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Germline *HLA* heterozygosity is associated with decreased lung cancer risk

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Summary

Heterozygosity at human leukocyte antigen (*HLA*) loci may improve lung cancer immunosurveillance by increasing recognition of the tumor by the immune system. Previous studies utilizing data from population-level biobanks, such as the United Kingdom Biobank and FinnGen, have identified an association between germline *HLA* class II (*HLA*-II) heterozygosity and reduced lung cancer risk in smokers. In the present study, we evaluate the association between *HLA* heterozygosity and lung cancer in a large case-control study (15,302 cases and 14,580 controls) with imputed *HLA* allele-type information, comparing differences in *HLA* heterozygosity between smokers and non-smokers, among lung cancer subtypes, and at 2- and 4-digit *HLA* allele resolution. We identify a strong protective association of *HLA*-II heterozygosity in smokers compared to non-smokers, particularly at the *HLA*-*DPB1* and *HLA*-*DPA1* loci, and provide subtype-specific resolution. Finally, analysis of the additive effects of *HLA* allele heterozygosity in smokers identified significant associations with several 4-digit *HLA* alleles, including *HLA*-*B**08:01, *HLA*-*A**01:01, *HLA*-*C**07:01, *HLA*-*DQA1**05:01, *HLA*-*DRB1**03:01, and *HLA*-*C**03:04. Our study provides additional evidence, with added histologic subtype information, that germline *HLA*-II heterozygosity is inversely associated with lung cancer risk.

Introduction

Lung cancer is the leading cause of global cancer-related mortality.^{1,2} Tobacco smoke is the primary risk factor for lung cancer development, but other factors contribute to

its risk, including environmental and occupational exposures (e.g., arsenic,³ radon,⁴ and asbestos⁴), chronic lung disease, and lung infections.⁵ Histologically, lung cancer tumors are divided into two main categories, specifically small-cell lung carcinoma (SCLC) and non-small-cell

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lung carcinoma (NSCLC), with the latter contributing to the majority of cases.⁶ The main subtypes of NSCLC include adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large-cell lung carcinoma (LCLC).⁶ A smaller group of neuroendocrine tumors also exists, with carcinoid tumors being the primary subset.

In addition to the increased lung cancer risk conferred by exogenous exposures, studies have also identified germline genetic risk variants that contribute to lung cancer pathogenesis.^{7–9} Variations in genes including *TP53*, *EGFR*, *CHRNA5*, *BRCA2*, and *TERT*, in addition to several others, have been implicated in lung cancer etiology.^{8,9} One set of associations, between polymorphisms in human leukocyte antigen (*HLA*) class I (*HLA-I*) and class II (*HLA-II*) genes and lung cancer development, has highlighted a potential role of the immune system in lung cancer development.^{8–10} The *HLA-I* and *HLA-II* genes are integral to the process of presenting endogenous and exogenous antigens, respectively, to T cells. Our previous fine-mapping study of the *HLA* region identified associations with *HLA-B*08:01* and *HLA-DQB1*06* in a European population and *HLA-DQB1*04:01* and *HLA-DRB1*07:01* in an Asian population.¹¹ The *HLA* genes have a diverse array of alleles, contributing to significant polymorphism that allows for the detection of a wide range of antigens.¹²

Increased *HLA* diversity may improve immunosurveillance of lung cancer, leading to earlier tumor recognition by the immune system.¹² Accordingly, the heterozygote advantage hypothesis¹³ suggests that heterozygosity at *HLA* loci improves protection from disease due to improved tumor antigen presentation.¹² Data from recent studies have supported this hypothesis in lung cancer, with studies identifying somatic *HLA-I* loss as a mechanism of immune evasion.^{14,15} One study identified that *HLA* loss of heterozygosity (LoH) occurs in 40% of NSCLC and is associated with a high subclonal neoantigen burden and enrichment in metastatic sites.¹⁵ Another study identified a higher incidence of *HLA-I* LoH in lung cancer with brain metastasis, in addition to reduced CD8⁺ T cell infiltration into these tumors.¹⁶ Taken together, these observations in the somatic setting suggest that germline *HLA* homozygosity may predispose an individual to NSCLC development, while heterozygosity may be protective. A recent study addressed this question using data from the United Kingdom Biobank (UKB) and FinnGen (FG), finding that *HLA-II* heterozygosity was associated with reduced lung cancer risk in current and

former, but not never, smokers.¹² The authors suggested that the differential results for smokers and non-smokers may result from smoking increasing the number of mutations and smoking-derived antigens, which increase the importance of the immune response to early neoplastic disease in this cohort of patients.¹²

In the current study, we sought to replicate the previously mentioned result in a larger sample to determine the association between heterozygosity at various *HLA* loci with lung cancer risk, stratified by smoking status. We evaluate this question in a large study of European ancestry ($N = 29,882$ with $n = 15,302$ cases), compare differences in *HLA* heterozygosity between smokers and non-smokers, highlight histologic subtype-specific effects, and provide granular information about *HLA* allelic subtypes to the 2- and 4-digit levels.

Material and methods

Sample collection and genotypes

The study sample, along with component studies, quality control, and processing steps, has been previously described¹¹ and will be described briefly here. Participants came from the OncoArray study, a collection of 30 case-control studies that form part of the Integrative Analysis of Lung Cancer Risk and Etiology (INTEGRAL) and the Lung Cancer Cohort Consortium (LC3).¹¹ Informed consent for all participants and institutional review board approval from each institution was obtained.¹¹ In the studied cohort, we analyzed 29,882 samples, including 15,302 cases, of European ancestry (Tables 1 and S1). Genotyping was performed using the OncoArray genotyping platform, a custom Illumina array focused on cancer and with additional coverage of the *HLA* region.¹¹ Sample and variant quality control metrics have been previously described, and in brief included the removal of samples with low genotyping success rates (<95%), mismatched genetically inferred and reported sex, or excess identity by descent sharing relative to other samples.¹¹

HLA imputation and validation

Utilizing the high coverage of the *HLA* region by the OncoArray SNP, genotyping data from 25 to 35 Mb at chromosome 6 was used to impute classical 2- and 4-digit *HLA* alleles.¹¹ The 2-digit *HLA* allele (e.g., *HLA-DRB1*03*) provides information about the broad serologic family, while the 4-digit *HLA* allele (e.g., *HLA-DRB1*03:01*) specifies the specific protein sequence of the peptide-binding region amino acids. Reference data for *HLA* imputation were collected by the Type 1 Diabetes Genetics Consortium (T1DGC) and includes 5,225 individuals of European origin with

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Table 1. Sample demographic and histologic information for controls and lung cancer cases

	Total cohort (N = 29,882)	Controls (n = 14,580)	Cases (n = 15,302)
Sex (male, %)	17,818 (59.6)	8,649 (59.3)	9,169 (59.9)
Age (median, IQR)	63 (56–70)	61 (55–68)	64 (57–71)
Smoking status (yes, %)	24,379 (81.6)	10,524 (72.2)	13,855 (90.5)
Lung cancer	–		15,302 (100)
Adenocarcinoma (%)			6,887 (45.0)
Squamous cell carcinoma (%)			4,075 (26.6)
Small cell lung cancer (%)			1,739 (11.4)
Non-small cell carcinoma (%)			991 (6.5)
Mixed (%)			915 (6.0)
Large cell lung cancer (%)			524 (3.4)
Carcinoids (%)			171 (1.1)

genotyping data for 8,534 SNPs and 424 classical *HLA-I* (*HLA-A*, *HLA-B*, and *HLA-C*) and *HLA-II* (*HLA-DRB1*, *HLA-DQB1*, *HLA-DQA1*, *HLA-DPB1*, and *HLA-DPA1*) genes.¹¹ Imputation was performed strictly within the HLA region (chr6:27970031–33965553) using the Michigan Imputation Server 4-digit HLA reference panel; no intensity data was used for HLA typing.¹¹ Using this approach, each participant had specific alleles at the HLA loci, which were used to define loss-of-*HLA*-allele (LoA), gain-of-*HLA*-allele (GoA), homozygosity, and heterozygosity (defined in the next section), utilizing a framework similar to that of the LoH in human leukocyte antigen (LOHHLA) tool.¹⁵ Validation of the *HLA*-imputed data was performed as previously described.¹¹

Statistical analysis

We defined *HLA* heterozygosity, GoA, and LoA as follows. *HLA* heterozygosity is defined as an individual who carries two different 4-digit HLA alleles (e.g., an individual who carries *HLA_A*01:01* and *HLA_A*01:02*). *HLA* GoA is defined as an individual who carries more than two copies of an *HLA* allele (e.g., an individual who carries *HLA_A*01:01*, *HLA_A*01:01*, and *HLA_A*01:02*). *HLA* LoA is defined as an individual who carries fewer than two copies of an *HLA* allele (e.g., an individual who carries only *HLA_A*01:01*). Several multivariable models were tested, varying the definitions of the *HLA* variable as described below. Each model was adjusted for principal components (PCs) 1–5, sex, age, and smoking status, and nominal significance was considered at $p < 0.05$.

Estimating effects of *HLA* gene heterozygosity—e.g., homozygous, heterozygous, GoA, and LoA—on lung cancer

To study how *HLA* heterozygosity contributes to lung cancer risk, we first stratified the dataset by smoking status for both cases and controls (24,379 smokers and 5,503 non-smokers). Among smokers, there were 13,855 cases and 10,524 controls. Among non-smokers, there were 1,447 cases and 4,056 controls. For each cohort, we then fitted a logistic regression to predict a binary disease status for each *HLA* gene factor variable, which consists of homozygous, heterozygous, GoA, or LoA. In each regression model, we controlled for the top five PCs, age, and sex. Treating *HLA* homozygosity as the baseline, we calculated the effects of heterozygosity, GoA, and LoA, and computed their p value and standard error with the Wald test. The odds ratio ($OR = \exp(\beta)$) was visualized together with the 95% confidence interval (CI), which was also on

the OR scale. Fixed-effect meta-analysis of ORs and 95% CIs was performed using the “metagen” function from the meta R package. Log(OR) and standard errors were pooled for each HLA locus, and summary effect estimates with heterogeneity statistics (I^2) were extracted. To test whether *HLA* heterozygosity demonstrated different effects among smokers and non-smokers, we conducted interaction analysis using the model $\text{logit}[\text{Disease}] = \text{HLA-gene} + \text{Smoke} + \text{HLA-gene} \times \text{Smoke} + 5 \text{ PCs} + \text{age} + \text{sex}$.

Estimating effects of *HLA* gene heterozygosity—e.g., homozygous, heterozygous, GoA, and LoA—on lung cancer subtypes

To study the association between *HLA* heterozygosity and each lung cancer subtype, we performed identical analyses stratified by lung cancer subtype. We constructed strata by combining all individuals with a specific type of lung cancer (e.g., adenocarcinoma) and compared them to all individuals without any form of lung cancer. We then performed logistic regression for each disease and smoking stratum, conditioning on the top five PCs, age, and sex. Analysis was not repeated for GoA and LoA owing to very small sample sizes.

Estimation of additive effects of 2-digit/4-digit *HLA* alleles on lung cancer subtypes

To estimate the additive effect of 2-digit and 4-digit *HLA* alleles, we also performed logistic regression for smokers and non-smokers. Importantly, besides the top five PCs, age, and sex, we also included the heterozygosity status of the indexing *HLA* gene as a covariate. For example, when regressing disease status on *HLA-A*01:01* allele numbers, we also included *HLA-A* status, such as homozygous, heterozygous, GoA, or LoA, as a covariate. We ran logistic regressions for 147 2-digit *HLA* alleles and 423 4-digit *HLA* alleles and visualized the p value using Manhattan plots. The Bonferroni correction was applied according to the number of alleles and plotted as a gray dashed line in the Manhattan plot (0.00034 threshold for 2-digit *HLA* alleles and 0.00019 threshold for 4-digit *HLA* alleles).

Results

Patients with lung cancer demonstrate less germline *HLA* heterozygosity

The demographic and histologic information for the 15,302 cases with lung cancer and 14,580 controls is

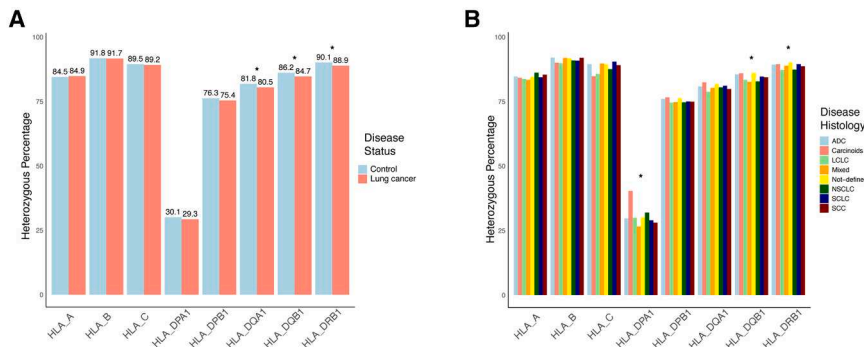


Figure 1. Demographic and histologic information stratified by HLA allele
Heterozygote percentage by (A) disease status and (B) disease histology. Significance established using the chi-squared test. * $p < 0.05$ for indicated HLA allele.

HLA heterozygosity has heterogeneous effects among smokers and non-smokers

We next tested whether HLA heterozygosity (including three HLA-I and five HLA-II genes) was significantly

presented in Tables 1 and S1. The predominant histologic subtype of lung cancer was ADC (45.0%), followed by SCC (26.6%) and SCLC (11.4%). In univariable analyses, no differences in HLA heterozygosity percentages were observed between cases and controls for HLA-A, HLA-B, HLA-C, HLA-DPA1, or HLA-DPB1 (Figure 1). Significant differences, however, were observed for HLA-DQA1, HLA-DQB1, and HLA-DRB1, with less HLA heterozygosity observed in the lung cancer cases. Upon analysis of the histologic subtypes, significant differences in HLA heterozygosity were observed for HLA-DPA1, HLA-DQB1, and HLA-DRB1, with ADC generally demonstrating increased heterozygosity compared to SCC.

Germline HLA heterozygosity is associated with overall lung cancer risk in multivariable analysis

Several models testing different underlying HLA variable constructs were implemented to analyze the association between germline HLA heterozygosity and overall lung cancer risk (Table 2). A protective effect of HLA heterozygosity was observed upon building an HLA variable counting the total number of heterozygotes per sample for all HLA loci (OR, 0.98; 95% CI, 0.96–0.99; $p = 2.72 \times 10^{-3}$), in addition to another HLA variable counting only samples with heterozygosity at all HLA loci (OR, 0.92; 95% CI, 0.86–0.98; $p = 8.86 \times 10^{-3}$). The effect appeared to be specific to major histocompatibility complex (MHC) class II loci, as significance was lost upon analysis building an HLA variable with just HLA-A, HLA-B, and HLA-C (OR, 1.00; 95% CI, 0.96–1.03; $p = 0.93$) but was retained when the HLA variable was based on HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and HLA-DRB1 (OR, 0.96; 95% CI, 0.94–0.98; $p = 2.33 \times 10^{-4}$). Furthermore, we performed a fixed-effect meta-analysis investigating the association between HLA heterozygosity with lung cancer in the overall cohort from our study with the data previously published by Krishna et al.¹² from the UKB and FG (Table S2). We demonstrated consistent effects across the studies, with no significant associations among HLA-I genes but significant associations with low heterogeneity in HLA-DPA1 (OR, 0.94; 95% CI, 0.90–0.99; $p = 0.01$; $I^2 = 0$) and HLA-DPB1 (OR, 0.93; 95% CI, 0.89–0.97; $p = 4.48 \times 10^{-4}$; $I^2 = 0.08$).

associated with lung cancer, stratified by smoking status ($n = 24,379$ smokers and $n = 5,503$ non-smokers). We found that among smokers, HLA-DPA1, HLA-DPB1, and HLA-DRB1 showed significant associations with disease development, whereas among non-smokers, only HLA-DQB1 heterozygosity was associated with reduced lung cancer risk (Figure 2). We also investigated the association between LoA and GoA with lung cancer risk (Figures S1A and S1B), although the sample size was very small. We additionally estimated the effects of heterozygosity by removing individuals with either GoA and LoA HLA genes (Figure S1C) and obtained similar results. To formally assess whether the HLA genes have different effects among smokers and non-smokers, we performed interaction analyses for eight HLA genes (Table S3). We found that HLA-DPA1 and HLA-DPB1 heterozygosity exhibited significantly different effects among smokers and non-smokers ($p = 0.0063$ for HLA-DPA1 and $p = 0.0089$ for HLA-DPB1).

HLA heterozygosity across different lung cancer subtypes

Next, we performed a stratified analysis to investigate the effect of HLA heterozygosity on lung cancer subtypes. We stratified the cohort by cancer subtype and conducted logistic regression separately for smokers and non-smokers (Figure 3). We found that all HLA-II genes exhibited protective effects for SCC among smokers but not for non-smokers. In contrast, heterozygosity of HLA-DPA1 showed risk associations for carcinoids and NSCLC among non-smokers. Interaction analysis indicated HLA-DPA1 has differential effects between smokers and non-smokers for ADC ($p = 0.0356$) and carcinoids ($p = 0.0471$) (Table S4). No significant differential effects between smokers and non-smokers were observed for SCC, possibly due to the small number of SCC cases among non-smokers.

Additive effects of HLA allele heterozygosity

Inspired by genome-wide association studies (GWASs), we further investigated additive effects of heterozygosity at HLA alleles on lung cancer risk. We typed 147 2-digit HLA alleles and 423 4-digit HLA alleles. We then performed stratified analysis for smokers and non-smokers (Figure 4 and Tables S5–S8). We performed logistic

Table 2. Multivariable logistic regression models varying HLA variable definition

	Model description	OR (95% CI)	p value
Model 1	HLA variable counts the total number of heterozygotes per sample for all HLA loci	0.98 (0.96, 0.99)	2.72×10^{-3}
Model 2	HLA variable counts only samples with heterozygosity at all HLA loci	0.92 (0.86, 0.98)	8.86×10^{-3}
Model 3	HLA variable counts the total number of heterozygotes per sample for HLA-A, HLA-B, and HLA-C	1.00 (0.96, 1.03)	0.93
Model 4	HLA variable counts the total number of heterozygotes per sample for HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and HLA-DRB1	0.96 (0.94, 0.98)	2.33×10^{-4}
Model 5	HLA variable counts the total number of losses of an HLA allele per sample	0.94 (0.83, 1.06)	0.32
Model 6	HLA variable counts the total number of gains of an HLA allele per sample	0.84 (0.53, 1.34)	0.46
Model 7	HLA variable counts the total number of LoA for HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and HLA-DRB1	1.03 (0.87, 1.23)	0.70
Model 8	HLA variable counts the total number of GoA for HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and HLA-DRB1	1.02 (0.45, 2.32)	0.95

Each model is adjusted for PCs 1–5, sex, age, and smoking status.

regression for each HLA allele while conditioning on covariates, including the top five PCs, age, sex, and heterozygosity, of the corresponding HLA gene. For instance, to estimate the additive effects of a 2-digit allele HLA-A*01, we performed logistic regression with $\text{logit}[\text{Disease}] = \text{HLA-A*01} + \text{HLA-A} + \text{PC} + \text{age} + \text{sex}$. Importantly, we condition on HLA-A (a factor variable including “Homozygous,” “Heterozygous,” “GoA,” and “LoA”) to remove gene-level effects, which produces more accurate additive effect estimates for HLA alleles.

We did not detect strong additive effects for HLA alleles among non-smokers, whereas several HLA alleles showed significant associations among smokers. For instance, HLA-B*08 (4-digit: HLA-B*08:01) and HLA-A*01 (4-digit: HLA-A*01:01) showed significant effects ($p = 5.42 \times 10^{-9}$ and $p = 9.99 \times 10^{-8}$, respectively). Compared to our previous study,¹¹ we replicated the top HLA alleles, including HLA-A*01:01, HLA-B*08:01, HLA-C*07:01, HLA-DQA1*05:01, and HLA-DRB1*03:01, and identified an association at HLA-C*03:04. We also found a significant association for HLA-DQB1*06, consistent with our prior work.¹¹ To identify independently associated HLA alleles, we performed conditional analyses by including all significant HLA alleles in a joint logistic regression (Table S9). HLA-A*01:01 and HLA-C*03:04 remained significant in the conditional analysis ($p = 1.25 \times 10^{-2}$ and $p = 9.91 \times 10^{-4}$, respectively). We repeated the analysis stratified by histology in SCC and ADC (Figures 5 and S2, respectively), which demonstrated no significant associations for ADC but significant 4-digit associations for the

following loci in SCC: HLA-A*01:01, HLA-B*07:02, HLA-B*08:01, HLA-C*03:04, HLA-C*07:01, HLA-C*07:02, HLA-DQA1*05:01, and HLA-DRB1*03:01. Epitopes associated with each of these 4-digit HLA types were queried in the Immune Epitope Database and Tools (IEDB; <https://www.iedb.org/>) database,¹⁷ and the top 50 results for each are displayed in Table S10.

Discussion

The present study characterizes the association between germline HLA region heterozygosity and lung cancer risk in a large consortium, identifying that patients with lung cancer have reduced germline HLA heterozygosity for class II loci. Furthermore, we identified differential effects when we stratified by both smoking status and histologic lung cancer subtype. Specifically, we identified a stronger protective effect of HLA-II heterozygosity in smokers compared to non-smokers, particularly at the HLA-DPB1 and HLA-DPA1 loci. Finally, analysis of the additive effects of HLA allele heterozygosity in smokers identified significant associations with several 4-digit HLA alleles, including HLA-B*08:01, HLA-A*01:01, HLA-C*07:01, HLA-DQA1*05:01, HLA-DRB1*03:01, and HLA-C*03:04.

The heterozygote advantage hypothesis suggests that heterozygosity at HLA loci may improve protection from disease due to improved tumor antigen presentation.^{12,13} The results from the present study are largely consistent

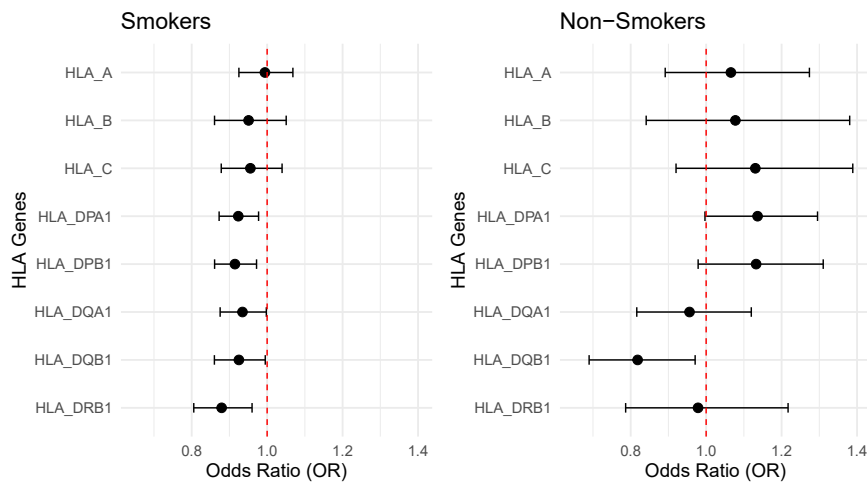


Figure 2. Effects of germline *HLA* heterozygosity on lung cancer risk among smokers and non-smokers

We ran logistic regression for each HLA gene using $\text{logit}[\text{Disease}] = \text{HLA-gene} + 5 \text{ PCs} + \text{age} + \text{sex}$. “Disease” is a binary variable to indicate if an individual has any form of lung cancer. “HLA-gene” is a factor variable that includes “Homozygosity,” “Heterozygosity,” “GoA,” and “LoA.” In this analysis, we calculated the OR for “Heterozygosity” by treating “Homozygosity” as the baseline. Results for “GoA” are shown in Figure S1A; results for “LoA” are shown in Figure S1B. 95% confidence intervals are shown in both panels.

with this hypothesis and previous work by Krishna et al.,¹² complementing their study with a larger sample size and additional histologic information. Krishna et al. analyzed participants from the UKB and FG, finding that germline *HLA*-II heterozygosity was associated with a decreased risk of lung cancer.¹² Interestingly, while *HLA*-I heterozygosity has been previously associated with other metastatic can-

cers,^{18–22} we replicate Krishna et al.’s¹² finding that *HLA*-II heterozygosity is primarily associated with lung cancer, despite identifying a small number of *HLA*-I associations that increase risk in our additive analysis. Furthermore, Krishna et al. demonstrated that *HLA*-II homozygosity conferred a higher lifetime risk of lung cancer, with 13.9% risk for current smokers who were also homozygous

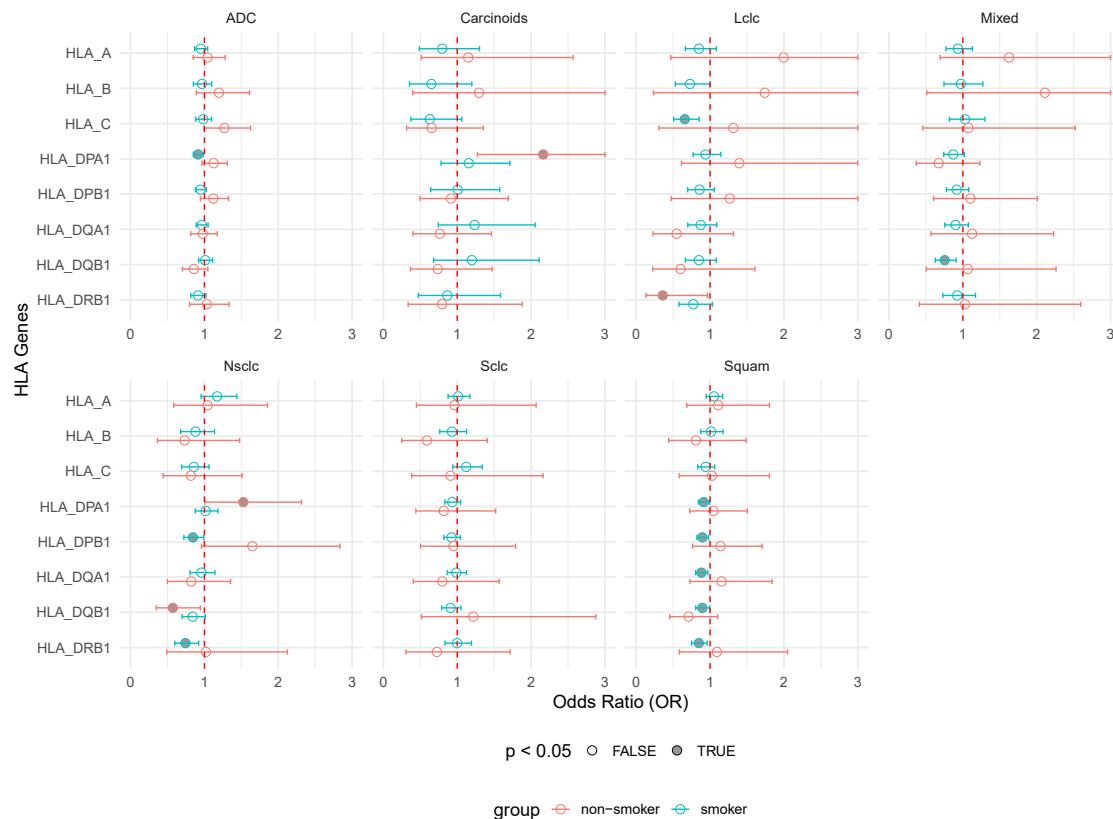


Figure 3. Effects of *HLA* heterozygosity on seven subtypes of lung cancer, stratified by smoking status

Confidence intervals are truncated at OR = 3 for visualization. Significant associations are denoted by filled circles. ADC, adenocarcinoma; LclC, large cell lung carcinoma; Mixed, multiple types; NsclC, non-small cell lung cancer; SclC, small cell lung cancer; Squam, squamous cell carcinoma. 95% confidence intervals are shown in all panels.

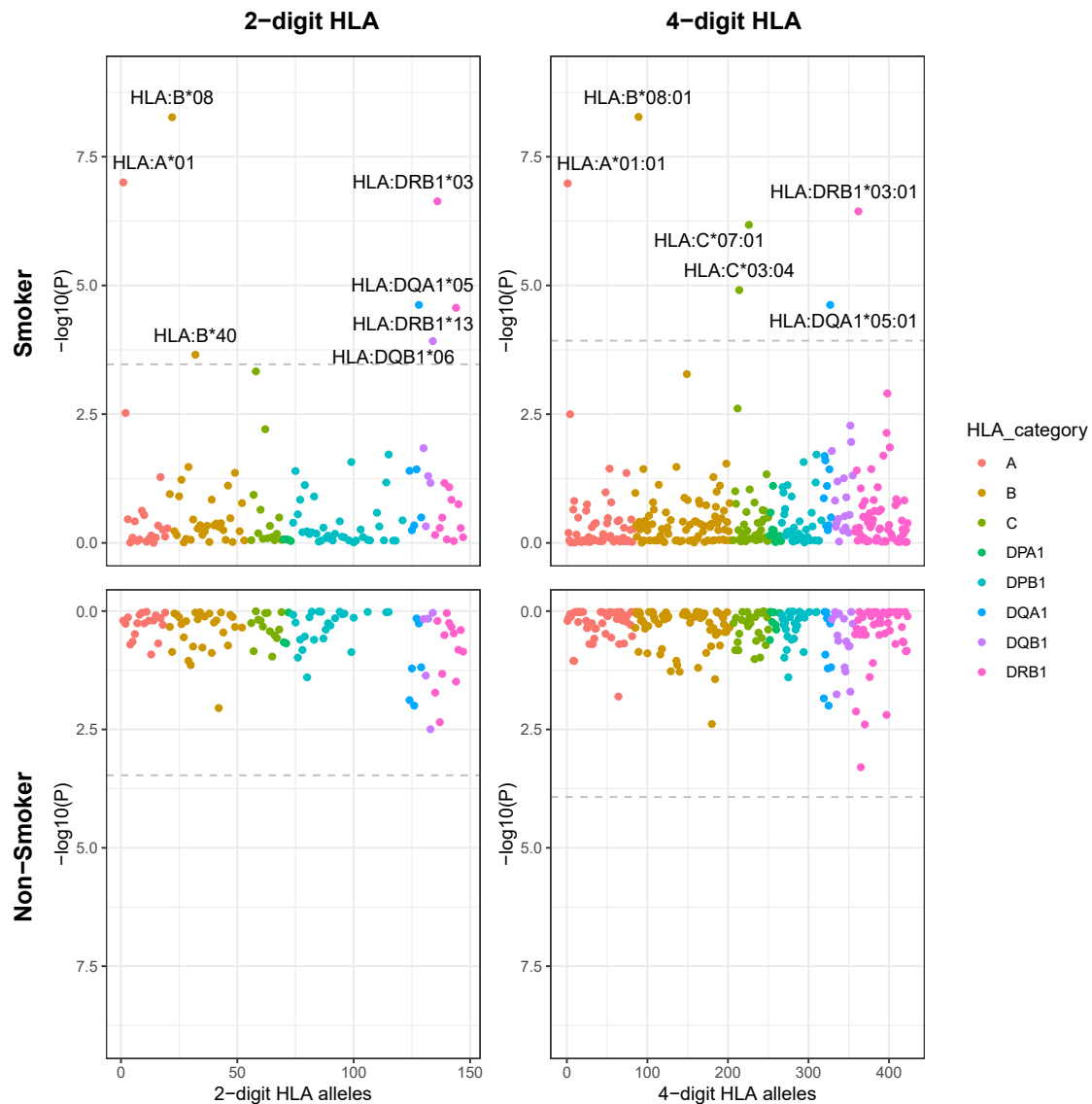


Figure 4. Logistic regression analysis for each 2-digit/4-digit HLA allele with aggregated disease status: $\text{logit}[\text{Disease}] = \text{HLA-allele} + \text{HLA-gene} + \text{PC} + \text{age} + \text{sex}$
 “HLA-allele” is the dosage of any 2-digit/4-digit HLA alleles (e.g., HLA:A*01 dosage, which consists of 0, 1, 2). “HLA-gene” is a factor variable for the corresponding HLA gene (e.g., HLA-A, which consists of “Homozygous,” “Heterozygous,” “GoA,” and “LoA”). The OR and p values are reported for all 2-digit/4-digit HLA alleles. The $-\log_{10}(p \text{ value})$ is visualized for each HLA allele (top row: smokers; bottom row: non-smokers; left column: 2-digit HLA alleles; right column: 4-digit HLA alleles).

at *HLA-DRB1*.¹² Molecular studies have provided additional evidence: (1) *HLA-II* peptide-binding groove amino acid heterozygosity is associated with reduced lung cancer risk, (2) single-cell RNA-sequencing data demonstrated alterations in *HLA-II* expressing lung macrophages and epithelial cells in smokers, and (3) neoantigen repertoire analysis demonstrated a loss of alleles with larger neopeptide repertoires in *HLA-II* LoH samples.¹² *HLA* heterozygosity research has also demonstrated that a careful balance between cancer and autoimmunity exists, with several studies demonstrating a complex association between *HLA* heterozygosity and immune-mediated disease risk as *HLA* heterozygosity may confer an increased risk of immune-mediated diseases.^{23–25}

Our study also aimed to characterize effects from specific *HLA-II* loci on risks for lung cancer. *HLA-DRB1* heterozygosity was replicated as an important locus for associated with increased (e.g., homozygous) or decreased (e.g., heterozygous) disease risk in our study, with the effect appearing for smokers and in the SCC and NSCLC subtypes. Our histology-specific results suggested that the unique protective effect of *HLA-II* heterozygosity exists among SCC patients with a history of smoking but not ADC patients with a history of smoking, consistent with previous work.¹¹ We also identified several *HLA* loci at 4-digit resolution associated with increased risk in the additive analysis, including *HLA-B*08:01*, *HLA-A*01:01*, *HLA-C*07:01*, *HLA-DQA1*05:01*, *HLA-DRB1*03:01*, and *HLA-C*03:04*. In our prior classical

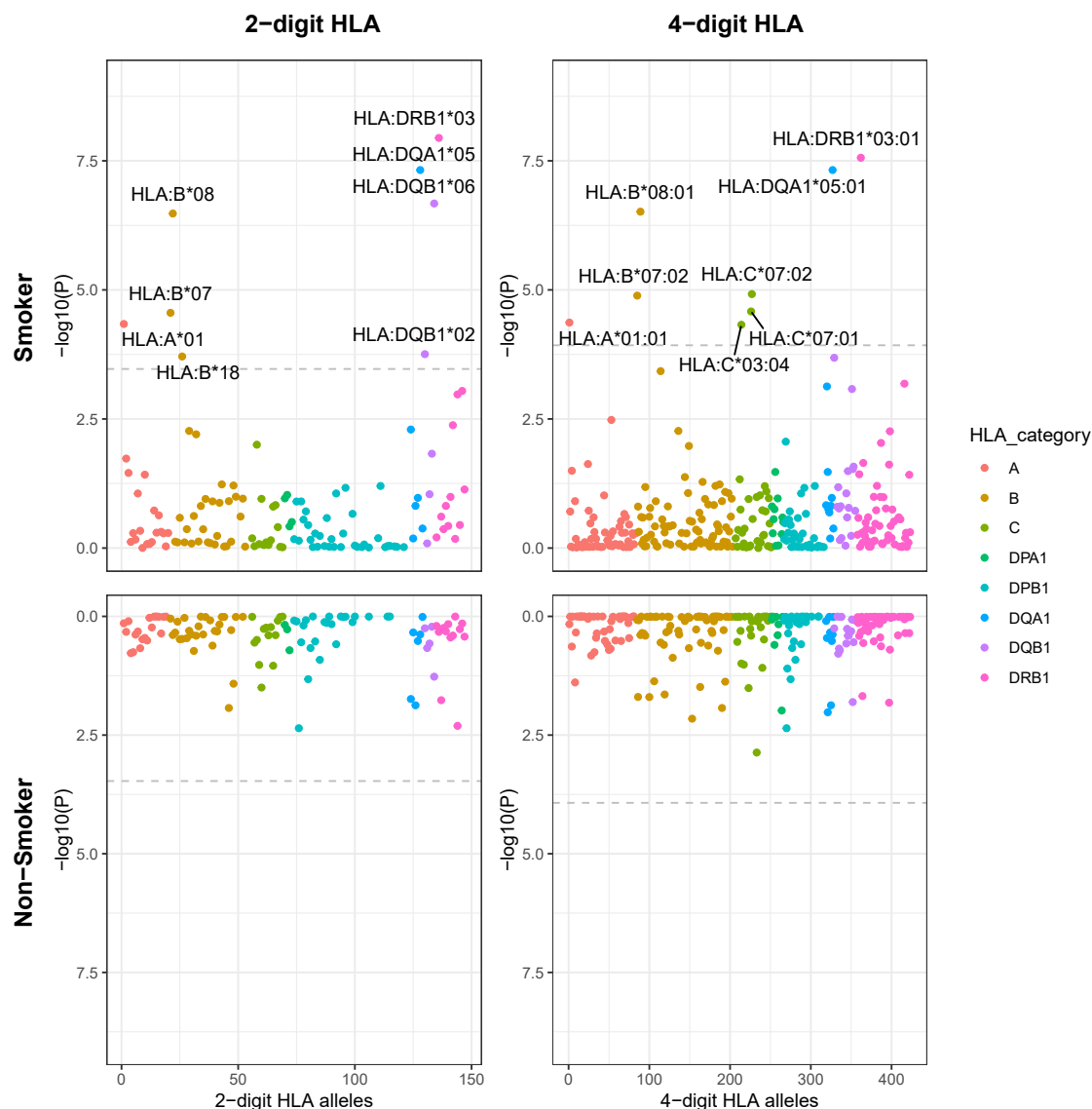


Figure 5. Logistic regression analysis for each 2-digit/4-digit HLA allele in SCC: $\text{logit}[\text{Disease}] = \text{HLA-allele} + \text{HLA-gene} + \text{PC} + \text{age} + \text{sex}$
 “HLA-allele” is the dosage of any 2-digit/4-digit HLA alleles (e.g., HLA-A*01 dosage, which consists of 0, 1, 2). “HLA-gene” is a factor variable for the corresponding HLA gene (e.g., HLA-A, which consists of “Homozygous,” “Heterozygous,” “GoA,” and “LoA”). The OR and p values are reported for all 2-digit/4-digit HLA alleles. The $-\log_{10}(p \text{ value})$ is visualized for each HLA allele (top row: smokers; bottom row: non-smokers; left column: 2-digit HLA alleles; right column: 4-digit HLA alleles).

GWAS, which tested the association between specific alleles and disease status, *HLA-B*08:01*, *HLA-A*01:01*, *HLA-C*07:01*, *HLA-DQA1*05:01*, and *HLA-DRB1*03:01* were identified.¹¹ *HLA-A*01:01*, *HLA-B*08:01*, *HLA-C*07:01*, and *HLA-DQA1*05:01* make up four of the five alleles (*HLA-DQB1*02:01*, the fifth, was not identified in our additive analysis) of the AH8.1 haplotype, which is a well-known and described Caucasian haplotype associated with immune-mediated diseases.^{26–28} Our study provides evidence that reduced heterozygosity at alleles in this haplotype are associated with an increased germline risk of developing lung cancer and is consistent with our previous GWAS analysis,¹¹ highlighting a significant association between these alleles and lung cancer.

While the association between lung cancer and smoking is well described,²⁹ our study and previous work¹² identified a unique protective effect of heterozygosity at *HLA-II* in smokers. Krishna et al.¹² also demonstrated that the effect was specific to current and former but not never-smokers, which we also found in our larger study. Krishna et al. hypothesize that in smokers, *HLA-II* heterozygosity may improve recognition of smoking-related antigens in developing tumors, which could be presented by alveolar macrophages or dendritic cells for CD4⁺ T cell recognition and development of an antitumor response.¹²

The present study has some limitations. First, the non-smoking analytical group had a much smaller sample size, limiting our ability to resolve effects of HLA

heterozygosity on lung cancer risk in this group. For most of the *HLA-II* region, heterozygosity showed little impact on risk for non-smokers, but *HLA-DQB1* heterozygosity was protective. Second, some cancer subtypes, such as mixed, LCLC, and carcinoids, demonstrated small sample sizes, also potentially preventing us from detecting significant effects. Finally, our study was focused on individuals of European ancestry, limiting the generalizability to other ancestral populations.

Taken together, our study validates a previous study of data from the UKB and FG, providing additional evidence that germline *HLA-II* heterozygosity is protective against developing lung cancer. Furthermore, our study highlights differences in protection across various *HLA-II* alleles at 2- and 4-digit resolution, in smokers and non-smokers, and across multiple histologic subtypes. Future research, as suggested by Krishna et al.,¹² may evaluate whether smokers who are homozygous at *HLA-II* may benefit from low-dose computed tomographic screening at an earlier age. Additional work may assess the relationship between immunotherapy response and *HLA* heterozygosity, as previous work in melanoma has demonstrated that a somatic loss of *HLA-I* heterozygosity was associated with poorer immune checkpoint blockade responses.²² Our results suggest that the evaluation of *HLA-II* heterozygosity as a prognostic biomarker may be evaluated in conjunction with other clinical variables in future prospective clinical trials.

Data and code availability

Genotype data for the lung cancer OncoArray study have been deposited at the database of Genotypes and Phenotypes (dbGaP) under accession phs001273.v1.p1. Model statements utilized for analyses are included throughout the article. Additional figure code is available upon reasonable request to the lead contact.

Web resources

Immune Epitope Database and Tools database (IEDB), <https://www.iedb.org/>

Michigan Imputation Server Reference Panels, <https://genepi.github.io/michigan-imputationserver/reference-panels/>

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Declaration of interests

The authors declare no competing interests.

Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.xhgg.2026.100567>.

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