAM171A2-ITGA2B	T	C	39.66	2kb upstream	–2.44 (–3.28, –1.59)	1.51×10^{-8}
rs6065	17: 4836381	GP1BA	C	T	08.53	missense	4.19 (2.96, 5.43)	2.92×10^{-11}

EAF, effect allele frequency; UTR, untranslated region

Table 2. Association between platelet count and risk of lung cancer using multiple IV analysis

SNP	Overall NSCLC		AC		SqCC		SCLC	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
IVW	1.62 (1.15, 2.27)	0.005	1.51 (0.92, 2.48)	0.11	1.59 (0.86, 2.92)	0.14	3.00 (1.27, 7.06)	0.01
Penalized IVW	1.62 (1.15, 2.27)	0.005	1.51 (0.92, 2.48)	0.11	1.59 (0.86, 2.92)	0.14	3.00 (1.27, 7.06)	0.01
Robust IVW	1.63 (1.26, 2.11)	<0.001	1.51 (0.90, 2.53)	0.12	1.54 (0.93, 2.56)	0.09	3.30 (1.52, 7.15)	0.003
MR-Egger	3.25 (1.16, 9.11)	0.03	6.06 (1.45, 25.27)	0.01	1.75 (0.22, 13.84)	0.59	3.29 (0.24, 45.11)	0.37
Penalized MR-Egger	3.25 (1.16, 9.11)	0.03	6.06 (1.45, 25.27)	0.01	1.75 (0.22, 13.84)	0.59	3.29 (0.24, 45.11)	0.37
Robust MR-Egger	3.23 (1.80, 5.78)	<0.001	5.88 (2.74, 12.61)	<0.001	1.70 (0.48, 6.08)	0.41	3.56 (1.25, 10.14)	0.02

OR, odds ratio of platelet count (PLT) on lung cancer risk per 100×10^9/L increment of PLT; NSCLC, non-small cell lung cancer; AC, adenocarcinoma; SqCC, squamous cell carcinoma; SCLC, small-cell carcinoma; IVW, inverse-variance weighted.

Figure Legends.

Figure 1. Diagram of Mendelian randomization (MR) analysis. MR aims to estimate the unbiased causal relationship between PLT and lung cancer risk by incorporating genetic variants as instrumental variables (IVs). Dashed line represents the association between instrumental variable (SNP) and outcome (risk of lung cancer), denoted using b_{YG} in log(odds ratio) scale and its standard error (SE_{YG}), which were obtained from GWAS. Estimates of quantitative trait loci relationship between SNP and phenotype (platelet count) were obtained from a recently published article, which were described by b_{XG} and SE_{XG}. Lung cancer risk was assessed for non-small cell lung cancer (NSCLC), adenocarcinoma (AC), squamous cell carcinoma (SqCC), and small cell carcinoma (SCLC).

Figure 2. Causal associations between platelet count and lung cancer risk. Forest plots of causal associations between platelet count (PLT) and risk of lung cancer using Mendelian randomization (MR) analysis incorporating different genetic variants as instrumental variables (IVs). Associations of PLT with risk of (A) non-small cell lung cancer (NSCLC), (B) adenocarcinoma (AC), (C) squamous cell carcinoma (SqCC), and (D) small-cell lung cancer (SCLC) were analyzed based on single IV or multiple IVs using inverse-variance weighted (IVW) analysis.

Figure 3. Assocations between SNPs and lung cancer risk. Scatter plots displaying estimates of the association between each SNP and risk of lung cancer against quantitative relationship of each SNP on platelet count (PLT) for (A) non-small cell lung cancer (NSCLC), (B) adenocarcinoma (AC), (C) squamous cell carcinoma (SqCC), and (D) small cell lung cancer (SCLC). Slope of the blue dashed line through the plot represents inverse-variance weighted (IVW) regression estimate for the causal effect of PLT on lung cancer risk.