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

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**Key Points**

**Question:** Platelets have a close connection with inflammation and , recently shown, with the lung, but is platelet count (PLT) a causal risk factor for lethal lung cancer?

**Findings:** Mendelian randomization analysis identified a causal relationship between elevated platelet count and increased risk of lung cancer, which showed a 62% and 200% increased risk of overall non-small cell lung cancer and small cell lung cancer, respectively, per 100×103/dL increment of platelet count.

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

**Abstract**

**Importance:** 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.

**Objective:** To evaluate the causal relationship of platelet count with lung cancer risk

**Design:** Using two-sample Mendelian Randomization analysis of a large consortium pooled genome-wide association studies, we performed single and combined multiple instrumental variable analysis using an inverse-weighted method, in addition to a series of sensitivity analyses.

**Setting:** Summary statistics from a recent publication and International Lung Cancer Consortium and Transdisciplinary Research in Cancer of the Lung data

**Participants:** We used 48,666 Caucasian Europeans to analyze associations between single nucleotide polymorphisms and platelet count. We used 29,266 cases and 56,450 controls to analyze associations between candidate single nucleotide polymorphisms (SNPs) and lung cancer risk.

**Exposure:** Platelet count

**Main Outcome:** Risk of non-small cell lung cancer, adenocarcinoma, squamous cell carcinoma, and small cell lung cancer, demonstrated by odds ratio per 100×103/dL increment of platelet count

**Results:** In single instrumental variable analysis, elevated platelet count was significantly associated with increased overall risk of non-small cell lung cancer (NSCLC) (ORrs6065, 2.92; 95%CI, 1.10–7.75; *P* = 0.03, ORrs210134, 1.76; 95%CI, 1.06–2.92; *P* = 0.03), a significant association with adenocarcinoma (OR210134, 2.10; 95%CI, 1.04–4.24; *P* = 0.04), and a borderline association with small cell lung cancer (ORrs210134, 3.53, 95%CI, 0.96–12.91, *P* = 0.06). In addition, rs17030845 showed a statistically significant association with risk of squamous cell carcinoma (OR, 8.03; 95%CI, 1.41–45.70; *P* = 0.02). Multiple instrumental variable analysis incorporating six SNPs further showed a 62% and 200% increased risk of overall NSCLC (OR, 1.62; 95%CI, 1.15–2.27; *P* = 0.005) and 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.

**Conclusion and Relevance:** 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.

**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](#_ENREF_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](#_ENREF_2) In addition, environmental–occupational exposures,[3](#_ENREF_3),[4](#_ENREF_4) lifestyle, and genetic variants[5](#_ENREF_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](#_ENREF_6) Platelets are an important element in coagulation and inflammation, and diverse mechanisms link activated platelets to cancer progression.[7](#_ENREF_7),[8](#_ENREF_8) Indeed, high platelet count (PLT) is associated with increased mortality in a variety of cancers, including malignant mesothelioma,[9](#_ENREF_9) gynecological malignancies,[10](#_ENREF_10) and breast cancer.[11](#_ENREF_11) In addition, platelet-to-lymphocyte ratio and mean platelet volume also add value in early diagnosis of lung cancer[12](#_ENREF_12) and prognosis prediction.[13](#_ENREF_13),[14](#_ENREF_14) 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[15](#_ENREF_15) 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.[16](#_ENREF_16) 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,[17](#_ENREF_17) such as the effect of higher adult height on escalated cancer risk.[18-22](#_ENREF_18)

In this study, we performed Mendelian randomization analysis 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).

**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.[23](#_ENREF_23) 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.[24](#_ENREF_24)

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.

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-ILCCO (Transdisciplinary Research in Cancer of Lung team of the International Lung Cancer Consortium) GWAS studies, which were detailed previously.[25](#_ENREF_25) Briefly, overall non-small cell lung cancer (NSCLC) samples were composed from Oncoarray and TRICL-ILCCO 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 (SCC) cases (Table S2).

**Mendelian Randomization (MR) analysis**

Mendelian randomization analysis with a single IV (one SNP at a time) was performed first. 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:[26](#_ENREF_26)

 (1)

Second, Mendelian Randomization analysis with multiple instrumental variables was also 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:[23](#_ENREF_23)

 (2)

In which *N* = 6 represents the number of instrumental variables included, and *bYX\_IVW*and *SEYX\_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 100×103/dL 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.[27](#_ENREF_27) 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%.

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

**Results**

Among 48,666 Europeans, 54 SNPs were quantitatively associated with platelet count with *P* ≤ 5×10-8 (Table S1).[24](#_ENREF_24) 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[25](#_ENREF_25) 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 were selected for MR analysis (Table 1), and 48 SNPs were excluded (Table S4).

For Mendelian Randomization analysis of overall NSCLC risk, single instrumental variable results incorporating missense SNP rs6065 in *GP1BA* or SNP rs210134 500B downstream of *BAK1* showed a statistically significant association of PLT on NSCLC risk (ORrs6065 per 100×103 increment of PLT, 2.92; 95%CI, 1.10–7.75; *P* = 0.03; ORrs210134, 1.76; 95%CI, 1.06–2.92; *P* = 0.03). Further, multiple IV analysis combining all six relatively independent SNPs situated in different chromosomes retained a statistically significant association, showing that each 100×103/dL 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, Mendelian Randomization analysis incorporating SNP rs210134 showed that platelet count had a statistically significant effect on risk of lung adenocarcinoma (AC) (OR, 2.10; 95%CI, 1.04–4.24; *P* = 0.04). Single instrumental variable analysis with SNP rs6065 showed a borderline association between platelet count and risk of lung adenocarcinoma (OR, 3.68; 95%CI, 0.96–14.15; *P* = 0.06), while multiple instrumental variable analysis with the inverse-variance weighted method showed weak evidence of this association (OR, 1.51; 95%CI, 0.92–2.48; *P* = 0.11) (Figure 2B and Figure 3B). However, MR-Egger sensitivity analysis revealed a significant association (OR, 6.06; 95%CI, 1.45–25.27; *P* = 0.01), consistent with results from robust or penalized MR-Egger methods.

For squamous cells carcinomas (SqCC), single instrumental variable analysis of SNP rs17030845 located in *THADA* showed an association between platelet count and risk of SqCC (OR, 8.03; 95%CI, 1.41–45.70; *P* = 0.02). However, multiple instrumental variable analysis yielded non-significant results, probably due to insufficient sample size (Figure 2C and Figure 3C).

Interestingly, single instrumental variable analysis of SNP rs210134 showed a borderline effect of platelet count on risk of small cell lung cancer (SCC) (ORrs210134, 3.53; 95%CI, 0.96–12.91; *P* = 0.06). Multiple instrumental variable analysis combining all six SNPs further confirmed this association (OR, 3.00; 95%CI, 1.27–7.06; *P* = 0.01) (Figure 2D and Figure 3D).

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 S5 and Figure S1).

**Discussion**

This Mendelian Randomization study suggests that each 100×103/dL 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 SqCC, although these results may be due to insufficient sample size.

Platelets have been studied for decades as an important regulator of inflammation and thrombosis[28](#_ENREF_28), which are broadly interrelated with human carcinogenesis.[11](#_ENREF_11) Platelets are also recognized as a stimulator of proangiogenic factors[11](#_ENREF_11) and a major source of vascular endothelial growth factor (VEGF),[29](#_ENREF_29) platelet-derived growth factor (PDGF),[30](#_ENREF_30),[31](#_ENREF_31) and basic fibroblast growth factor (bFGF),[31](#_ENREF_31) which act as promoters of tumor growth in lung.[32-38](#_ENREF_32) New evidence suggests that platelets are relevant to defensive, physiological immune responses of the lungs and to inflammatory lung diseases.[39](#_ENREF_39) 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.[40](#_ENREF_40) 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.[41](#_ENREF_41) 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.[42](#_ENREF_42) 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[43](#_ENREF_43),[44](#_ENREF_44) and clopidogrel, as a therapeutic strategy against cancer.[45](#_ENREF_45),[46](#_ENREF_46) 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.[47](#_ENREF_47) 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.[48](#_ENREF_48) 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.

**Author Contributions**

Drafting of the manuscript: Y.W., Ying Z., R.Z., F.C. Project coordination: C.I.A., R.J.H., D.C.C. Statistical analysis: Y.W., Y.Z., R.Z., X.D., S.S., J.B., Yang Z. Assessment of the association between candidate variants and risk of lung cancer: Y.H., C.I.A. Sample collection, development of epidemiological studies and data management for each site: D.A., N.C., M.T.L., B.Z., S.C., F.G., S.L., M.-S.T., F.A.S., A.T., A.F.-S., G.F.-T., C.C., M.B., J.D., S.E.B., M.J., P.B., J.D.M., R.C.-T., A.R., T.M., H-E.W., H.B., Albert R., G.R., S.A., J.K.F., M.D., M.W.M., X.W., Y.Y., L.L.M., L.W., O.M., J.M., Hans B., G.L., Y.B., L.K., A.A., E.D., L.A.K., H.F.M.H., A.H., V.S., S.Z., K.G., Mikael J., P.W., A.C., F.T., M.D.T., P.L., M.B.S., M.C.A., R.H., John M., Victoria S., T.R., T.E.T., G.W.R., K.S., H.S., Z.H., Jun D., C.I.A., Y.H., D.Z., G.G., R.J.H., D.C.C. Study supervision: F.C., D.C.C. Manuscript revision and approval of the final manuscript: all authors.

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**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. 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 (SCC).

**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 (SCC) were analyzed based on single IVs 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.

**Table 1. SNPs of specific platelet-related genes**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SNP | Chr:Position(hg19) | Gene | Referenceallele | Effectallele | 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 | 500B 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 Mendelian Randomization analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| SNP | Overall NSCLC | AC | SqCC | SCC |
| OR (95%CI) | *P* | OR (95%CI) |  *P* | OR (95%CI) | *P* | OR (95%CI) | *P* |
| **Single IV analysis** |  |
| rs17030845 | 1.81 (0.62, 5.28) | 0.28 | 1.13 (0.26, 4.91) | 0.87 | 8.03 (1.41, 45.70) | 0.02 | 0.41 (0.03, 5.69) | 0.50 |
| rs6141 | 1.84 (0.67, 5.04) | 0.23 | 1.05 (0.26, 4.29) | 0.95 | 3.63 (0.72, 18.27) | 0.12 | 1.92 (0.14, 25.65) | 0.62 |
| rs3792366 | 0.93 (0.31, 2.76) | 0.90 | 0.77 (0.17, 3.44) | 0.73 | 0.52 (0.09, 2.98) | 0.47 | 3.19 (0.21, 49.22) | 0.41 |
| rs210134 | 1.76 (1.06, 2.92) | 0.03 | 2.10 (1.04, 4.24) | 0.04 | 1.27 (0.57, 2.86) | 0.56 | 3.53 (0.96, 12.91) | 0.06 |
| rs708382 | 0.86 (0.33, 2.23) | 0.76 | 0.59 (0.16, 2.20) | 0.43 | 1.27 (0.28, 5.88) | 0.76 | 5.96 (0.53, 67.42) | 0.15 |
| rs6065 | 2.92 (1.10, 7.75) | 0.03 | 3.68 (0.96, 14.15) | 0.06 | 1.38 (0.29, 6.59) | 0.69 | 6.39 (0.58, 70.94) | 0.13 |
| **Multiple IV analysis** |  |  |  |  |  |  |  |  |
| 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 100×103/L increment of PLT; NSCLC, non-small cell lung cancer; AC, adenocarcinoma; SqCC, squamous cell carcinoma; SCC, small-cell carcinoma; IV, instrumental variable; IVW, inverse-variance weighted; MR, Mendelian randomization