Obesity, metabolic factors and risk of different histological types of lung cancer: A Mendelian randomization study

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation

Published Version
doi:10.1371/journal.pone.0177875

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:33490976

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
RESEARCH ARTICLE

Obesity, metabolic factors and risk of different histological types of lung cancer: A Mendelian randomization study

Robert Carreras-Torres¹, Mattias Johansson¹, Philip C. Haycock², Kaitlin H. Wade², Caroline L. Relton², Richard M. Martin²,³, George Davey Smith², Demetrius Albanes⁴, Melinda C. Aldrich⁵, Angeline Andrew⁶, Susanne M. Arnold⁷, Heike Bickeböller⁸, Stig E. Bojesen⁹, Hans Brunström¹⁰, Jonas Manjer¹⁰, Irene Brüské¹¹, Neil E. Caporaso¹², Chu Chen¹³, David C. Christiani¹⁴, W. Jay Christian¹⁵, Jennifer A. Doherty¹⁶, Eric J. Duell¹⁷, John K. Field¹⁸, Michael P. A. Davies¹⁸, Michael W. Marcus¹⁸, Gary E. Goodman¹⁹, Kjell Grankvist²⁰, Aage Haugen²¹, Yun-Chul Hong²², Lambertus A. Kiemeney²³, Erik H. F. M. van der Heijden²³, Peter Kraft²⁴, Mikael B. Johansson²⁰, Stephen Lam²⁵, Maria Teresa Landi¹², Philip Lazarus²⁶, Loïc Le Marchand²⁷, Geoffrey Liu²⁸, Olle Melander²,³,²⁹, Sungshim L. Park³¹, Gad Rennert³², Angela Risch³³, Eric B. Haura³⁴, Ghislaine Scelo³¹, David Zaridze³⁶, Anush Mukeria³⁵, Milan Savić³⁶, Jolanta Lissowska³⁷, Beata Sliwińska³⁷, Vladimir Janout³⁸, Ivana Holcátova³⁹, Dana Mates³⁸, Matthew B. Schabath⁴, Hongbing Shen⁴², Adonina Tardon⁴³, M Dawn Teare⁴,³³, Penella Woll⁴⁴, Ming-Sound Tsao⁴⁵, Xifeng Wu⁴⁶, Jian-Min Yuan⁴⁶, Rayjean J. Hung⁴⁶, Christopher I. Amos⁴⁶,⁵⁰, James McKay⁴⁷, Paul Brennan⁴⁷

1 Section of Genetics, International Agency for Research on Cancer (IARC), Lyon, France, 2 MRC Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom, 3 National Institute for Health Research Biomedical Research Unit in Nutrition, Diet and Lifestyle at University Hospitals Bristol NHS Foundation Trust and the University of Bristol, Bristol, United Kingdom, 4 Metabolic Epidemiology Branch, DCEG, National Cancer Institute, NIH, Rockville, Maryland, United States of America, 5 Department of Thoracic Surgery, Division of Epidemiology, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 6 Norris Cotton Cancer Center, Lebanon, New Hampshire, United States of America, 7 University of Kentucky Markey Cancer Center, Lexington, Kentucky, United States of America, 8 University Medical Center Göteborg, Göteborg, Sweden, 9 Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 10 Laboratory Medicine Region Skåne, Department of Clinical Sciences Lund, Lund University, Lund, Sweden, 11 Helmholtz Zentrum München, Munich, Germany, 12 Genetic Epidemiology Branch, DCEG, National Cancer Institute, NIH, Rockville, Maryland, United States of America, 13 Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America, 14 Departments of Environmental Health and Epidemiology, Harvard T. H. Chan School of Public Health and Department of Medicine, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts, United States of America, 15 Department of Epidemiology, College of Public Health, University of Kentucky, Lexington, Kentucky, United States of America, 16 Department of Epidemiology, Geisel School of Medicine, Dartmouth College, Lebanon, New Hampshire, United States of America, 17 Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalán Institute of Oncology (ICO-IDIBELL), Barcelona, Spain, 18 Roy Castle Lung Cancer Research Programme, Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine, The University of Liverpool, Liverpool, United Kingdom, 19 Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America, 20 Department of Medical Biosciences, Umeå University, Umeå, Sweden, 21 National Institute of Occupational Health, Oslo, Norway, 22 Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea, 23 Radboud University Medical Center, Nijmegen, The Netherlands, 24 Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, United States of America, 25 British Columbia Cancer Agency, Vancouver, British Columbia, Canada, 26 Washington State University College of Pharmacy, Spokane, Washington, United States of America, 27 Epidemiology Program, University of Hawaii Cancer Center, Honolulu, Hawaii, United States of America, 28 Ontario Cancer Institute, Princess Margaret Cancer Center, Toronto, Ontario, Canada, 29 Department of Clinical Sciences Malmö, Lund University, Lund, Sweden, 30 Department of Internal Medicine, Skåne University Hospital, Malmö, Sweden, 31 Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, United States of America, 32 Clallit National Cancer Control Centre and Department of Community Medicine and Epidemiology, Carmel Medical Center and B. Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, and 33 综合性医疗中心，以色列特拉维夫大学，以色列

OPEN ACCESS


Editor: Cheng Hu, Shanghai Diabetes Institute, China

Received: April 20, 2017
Accepted: May 4, 2017
Published: June 8, 2017

Copyright: This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Data Availability Statement: All relevant data are within the paper and its Supporting information files.

Funding: RCT, MJ, PCH, KHW, CR, RMM, GDS, and PB are investigators or researchers on a Cancer Research UK (C18281/A19169) Programme Grant (the Integrative Cancer Epidemiology Programme). RMM is supported by the National Institute for Health Research (NIHR) Bristol Nutritional Biomedical Research Unit based
Causality appraisal of metabolic factors on risk of lung cancer subtypes

The authors have declared that no competing interests exist.

Abstract

Background

Assessing the relationship between lung cancer and metabolic conditions is challenging because of the confounding effect of tobacco. Mendelian randomization (MR), or the use of genetic instrumental variables to assess causality, may help to identify the metabolic drivers of lung cancer.

Methods and findings

We identified genetic instruments for potential metabolic risk factors and evaluated these in relation to risk using 29,266 lung cancer cases (including 11,273 adenocarcinomas, 7,426 squamous cell and 2,664 small cell cases) and 56,450 controls. The MR risk analysis suggested a causal effect of body mass index (BMI) on lung cancer risk for two of the three major histological subtypes, with evidence of a risk increase for squamous cell carcinoma (odds ratio (OR) [95% confidence interval (CI)] = 1.20 [1.01–1.43] and for small cell lung cancer (OR [95%CI] = 0.93 [0.79–1.08]) (P heterogeneity = 0.34). Additional analysis using a genetic instrument for BMI showed that each SD increase in BMI increased cigarette consumption by 1.27 cigarettes per day (P = 2.1x10^-3), but not for adenocarcinoma (OR [95%CI] = 0.93 [0.79–1.08]) (P heterogeneity = 0.34). There was also evidence that low-density lipoprotein cholesterol was inversely associated with lung cancer overall risk (OR [95%CI] = 0.90 [0.84–0.97] per SD of 38 mg/dl), while fasting insulin was positively associated (OR [95%CI] = 1.63 [1.25–2.13] per SD of 44.4 pmol/l). Sensitivity analyses including a weighted-median approach and MR-Egger test did not detect other pleiotropic effects biasing the main results.
Conclusions

Our results are consistent with a causal role of fasting insulin and low-density lipoprotein cholesterol in lung cancer etiology, as well as for BMI in squamous cell and small cell carcinoma. The latter relation may be mediated by a previously unrecognized effect of obesity on smoking behavior.

Introduction

Lung cancer is the leading cause of cancer mortality [1]. Most lung cancers are caused by tobacco smoking [2], although associations have also been reported with a range of inflammatory and metabolic conditions. Observational studies have indicated an inverse relationship for both body mass index (BMI) [3–7] and lipid levels [8,9], as well as a positive correlation with dietary glycemic index [10] and insulin levels [11], with lung cancer risk. However, given the strong effect of tobacco smoking on lung cancer risk, and the well described association between tobacco consumption and alterations in body weight [12–14], traditional observational studies are unlikely to fully account for the confounding effect of tobacco exposure when describing the relationship between lung cancer and obesity or metabolic conditions.

Mendelian randomization (MR) is an analytical approach based on genetic markers of an exposure (i.e. an instrumental variable) that is less sensitive to reverse causation and confounding than traditional regression analyses in observational studies [15]. Inherited gene variants associated with the risk factors of interest should act as unconfounded markers of those risk factors, assuming an absence of pleiotropy [16]. In this instance, an association between the genetic variant and the outcome implies that the risk factor of interest may have a causal effect on the outcome [17]. Previous MR analyses on lung cancer risk showed that a genetic score for increased BMI raised the risk for lung cancer, especially for squamous cell carcinoma and small cell lung cancer [18,19]. These results are in contradiction to observational findings, confirming the utility of MR analyses for this exposure.

The goal of the current study was to use genetic variations associated with a range of metabolic factors, including obesity, body shape, dyslipidemia and hyperglycemia, to further investigate the causal relationship between these metabolic exposures and lung cancer. The method applied in this study is called two-sample MR, which combines summary statistics on genetic variant-exposure and genetic variant-outcome associations from different samples [20,21]. Furthermore, we sought to confirm that the genetic control of these metabolic phenotypes did not influence cigarette smoking behavior.

Methods

Genetic instruments for obesity and metabolic parameters

Genetic instruments for potential risk factors were independent (linkage disequilibrium (LD) $R^2 < 0.01$ in European 1000 Genomes Phase3 samples [22]) single nucleotide polymorphisms (SNPs) that were associated with the trait of interest in the most recent genome-wide association study (GWAS) ($P < 5 \times 10^{-8}$) on European ancestry samples. Results from the Genetic Investigation of ANthropometric Traits (GIANT) consortium were used to identify genetic proxies for body mass index (BMI) [23], and waist to hip ratio [24]. High-density and low-density lipoprotein cholesterol (HDL and LDL), total cholesterol and triglycerides were selected as lipid profile components. Genetic loci influencing bloodstream levels of these lipids were
identified from GWA data provided by the Global Lipids Genetic Consortium (GLGC) [25]. Additionally, genetic instruments of low frequency were also identified for HDL, LDL cholesterol and triglycerides [26]. Finally, hyperglycemia and hyperinsulinemia parameters were reported within the Meta-Analysis of Glucose and Insulin related traits Consortium (MAGIC) at fasting levels and during an oral glucose tolerance test (OGTT). Genetic instruments were identified for plasma levels of fasting glucose, fasting insulin and 2-hour post-challenge glucose [27]. For these metabolic parameters, in order to reduce potential pleiotropic effect from BMI, SNPs that were not associated with the phenotype once adjusted for BMI (P > 0.05) were excluded from our analyses. For each identified SNP, the reported effect was for the increasing trait allele expressed in standard deviations (SD) of the trait per-allele (βGP) along with the standard error (SEGP). For the studies in which the genetic effects were not originally reported in SDs of the trait, they were recalibrated according to the mean SD, weighted for sample size, reported across different case-control samples. SNPs with ambiguous strand codification (A/T or C/G) were replaced by SNPs in tight genetic linkage (R² > 0.8) using the proxysnps R package (European populations) (R Project) or removed from the analyses. Details on i) the number of SNPs identified for each potential risk trait, ii) the mean and SD of the traits in the discovery GWAS, and iii) the proportion of trait variance explained by the genetic proxies are presented in Table 1.

### Lung cancer association results

GWAS results on overall lung cancer risk and by histology type were obtained from the recently completed meta-analyses [28] comprising the previous TRICL-ILCCO lung cancer GWAS (11,177 lung cancer cases and 40,396 controls) [29] and an additional 18,089 lung cancer samples and 16,054 controls genotyped using the Illumina Infinium OncoArray-500K BeadChip (Illumina Inc. San Diego, CA). The overall sample size was 29,266 lung cancer cases and 56,450 controls. GWAS analyses were stratified by histology, including 11,273 adenocarcinomas, 7,426 squamous cell carcinomas and 2,664 small cell lung cancers. Additionally, analyses were performed stratified by smoking status defined as ever smokers (current and former smokers; 23,223 cases and 16,964 controls) and never smokers (2,355 cases and 7,504 controls). SNPs with imputation quality score (R²) less than 0.7 were removed from the datasets. For all SNPs used as instruments in this report, SNP to phenotype effect (βGP) and SNP to disease effect (βGD) for each lung cancer subgroup can be observed in S1 Table.
Power assessment

Power calculations on MR analyses were performed according to Burgess [30], given the number of cases and controls of the lung cancer subgroups, for five genetic instruments accounting for different proportion of explained variance (1%, 2.5%, 5%, 10% and 15%) within the range of our genetic instrument sets (see Table 1). Power calculations are presented in S1 Fig.

Mendelian randomization analyses on lung cancer

The causal risk effects of metabolic factors on lung cancer overall, as well as by histology and other subgroups were estimated using a likelihood-based approach, which is considered the most accurate method to estimate causal effects when there is a continuous log-linear association between risk factor and disease risk [20]. The resulting odds ratio (OR) and 95% confidence interval (CI) provide an estimate of relative risk caused by each SD increase in the exposure. We investigated the heterogeneity of causal effects by histology and by smoking status, estimating the $I^2$ statistic assuming a fixed-effect model using the meta R package (R project). We also provide OR estimates using the complementary weighted median method wherein the causal effect estimate is weighted towards the median of the distribution of instrumental SNPs [31]. This approach is less sensitive to individual instrumental SNPs behaving as outliers. Furthermore, because OR estimates can be confounded if the instrumental genetic variants are also associated with other causal risk factors, i.e. through directional pleiotropy, we also performed MR-Egger [32]. This approach involves a weighted linear regression of the SNP to disease effects ($\beta_{GD}$) on the SNP to phenotype effects ($\beta_{GP}$), and provides a direct estimate of any directional pleiotropy under the assumption that pleiotropic biases affecting the overall MR risk estimates are acting in the same direction [33]. Additionally, MR-Egger regression is able to identify SNPs behaving as genetic outliers, providing Bonferroni corrected P-values. We also provide a funnel plot of the fold risk increase ($\exp(\beta_{GD}/\beta_{GP})$) vs. the instrumental strength of each SNP ($\beta_{GP}/SE_{GD}^2$) to allow visual assessment of asymmetry of instrumental causal estimates. These plots were generated using the ggplot2 R package (R Project).

Assessment of association between the genetic instruments and other phenotypes

To evaluate whether MR lung cancer risk estimates could be generated by other phenotypes than the instrumented ones, the association of genetic instruments with other phenotypes was assessed. The effects of our genetic instruments on cigarette smoking was evaluated using a novel online platform for Mendelian randomization based on summary genetic association data from GWAS (MR-Base; http://www.mrbase.org/) [34]. Genetic effects on cigarette smoking (cigarettes per day) were obtained from the Tobacco and Genetics (TAG) consortium [35]. TAG data were generated from 16 independent studies which included 38,181 smokers with information on number of smoked cigarettes per day. We also assessed the association of dyslipidemia and hyperglycemia genetic instruments with BMI using data from the GIANT consortium [23].

Results

Obesity and body shape parameters

Based on 72 SNPs used as genetic instruments for BMI, we estimated an overall OR (per SD increase in BMI (4.6 kg/m$^2$)) of 1.07 (95%CI = 0.96–1.20) for lung cancer risk (Fig 1). However, stratified analyses by histology subtypes revealed heterogeneity in the causal effect estimates ($P_{heterogeneity} = 4.3x10^{-3}$), with no effect on adenocarcinoma (OR [95%CI] = 0.93 [0.79–
but an increased risk for squamous cell carcinoma (OR [95%CI] = 1.20 [1.01–1.43]) and small cell lung cancer (OR [95%CI] = 1.52 [1.15–2.00]) (Fig 2). Analyses stratified by smoking status showed a null effect of BMI in never smokers (OR [95%CI] = 0.83 [0.60–1.16]) and in ever smokers (OR [95%CI] = 1.12 [0.97–1.29]) (P heterogeneity = 0.10) (Fig 2). Weighted median MR approach provided similar risk estimates for the different lung cancer types (S2 Table). The MR-Egger intercept test revealed the presence of bias on the initial risk estimation on lung cancer overall and in ever-smokers (S3 Table), with MR-Egger risk estimates indicating no effect of BMI on lung cancer overall. Potential asymmetry of the SNP risk estimates reflected the presence of directional pleiotropy for lung cancer overall (Fig 3A). Funnel plots for the other histological types can be observed in the funnel plots included in Fig 3B–3F. Regarding waist-to-hip ratio, there was no evidence of causal effect on lung cancer overall (OR [95%CI] = 1.04 [0.87–1.25]) (Fig 1) or histology subgroups (S2 Fig). Sensitivity analyses did not detect any effect biasing the initial risk estimates (S2 and S3 Tables). Funnel plots can be observed in S3 Fig.

**Blood lipid levels**

There was no consistent evidence of a causal effect of HDL on lung cancer overall (OR [95%CI] = 1.01 [0.94–1.07] for instrumental common SNPs, and OR [95%CI] = 1.07 [0.95–1.21] for instrumental rare SNPs) (Fig 1), nor any lung cancer subgroup (S4–S7 Figs).
Triglyceride levels did not indicate any effect on lung cancer overall; (OR [95%CI] = 0.98 [0.91–1.06] for instrumental common SNPs and OR [95%CI] = 0.87 [0.70–1.08] for instrumental rare SNPs) (Fig 1), or for lung cancer subtypes (S8–S11 Figs). An inverse relationship was observed with overall lung cancer risk for each SD increase in LDL (approximately 38.0 mg/dl) (OR [95%CI] = 0.90 [0.84–0.97] for instrumental common SNPs (Fig 1), although stratified analyses did not provided a consistent inverse association among histology types (OR of 0.93 for adenocarcinoma (95%CI = 0.85–1.03), 0.88 for squamous cell carcinoma (95%CI = 0.79–0.98), and 0.96 for small cell lung cancer (95%CI = 0.81–1.14) (Fig 4 and S12 Fig for funnel plots). Additionally, LDL instrumental rare SNPs did not provide evidence of association with lung cancer overall (OR [95%CI] = 1.09 [0.93–1.27]) (Fig 1) or subtypes (S13 and S14 Figs for funnel plots). Finally, total cholesterol showed a similar pattern of association with lung cancer overall and with subtypes that LDL common SNPs (OR of 0.94 for lung cancer overall (95%CI = 0.88–1.01), OR of 1.01 for adenocarcinoma (95%CI = 0.92–1.10), 0.89 for squamous cell carcinoma (95%CI = 0.80–1.00), and 0.93 for small cell lung cancer (95%CI = 0.79–1.10)) (S15 and S16 Figs for funnel plots).

**Insulin resistance parameters**

Among glucose and insulin parameters, fasting insulin was associated with an increased risk in overall lung cancer (OR [95%CI] = 1.63 [1.25–2.13] per each SD increase [44.4 pmol/l]) (Fig 1). Stratified analyses provided consistent associations with all histology subtypes (Fig 5), with a risk increase of 1.60 for adenocarcinoma (95%CI = 1.12–2.31), 1.84 for squamous cell carcinoma (95%CI = 1.20–2.81), and 2.46 for small cell lung cancer (95%CI = 1.26–4.81). Stratified analyses by smoking status showed some heterogeneity in causal effects ($P_{\text{heterogeneity}} = 0.16$) (Fig 5), with a clear increased risk among ever smokers (OR [95%CI] = 1.86 [1.33–2.60]), but no association among never smokers (OR [95%CI] = 1.01 [0.46–2.20]). Sensitivity tests identified rs4865796 as an outlier SNP in the analyses for lung adenocarcinoma subtype and lung cancer overall in ever smokers. Removing this SNP, the analyses indicated slightly higher levels of risk associated with this genetic instrument (Funnel plots in S17 Fig).
Fig 3. Funnel plots for the distribution of risk estimates of BMI instrumental SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value.

https://doi.org/10.1371/journal.pone.0177875.g003
Fasting glucose showed little evidence for an association with overall lung cancer risk (OR per SD of 0.8 mmol/l = 1.10; 95%CI = 0.96–1.26) (Fig 1), nor for adenocarcinoma (OR [95% CI] = 1.03 [0.85–1.24] or small cell lung cancer (OR [95%CI] = 1.19 [0.83–1.68]), where a positive association with risk was seen for squamous cell carcinoma (OR [95%CI] = 1.32 [1.06–1.65]) (S18 and S19 Figs for funnel plots). Finally, 2-hour post-challenge glucose showed no evidence of causality for lung cancer overall (OR [95%CI] = 0.96 [0.77–1.20]) (Fig 1) or any lung cancer subgroup (S20 and S21 Figs for funnel plots).

<table>
<thead>
<tr>
<th>Lung Cancer</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>29,266</td>
<td>56,450</td>
<td>0.90</td>
<td>0.84 - 0.97</td>
<td>3.8x10^{-3}</td>
</tr>
<tr>
<td><strong>Histology type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>11,273</td>
<td>55,483</td>
<td>0.93</td>
<td>0.85 - 1.03</td>
<td>0.15</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>7,426</td>
<td>55,627</td>
<td>0.88</td>
<td>0.79 - 0.98</td>
<td>0.02</td>
</tr>
<tr>
<td>Small cell</td>
<td>2,664</td>
<td>21,444</td>
<td>0.96</td>
<td>0.81 - 1.14</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Smoking group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers</td>
<td>2,355</td>
<td>7,504</td>
<td>0.88</td>
<td>0.72 - 1.08</td>
<td>0.24</td>
</tr>
<tr>
<td>Ever smokers</td>
<td>23,223</td>
<td>16,964</td>
<td>0.92</td>
<td>0.84 - 1.00</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Fig 4. Forest plot of lung cancer risk for each SD increase in LDL (approximately 38.0 mg/dl) observed in the likelihood-based MR approach using the instrument set of common SNPs. 95%CI: 95% Confidence Interval; P: P value. I^2: between-strata heterogeneity. PHet: P value of between-strata heterogeneity.

https://doi.org/10.1371/journal.pone.0177875.g004

Fasting glucose showed little evidence for an association with overall lung cancer risk (OR per SD of 0.8 mmol/l = 1.10; 95%CI = 0.96–1.26) (Fig 1), nor for adenocarcinoma (OR [95% CI] = 1.03 [0.85–1.24] or small cell lung cancer (OR [95%CI] = 1.19 [0.83–1.68]), where a positive association with risk was seen for squamous cell carcinoma (OR [95%CI] = 1.32 [1.06–1.65]) (S18 and S19 Figs for funnel plots). Finally, 2-hour post-challenge glucose showed no evidence of causality for lung cancer overall (OR [95%CI] = 0.96 [0.77–1.20]) (Fig 1) or any lung cancer subgroup (S20 and S21 Figs for funnel plots).

<table>
<thead>
<tr>
<th>Lung Cancer</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>29,266</td>
<td>56,450</td>
<td>1.63</td>
<td>1.25 - 2.13</td>
<td>3.2x10^{-4}</td>
</tr>
<tr>
<td><strong>Histology type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>11,273</td>
<td>55,483</td>
<td>1.60</td>
<td>1.12 - 2.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Squamous cell</td>
<td>7,426</td>
<td>55,627</td>
<td>1.84</td>
<td>1.20 - 2.81</td>
<td>5.1x10^{-3}</td>
</tr>
<tr>
<td>Small cell</td>
<td>2,664</td>
<td>21,444</td>
<td>2.46</td>
<td>1.26 - 4.81</td>
<td>8.5x10^{-3}</td>
</tr>
<tr>
<td><strong>Smoking group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers</td>
<td>2,355</td>
<td>7,504</td>
<td>1.01</td>
<td>0.46 - 2.20</td>
<td>0.98</td>
</tr>
<tr>
<td>Ever smokers</td>
<td>23,223</td>
<td>16,964</td>
<td>1.86</td>
<td>1.33 - 2.60</td>
<td>3.2x10^{-4}</td>
</tr>
</tbody>
</table>

Fig 5. Forest plot of lung cancer risk for each SD increase in fasting insulin (approximately 44.4 pmol/L) observed in the likelihood-based MR approach. 95%CI: 95% Confidence Interval; P: P value. I^2: between-strata heterogeneity. PHet: P value of between-strata heterogeneity.

https://doi.org/10.1371/journal.pone.0177875.g005
Assessment of association between the genetic instruments and other phenotypes

Using MR-Base, we also evaluated the association between cigarette smoking for each genetic instrument associated with lung cancer risk, including BMI, LDL, and fasting insulin. Each SD increase in BMI increased cigarette consumption by 1.27 cigarettes (95%CI = 0.46:2.07; P = 2.1x10^{-3}; MR likelihood-based approach), while the other metabolic genetic instruments were not associated with cigarette smoking (MR likelihood-based estimate per SD increase [95%CI] = -0.35 [-0.92:0.22] for LDL and 1.21 [-0.67:3.08] for fasting insulin). Additionally, each SD increase in LDL was inversely associated with BMI (-0.02 SD in BMI (95%CI = -0.04:3.8x10^{-3}; P = 0.02), while each SD increase in fasting was not associated with BMI (0.07 SD in BMI (95%CI = -0.01:0.15; P = 0.10)).

Discussion

We have used existing data from large GWAS on metabolic factors to identify potentially causal risk factors of lung cancer that are amenable to intervention. Our results suggested that higher BMI is associated with an increase in risk of squamous and small cell carcinoma of the lung, and that this relation may be mediated by altered smoking behavior. Additionally, we found consistent evidence of a positive association between fasting insulin and overall lung cancer risk, as well as an inverse relationship between LDL levels and lung cancer risk.

Obesity and related metabolic factors are associated with risk of several cancers [36], but traditional observational studies are not reliable for assessing the association between metabolic factors and cancers strongly associated with smoking. The strength of the association between BMI and lung cancer subtypes found in this study, were weaker than those reported in previous MR analyses (45%-81% of risk increase) [18,19]. These previous studies were based on smaller sample sizes (12,160 and 16,572 lung cancer cases, respectively), whereas our current study provides causal estimates from 29,266 lung cancer cases and 56,450 controls, including the samples of previous studies. Our analysis also detected a strong positive effect of each unit increase in BMI on number of cigarettes smoked per day, indicating that the associations with risk of squamous and small cell carcinoma lung cancer are caused by an influence of BMI on smoking behavior. Given that this association was derived from genetic data on BMI, and therefore not influenced by any inverse effect that tobacco consumption may have on BMI levels, it has important implications beyond this study on the smoking behavior and smoking prevention.

Our results lend further support to epidemiological studies showing an increased lung cancer risk for the effect of reduced lipid levels [9] and elevated circulating insulin [11]. Several studies have reported an inverse relationship between total cholesterol levels and cancer risk [37], which has been previously interpreted as an effect of preclinical cancer due to an increased uptake of cholesterol by tumor cells [9]. In contrast, our study suggests a potential relationship between cholesterol levels and lung cancer. However, cholesterol genetic instruments using common and rare SNPs did not provide homogeneous results. This could be explained by different observations: by the inverse relationship between the instrument set of common SNPs for cholesterol and BMI revealed by a sensitivity analysis using MR-Base, by a multiple testing effect, but also by the different proportion of exposure that genetic instruments represent (instrument sets of rare SNPs were weaker and had less power to detect potential causal associations). Further MR mediation analyses are needed in order to clarify the causal relationship between lipid levels and lung cancer accounting for the potential effect of BMI and smoking. Finally, the risk increase observed by fasting glucose and fasting insulin is unlikely to be driven by cigarette smoking or BMI, since the relevant instrumental sets are not
associated with smoking or BMI. A potential causal relationship between increased insulin and cancer risk using genetic instruments has also been reported for endometrial cancer [38] and pancreatic cancer [39]. These results lend further support for an important role of the insulin-pathway in cancer aetiology [40–42].

One of the main caveats in Mendelian randomization studies is the potential violation of methodological assumptions, in particular the lack of directional pleiotropic effects biasing association estimates. In the current study we evaluated the extent to which pleiotropy may have affected our risk estimates using MR-Egger regression, as well as the MR-Base platform to directly assess the relation between genetic instruments and potential confounders.

In conclusion, using a two-sample Mendelian randomization approach, this study assessed a range of metabolic factors in relation to lung cancer risk. Our results revealed that genetic associations between BMI and lung cancer subtypes could be mediated by a positive relationship between BMI and cigarette consumption. Our results are also consistent with observational studies that indicate an inverse relationship between lipid levels and lung cancer risk, and an increasing risk caused by elevated fasting insulin levels.

Supporting information
S1 Fig. Power calculations for Mendelian randomization analyses on lung cancer using genetic instruments accounting for different proportion of phenotypic variance (15.0, 10.0, 5.0, 2.5, and 1.0%).
(PDF)

S2 Fig. Forest plot of lung cancer risk for each SD increase of waist-to-hip ratio observed in a likelihood-based MR approach. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity.
(PDF)

S3 Fig. Funnel plots for the distribution of risk estimates of waist-to-hip ratio-instrumental SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value.
(PDF)

S4 Fig. Forest plot of lung cancer risk for each SD increase in HDL observed in a likelihood-based MR approach using the instrument set of common SNPs. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity.
(PDF)

S5 Fig. Forest plot of lung cancer risk for each SD increase in HDL observed in a likelihood-based MR approach using the instrument set of rare SNPs. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity.
(PDF)

S6 Fig. Funnel plots for the distribution of risk estimates of HDL instrumental common SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value.
(PDF)

S7 Fig. Funnel plots for the distribution of risk estimates of HDL instrumental rare SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value.
(PDF)
S8 Fig. Forest plot of lung cancer risk for each SD increase in triglycerides observed in a likelihood-based MR approach using the main instrument set of common SNPs. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity. (PDF)

S9 Fig. Forest plot of lung cancer risk for each SD increase in triglycerides observed in a likelihood-based MR approach using the instrument set of rare SNPs. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity. (PDF)

S10 Fig. Funnel plots for the distribution of risk estimates of triglycerides instrumental common SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value. (PDF)

S11 Fig. Funnel plots for the distribution of risk estimates of triglycerides instrumental rare SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value. (PDF)

S12 Fig. Funnel plots for the distribution of risk estimates of LDL instrumental common SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value. (PDF)

S13 Fig. Forest plot of lung cancer risk for each SD increase in LDL observed in a likelihood-based MR approach using the instrument set of rare SNPs. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity. (PDF)

S14 Fig. Funnel plots for the distribution of risk estimates of LDL instrumental rare SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value. (PDF)

S15 Fig. Forest plot of lung cancer risk for each SD increase in total cholesterol observed in a likelihood-based MR approach. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity. (PDF)

S16 Fig. Funnel plots for the distribution of risk estimates of total cholesterol instrumental SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value. (PDF)

S17 Fig. Funnel plots for the distribution of risk estimates of fasting insulin instrumental SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value. (PDF)

S18 Fig. Forest plot of lung cancer risk for each SD increase in fasting glucose observed in a likelihood-based MR approach. 95%CI: 95% Confidence Interval; P: P value. I²: between-
strata heterogeneity. PHet: P value of between-strata heterogeneity.

S19 Fig. Funnel plots for the distribution of risk estimates of fasting glucose instrumental SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value.

S20 Fig. Forest plot of lung cancer risk for each SD increase in glucose 2h post-challenge observed in a likelihood-based MR approach. 95%CI: 95% Confidence Interval; P: P value. I²: between-strata heterogeneity. PHet: P value of between-strata heterogeneity.

S21 Fig. Funnel plots for the distribution of risk estimates of glucose 2h post-challenge instrumental SNPs along with MR causal effect lung cancer subtypes. OR: Odds ratio; Int: Intercept; P: P value.

S1 Table. Association parameters of instrumental SNPs for the corresponding metabolic factor and for different lung cancer groups. CHR: Chromosome. BP: Base pair. SE: Standard error. BMI: Body mass index. HDL: High-density lipoprotein, LDL: Low-density lipoprotein.

S2 Table. Risk increase on lung cancer phenotypes for each standard deviation increase in the phenotype provided by weighted median MR approach. HDL: High-density lipoprotein, LDL: Low-density lipoprotein. Chol: Cholesterol. OR: Odds ratio. LCI: Lower confidence interval. UCI: Upper confidence interval. P: P value.


Author Contributions

Conceptualization: MJ JMK PB.
Data curation: RCT.
Formal analysis: RCT.
Funding acquisition: MJ JMK PB PCH KHW CLR RMM GDS.
Investigation: DA MCA AA SMA H. Bickebøller SEB H. Brunnström JM IB NEC CC DCC WJC JAD EJD JKF MPAD MWM GEG KG AH YCH LAK EHFMH PK MBJ SL MTL PL LLM GL OM SLP GR AR EBH MBS HS AT MDT PW MST XW JMY RJH CIA GS DZ AM MS JL BS VJ IH DM.
Methodology: PCH KHW CLR RMM GDS.
Project administration: MJ JMK PB.
Supervision: MJ JMK PB.
Writing – original draft: RCT MJ JMK PB.

Writing – review & editing: PCH KHW CLR RMM GDS DA MCA AA SMA H. Bickebøller SEB H. Brunström JM IB NEC CC DCC WJC JAD EJD JKF MPAD MWM GEG KG AH YCH LAK EHF MH PK MBJ SL ML PL LLM GL OM SLP GR AR EBH MBS HS AT MDT PW MST XW JMY RJH CIA GS DZ AM MS JL BS VJ IH DM.

References


Causality appraisal of metabolic factors on risk of lung cancer subtypes


