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Genetically defined causal effects of natural killer cells related traits in risk of infection: a Mendelian randomization study

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Abstract

Background The intricate interplay between genetics and immunology often dictates the host's susceptibility to various diseases. This study explored the genetic causal relationship between natural killer (NK) cell-related traits and the risk of infection.

Methods Single-nucleotide polymorphisms (SNPs) significantly associated with NK cell-related traits were selected as instrumental variables to estimate their genetic causal effects on infection. SNPs from a genome-wide association study (GWAS) on NK cell-related traits, including absolute cell counts, median fluorescence intensities reflecting surface antigen levels, and relative cell counts, were used as exposure instruments. Summary-level GWAS statistics of four phenotypes of infection were used as the outcome data. The exposure and outcome data were analyzed via the two-sample Mendelian randomization method.

Results Each one standard deviation increase in the expression level of human leukocyte antigen (HLA)-DR on HLA-DR$^+$ NK cells was associated with a lower risk of pneumonia ($P < 0.05$). An increased HLA-DR$^+$ NK/CD3$^-$ lymphocyte ratio was related to a lower of risk of pneumonia ($P < 0.05$). Each one standard deviation increase in the absolute count of HLA-DR$^+$ NK cells was associated with a lower risk of both bacterial pneumonia and pneumonia ($P < 0.05$). An increased HLA-DR$^+$ NK/NK ratio was associated with a decreased risk of both pneumonia and bacterial pneumonia ($P < 0.05$). The results were robust under all sensitivity analyses. No evidence for heterogeneity, pleiotropy, or potential reverse causality was detected. Notably, our analysis did not reveal any significant associations between NK cell-related traits and other phenotypes of infection, including cellulitis, cystitis, and intestinal infection.

Conclusions HLA-DR$^+$ NK cells could be a novel immune cell trait associated with a lower risk of bacterial pneumonia or pneumonia.

Keywords Infection, Mendelian randomization (MR), Genome-wide association study (GWAS), Bacterial pneumonia, Pneumonia, HLA-DR$^+$ NK cell

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Background

Infection, which ranges from mild to life-threatening, is a persistent challenge in global health [1, 2]. The multifaceted nature of infectious agents and their continuous evolution necessitate a deeper understanding of host factors that modulate susceptibility. In addition to mere exposure, this risk is closely intertwined with the host's immune response, underscoring the need to elucidate the intricacies of our immune defenses [3].

Natural killer (NK) cells are an important type of innate immune cell and are defined as "cytotoxic innate lymphocytes with 'natural killing' capacity and antibody-dependent cell-mediated cytotoxicity" [4–6]. Unlike NK cells, whose protective effects against tumors and acute viral infections are well established in the literature [4, 7], their role against bacterial infections remains controversial. Evidence from animal models has revealed a complex and conflicting role for NK cells in the immuno-pathogenesis of bacterial infections [8, 9]. NK and NK T-Cell depletion resulted in increased bacterial burdens and increased levels of serum and splenocyte interferon-γ (IFN-γ) in *Streptococcus pneumoniae* infection mouse model [10]. In mice infected with pulmonary non-tuberculous mycobacteria, NK cell depletion increased bacterial burden and mortality [11]. Another study revealed that NK cell deficient mice had higher survival rates and lower levels of proinflammatory cytokines than wild-type mice did [12]. On the basis of clinical evidence in humans, researchers have been unable to determine whether NK cells have a positive or negative effect on infection. A decease in the NK cells count is related an increased risk of severe infection [13], increased severity of infection [14] and a poor survival rate [15]. In addition, a lower level of IFN-γ secretion by NK cells is associated with a greater risk of severe infection [13]. In contrast, an increased number of circulating NK cells was associated with early mortality in patients with severe infection [16]. Patients with severe infection due to gram-negative bacteria and septic shock suffering from multi-organ dysfunction and an increased mortality rate had elevated levels of granzyme proteins in their NK cells [17, 18]. Further research with advanced analytical techniques reveals that NK cells actually exhibit a high degree of heterogeneity [19]. This heterogeneity may explain their complex role in immune regulation [20, 21]. Thus, the role of NK cells in infection is controversial, and our understanding remains incomplete. Efforts are needed to fully elucidate and precisely define the contribution of NK cells to the development of infection.

Mendelian randomization (MR) represents a methodology for the evaluation of etiological inferences in epidemiological and genetic studies. The method employs genetic variants that are strongly correlated with exposure factors as instrumental variables, with the objective of assessing the genetic causal relationship between exposure factors and outcomes. The method exploits the random distribution of genetic factors in the human body and the fact that genetic factors are fixed at the time of fertilization to explore the genetic causal relationship between exposure factors and outcomes in a way that approximates the principles of randomized controlled trials (RCTs). This addresses the limitations of RCTs, which are currently regarded as the gold standard for determining causality. These limitations include cost-effectiveness, time efficiency and ethical concerns.

By harnessing genetic variations as a tool, MR enables us to navigate the complex web of causality, offering robust insights into the relationship between NK cell populations and infection risk. We sought to test whether genetically altered NK cell-related traits were associated with infection phenotypes predominantly caused by bacteria via a two-sample summary-level MR analysis. In our study, we focused on eleven NK cell traits identified from the most recent high-quality genome-wide association study (GWAS) on immune cells. We assessed four distinct bacterial infection phenotypes: intestinal infections, pneumonia, cellulitis, and cystitis. These infection phenotypes were selected on the basis of disease diagnosis codes reported in the latest comprehensive GWAS on bacterial infections.

Methods

Study Design

This MR study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology - Mendelian Randomization reporting guidelines [22] (Supplementary file 1). Figure 1 provides a schematic representation of the study design. We conducted a two-sample MR analysis using publicly available summary statistics from fifteen GWASs: eleven for exposures and four for outcomes. To mitigate bias from population stratification, both exposure and outcome cohorts were restricted to individuals of European ancestry [23].

This study aimed to assess the impact of circulating NK cell immune traits on infection risk. Exposures included NK cell-related traits (described in Supplementary file 3: Table S1A; instruments detailed in Supplementary file 3: Table S2A), whereas outcomes included traits associated with intestinal infection, pneumonia, cellulitis, and cystitis (Supplementary file 3: Table S1B). All the data were sourced from publicly accessible repositories with existing ethical approval, obviating the need for additional ethical clearance.

Data sources

Exposure: The NK cell-related traits were derived from the SardiNIA project, which was accessible via the GWAS Catalog [24] (see Supplementary file 3: Table

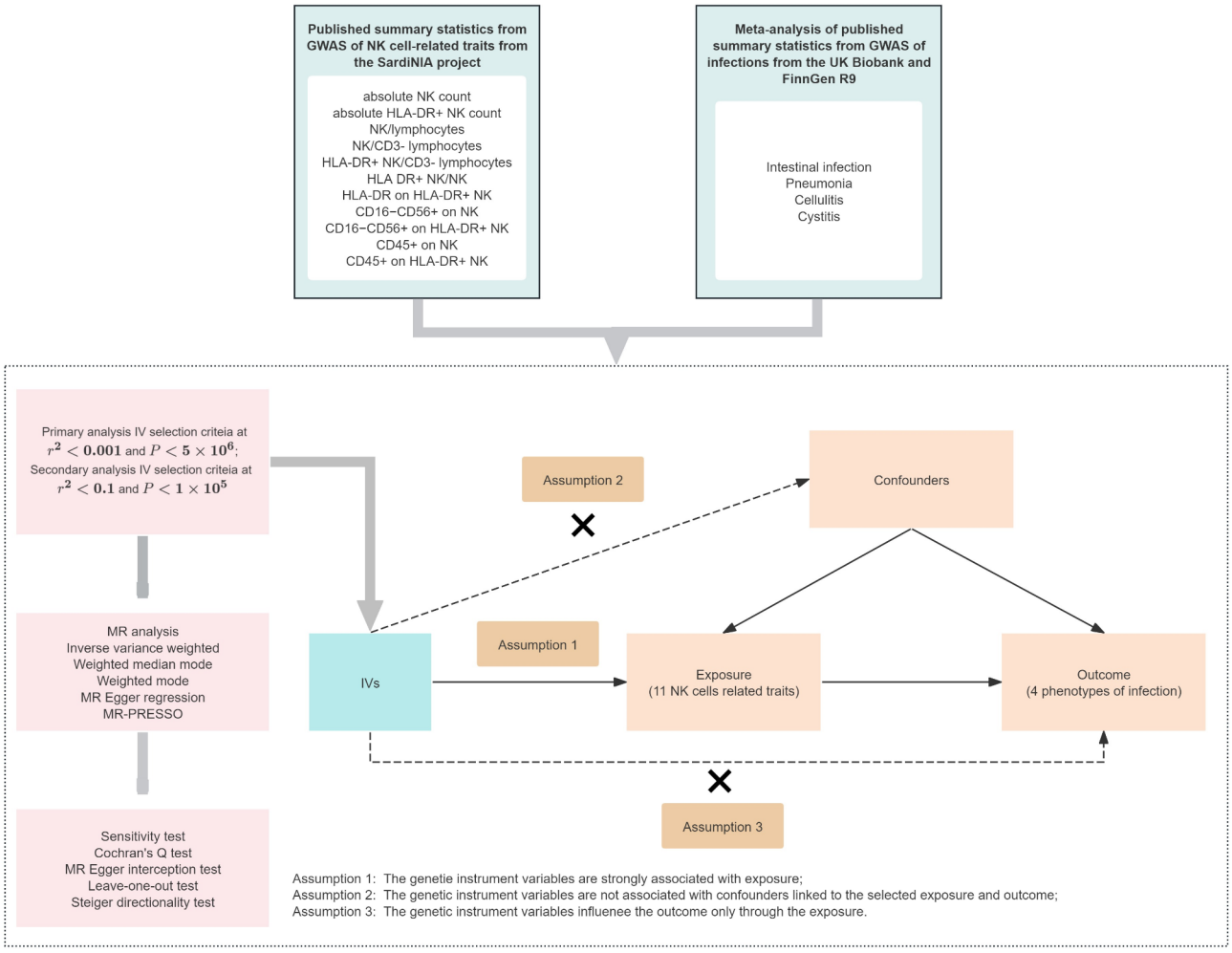


Fig. 1 An overview of two-sample Mendelian randomization study. Abbreviations: GWAS genome-wide association study; NK, natural killer; IV, instrument variable; MR, Mendelian randomization; IVW, inverse variance weighted; MR-PRESSO Mendelian Randomization Pleiotropy RESidual Sum and Outlier

S1A). These traits included the absolute NK count, absolute human leukocyte antigen (HLA)-DR $^+$ NK count, the ratio of NK/lymphocytes, NK/CD3$^-$ lymphocytes, HLA-DR $^+$ NK/NK, and HLA-DR$^+$ NK/CD3$^-$ lymphocytes, as well as the median fluorescence intensities of CD16$^-$CD56$^+$ and CD45$^+$ on both NK and HLA-DR$^+$ NK cells and HLA-DR on HLA-DR$^+$ NK cells. NK cells were identified as CD16+or CD56+within the CD3-CD45+population. The HLA-DR positivity of NK cells served as an activation marker [24]. The SardiNIA study enrolled 3,757 native Sardinians without overlap [24]. Approximately 22 million single-nucleotide polymorphisms (SNPs) were imputed via a Sardinian reference panel and tested for associations, adjusting for sex, age, and age-squared [24, 25].

Outcome: Four common infection phenotypes were evaluated on the basis of disease incidence and available summary statistics from two independent European ancestry cohorts: UK Biobank [26] and FinnGen Release 9 [27] (Supplementary file 3: Table S1B). These cohorts had no overlap. The UK Biobank GWAS employed Scalable and Accurate Implementation of GEneralized mixed model (SAIGE), adjusting for relatedness, sex, birth year, and principal components [26]. The FinnGen GWAS also used SAIGE, adjusting for sex, age, principal components, and batch [27]. Phenotypes of the UK Biobank were extracted from International Classification of Diseases (ICD) billing codes derived from electronic health records, whereas phenotypes of the FinnGen population were structured according to the hierarchical subtyping system of the ICD-10 classification.

Instrument selection

In the primary MR analyses, SNPs were selected as instrumental variables on the basis of statistical criteria. Although the conventional *P* value threshold for instrument selection in MR studies is 5×10^{-8}, this approach results in an insufficient number of instruments exceeding this threshold, which leads to underpowered analyses or inflated results [28]. To obtain a minimum of five

eligible instruments, the threshold was increased by a factor of ten in a sequential manner. The final range of *P* values employed for instrument inclusion across all traits extended from 5×10^{-8} to 5×10^{-6} in the primary analysis [29, 30]. The TwoSampleMR package identified independent variants with $r^2<0.001$ in the 1000 Genomes European ancestry data. When SNPs were not shared between exposure and outcome, we supplemented instrumental variables with proxies from the 1000G EUR dataset that met a $r^2>0.8$ threshold. F statistics were calculated via the variance in exposure explained by SNPs to assess instrumental strength. The exposure variance explained (R^2) for binary traits and variable phenotype effects was calculated via the TwoSampleMR package, which used the minor allele frequency and SNP exposure effect size [31]. The F statistic followed the formula $F=R^2(n-k-1)/(k(1-R^2))$, where n was the sample size and k was the number of instrumental variables [32]. Only SNPs with an F statistic greater than ten were retained in the primary analyses [33, 34]. We excluded potentially weak instruments (F statistic < 10) and reduced the risk of bias [35]. Steiger filtering was performed to identify instrumental SNPs that explain more significant variation in an outcome than in an exposure, which may indicate potential reverse genetic causal relationships in subsequent MR analyses.

To complement our primary analyses and potentially improve statistical power, we conducted secondary analyses using less stringent criteria, as reported in previous literature. Specifically, we applied a *P* value threshold of 1×10^{-5} for SNP inclusion. Additionally, we used the linkage disequilibrium pruning criterion of $r^2<0.1$ within a 500 kb window to ensure a degree of independence among the selected SNPs [24, 29, 30, 36]. This approach allowed us to balance rigorous statistical standards in our primary analyses with a more inclusive strategy in our secondary analyses, potentially capturing additional genetic signals that might contribute to the relationship between NK cell traits and infection risk.

Primary MR analysis

In our primary MR analysis, we employed a range of methods tailored to the number of instrumental SNPs. In the case of instruments comprising a single SNP, the Wald ratio was employed to derive MR effect estimates. In contrast, for instruments comprising two or more SNPs, the inverse-variance weighted (IVW) method was employed for the meta-analysis of Wald ratios, as outlined in reference [37]. For NK cell-related traits with more than three available SNPs, a variety of MR methods, including the weighted median [38], weighted mode [39], MR Egger regression [40], and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) have been employed [41]. All reported associations were presented as odds ratios (ORs) for the outcome per standard deviation increase in genetically predicted circulating immunophenotype levels. MR analyses were conducted for the outcome of GWASs from the UK Biobank and the FinnGen project, as well as a meta-analysis of both cohorts. Unless otherwise stated, the meta-analysis was employed as the primary outcome study.

Sensitivity analysis

In our study, we adhered to three fundamental assumptions to establish a genetic causal relationship: (1) the instrument must be robustly associated with the exposure; (2) it cannot influence exposure-outcome confounders; and (3) it can only affect the outcome through the risk factor. To validate these assumptions and reinforce the robustness of our findings, we conducted a series of sensitivity analyses(Fig. 1).

To ascertain whether the observed differences in instrument effect size could be attributed to pleiotropy rather than mere chance, we employed Cochran's Q test, applying a significance threshold of $P<0.05$ when at least two variants were available [37]. Furthermore, we conducted leave-one-out analyses to assess the heterogeneity of our results [42]. Finally, we conducted a Steiger directionality test to validate the assumption that exposure causes outcomes [43]. The comprehensive sensitivity analyses permitted an evaluation of the robustness of our primary findings and addressed potential violations of MR assumptions.

Statistical analysis

All MR analyses were performed via the TwoSampleMR package (version 0.5.7) in R (version 4.2.2) [44], whereas METAL (version 2020-05-05) in Linux (version Ubuntu 22.04) was used to perform meta-analyses of the MR results [45]. We set a significance threshold of $P_{FDR} < 0.05$ after correction for the false discovery rate (FDR) on the basis of the eleven multiple tests analyzed. The MR results were considered robust if they were confirmed by at least two different methods.

Results

Overview

Figure 1 provides a schematic representation of the study design. Our analysis focused on eleven NK cell-related traits selected after harmonizing the instrumental variables. All the SNPs had F statistics greater than 10, confirming their effectiveness as robust instruments (see Supplementary file 3: Tables S2A). From these, we identified four exposure-outcome pairs that showed robust genetic causal relationships (Table 1; Fig. 2). We performed separate MR analyses for the outcome GWASs from the UK Biobank and FinnGen, as well as a meta-analysis combining both cohorts. Unless otherwise

Table 1 Primary inverse variance weighted Mendelian randomization for natural killer cell related traits by infection phenotypes

Exposure		Cellulitis			Cystitis			Intestinal infection			Pneumonia		
	Number of IVs	OR (95% CI)	*P* value	P_{FDR}	OR (95% CI)	*P* value	P_{FDR}	OR (95% CI)	*P* value	P_{FDR}	OR (95% CI)	*P* value	P_{FDR}
NK Absolute Count	15	0.99 (0.93, 1.05)	0.733	0.733	1.03 (0.99, 1.08)	0.13	0.455	1.02 (0.99, 1.06)	0.167	0.866	1.01 (0.97, 1.05)	0.656	0.902
NK /lymphocyte	15	0.98 (0.96, 1.00)	0.12	0.44	1.00 (0.98, 1.03)	0.72	0.799	1.00 (0.98, 1.01)	0.63	0.866	1.00 (0.99, 1.02)	0.857	0.943
NK /CD3- lymphocyte	23	0.99 (0.97, 1.01)	0.276	0.657	1.01 (0.99, 1.02)	0.289	0.455	0.99 (0.98, 1.01)	0.361	0.866	1.00 (0.99, 1.01)	0.844	0.943
HLA-DR + NK Absolute Count	8	0.96 (0.91, 1.01)	0.086	0.44	1.02 (0.98, 1.06)	0.275	0.455	0.99 (0.96, 1.02)	0.593	0.866	0.96 (0.94, 0.99)	0.002	0.011
HLA-DR + NK /NK	17	0.97 (0.94, 1.00)	0.054	0.44	1.00 (0.97, 1.03)	0.911	0.911	0.99 (0.98, 1.01)	0.462	0.866	0.96 (0.95, 0.98)	<0.001	<0.001
HLA-DR + NK /CD3- lymphocyte	15	0.99 (0.95, 1.03)	0.555	0.678	1.02 (0.99, 1.05)	0.257	0.455	1.00 (0.98, 1.02)	0.891	0.928	0.97 (0.95, 0.99)	0.013	0.037
HLA-DR on HLA-DR + NK	17	0.99 (0.96, 1.01)	0.366	0.67	1.01 (0.98, 1.03)	0.596	0.799	1.00 (0.98, 1.02)	0.928	0.928	0.98 (0.97, 0.99)	0.006	0.022
CD45 on NK	18	1.01 (0.97, 1.04)	0.694	0.733	1.02 (1.00, 1.05)	0.106	0.455	1.01 (0.99, 1.03)	0.35	0.866	1.00 (0.98, 1.02)	0.975	0.975
CD45 on HLA-DR + NK	6	1.03 (0.95, 1.12)	0.49	0.674	0.99 (0.92, 1.06)	0.726	0.799	1.02 (0.98, 1.07)	0.318	0.866	0.99 (0.95, 1.03)	0.519	0.902
CD16-CD56 on NK	20	1.01 (0.98, 1.04)	0.459	0.674	0.98 (0.95, 1.00)	0.103	0.455	1.00 (0.98, 1.02)	0.919	0.928	1.00 (0.99, 1.02)	0.607	0.902
CD16-CD56 on HLA-DR + NK	14	0.98 (0.96, 1.01)	0.299	0.657	1.02 (0.99, 1.04)	0.169	0.455	1.00 (0.99, 1.02)	0.489	0.866	1.01 (0.99, 1.03)	0.441	0.902

The results presented here were based on meta-analyses combining data from the UK Biobank and the FinnGen project. The primary analysis employed the IVW method with an instrument inclusion threshold of 5×10^{-6}.
Abbreviations: NK, natural killer; IV, instrument variable; OR, odd ratio; CI, confidence interval; FDR, false discovery rate; IVW inverse variance weighted

stated, the results reported in our study were based on meta-analyses.

Primary MR analysis

To elucidate the genetic causal relationships between the immunophenotypes and infection risk, we conducted a two-sample MR analysis (Table 1; detailed results in Supplementary file 3: Table S2B, Table S2C, and Table S2D). The primary analysis utilized the IVW method with an instrument inclusion threshold of 5×10^{-6}.

After FDR adjustment, four significant associations between NK cell-related traits and pneumonia were identified (Fig. 2). An increased HLA-DR$^+$ NK absolute count was associated with a decreased risk of pneumonia (OR = 0.96, 95% confidence interval (CI) = 0.94–0.99, $P = 1.96 \times 10^{-3}$, $P_{FDR} = 0.011$). Similarly, an elevated HLA-DR$^+$ NK/NK ratio was correlated with a reduced pneumonia risk (OR = 0.96, 95% CI = 0.95–0.98, $P = 2.01 \times 10^{-6}$, P_{FDR} <0.001). The HLA-DR$^+$ NK/CD3- lymphocyte ratio also demonstrated an inverse association with pneumonia risk (OR = 0.97, 95% CI = 0.95–0.98, $P = 1.34 \times 10^{-2}$, P_{FDR} = 0.037). Additionally, HLA-DR expression on HLA-DR$^+$ NK cells was identified as a protective factor against pneumonia (OR = 0.98, 95% CI = 0.97–0.99, $P = 5.95 \times 10^{-3}$, $P_{FDR} = 0.022$).

Furthermore, our study revealed two NK cell-related traits as protective factors specifically against bacterial pneumonia (Fig. 3). The HLA-DR$^+$ NK absolute count was inversely associated with bacterial pneumonia risk (OR = 0.94, 95% CI = 0.91–0.97, $P = 6.15 \times 10^{-4}$, P_{FDR} = 0.003). Similarly, an elevated HLA-DR$^+$ NK/NK ratio was associated with a reduced likelihood of bacterial pneumonia (OR = 0.96, 95% CI = 0.94–0.98, $P = 3.00 \times 10^{-4}$, $P_{FDR} = 0.003$).

To further validate the genetic causal relationships between significant NK cell-related immunophenotypes and bacterial pneumonia or pneumonia in both primary and secondary analyses, additional MR methods, including weighted mode, weighted median, and MR-PRESSO analyses were employed (Fig. 3).

Notably, our analysis did not reveal any significant associations between NK cell-related traits and other phenotypes of infections, including cellulitis, cystitis, and intestinal infection (Fig. 2; Table 1).

Sensitivity analysis

For all four exposure-outcome pairs in primary analysis, no evidence of horizontal pleiotropy was identified (all $P > 0.05$). The MR Egger intercept test indicated the absence of directional pleiotropy (Table 2). The Cochran's Q test indicated the absence of significant heterogeneity (Qpval > 0.05 for both the IVW and MR Egger models) (Table 2). No outliers were identified in the course of the analysis.

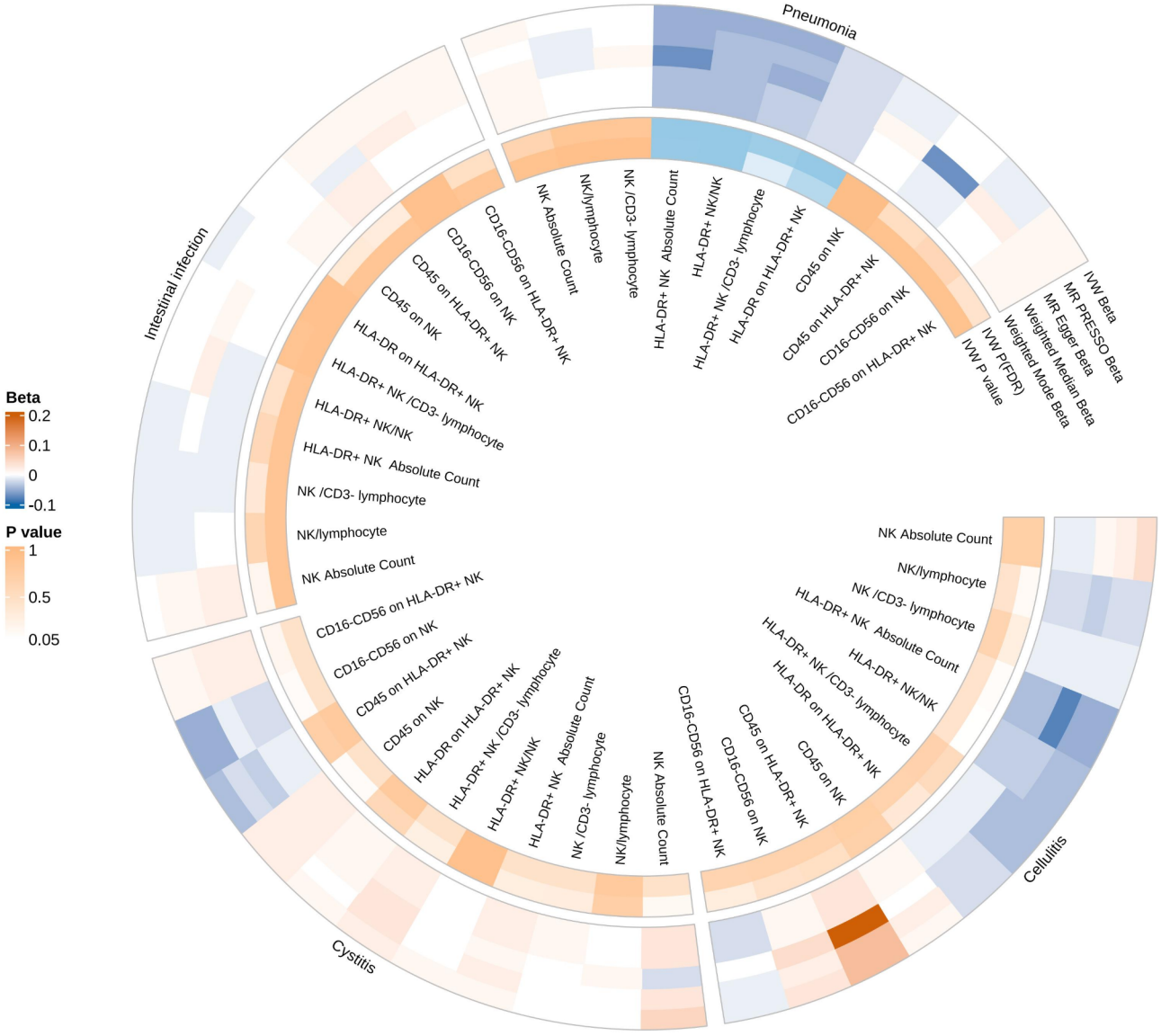


Fig. 2 A heatmap of Mendelian randomization for natural killer cell related traits on the risk of infection. The MR estimates and *P* values were illustrated in the plot. The outer circle employed a color scheme to represent the direction of the estimate effect (beta), whereas the inner circle utilized a different color scheme to indicate the *P* values and *P* values with FDR correction of IVW analyses. The results were derived from a meta-analysis of data from the UK Biobank and the FinnGen project. The primary analysis employed the IVW method with an instrument inclusion threshold of 5×10^{-6}. Abbreviations: NK, natural killer; MR, Mendelian randomization; IVW, inverse variance weighted; MR-PRESSO Mendelian Randomization Pleiotropy RESidual Sum and Outlier; FDR, false discovery rate.

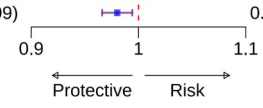
Sensitivity analyses provided further confirmation of the robustness of the observed genetic causal effects of genetic components (Table 2; Supplementary file 3: Table S2D). The stability of the results was further corroborated by scatter plots, density plots, and funnel plots (see Supplementary file 2 S1–S3). The results of the leave-one-out analyses are presented in Supplementary file 2 S4.

Secondary MR analysis

We also conducted a secondary analysis via the IVW method with less stringent criteria for instrumental variable selection ($P<1\times10^{-6}$), revealed several associations after adjustment for FDR (detailed in Supplementary file 3: Tables S3A-3E). Our secondary analysis revealed three significant associations between NK cell-related traits and cellulitis, and one significant association with pneumonia. Specifically, an increased HLA-DR$^+$ NK absolute count was associated with a decreased risk of cellulitis (OR=0.96, 95% CI=0.93–0.98, P_{FDR} = 0.012), whereas an increased HLA-DR$^+$ NK/NK ratio was also correlated with a decreased risk of cellulitis (OR=0.97, 95% CI=0.94–0.99, P_{FDR} = 0.018). For pneumonia, the

A Pneumonia

exposure	Number of IVs	OR (95% CI)	P value	P value(FDR)
HLA-DR+ NK Absolute Count	8			
Inverse variance weighted		0.96 (0.94,0.99)	0.002	0.011
MR Egger		0.93 (0.90,0.97)	0.008	
Weighted median		0.95 (0.93,0.98)	<0.001	
Weighted mode		0.95 (0.93,0.97)	0.003	
MR-PRESSO		0.96 (0.94,0.99)	0.017	
HLA-DR+ NK/NK	17			
Inverse variance weighted		0.96 (0.95,0.98)	<0.001	<0.001
MR Egger		0.96 (0.94,0.98)	0.007	
Weighted median		0.96 (0.94,0.98)	0.001	
Weighted mode		0.95 (0.93,0.98)	0.002	
MR-PRESSO		0.96 (0.95,0.98)	<0.001	
HLA-DR+ NK /CD3- lymphocyte	15			
Inverse variance weighted		0.97 (0.95,0.99)	0.013	0.037
MR Egger		0.95 (0.92,0.99)	0.017	
Weighted median		0.96 (0.93,0.98)	<0.001	
Weighted mode		0.95 (0.93,0.98)	0.002	
MR-PRESSO		0.97 (0.95,0.99)	0.027	
HLA-DR on HLA-DR+ NK	17			
Inverse variance weighted		0.98 (0.97,0.99)	0.006	0.022
MR Egger		0.98 (0.96,1.00)	0.093	
Weighted median		0.98 (0.96,1.00)	0.119	
Weighted mode		0.98 (0.97,1.00)	0.133	
MR-PRESSO		0.98 (0.97,0.99)	0.014	



B Bacterial pneumonia

exposure	Number of IVs	OR (95% CI)	P value	P value(FDR)
HLA-DR+ NK Absolute Count	8			
Inverse variance weighted		0.94 (0.91,0.97)	0.001	0.003
MR Egger		0.93 (0.87,0.98)	0.042	
Weighted median		0.94 (0.90,0.97)	0.001	
Weighted mode		0.94 (0.90,0.97)	0.008	
MR-PRESSO		0.94 (0.91,0.97)	0.011	
HLA-DR+ NK/NK	17			
Inverse variance weighted		0.96 (0.94,0.98)	<0.001	0.003
MR Egger		0.95 (0.91,0.98)	0.012	
Weighted median		0.95 (0.92,0.98)	0.003	
Weighted mode		0.94 (0.91,0.98)	0.01	
MR-PRESSO		0.96 (0.94,0.98)	0.002	
HLA-DR+ NK /CD3- lymphocyte	15			
Inverse variance weighted		0.96 (0.93,1.00)	0.039	0.111
MR Egger		0.93 (0.88,0.99)	0.042	
Weighted median		0.94 (0.90,0.97)	0.001	
Weighted mode		0.94 (0.90,0.97)	0.003	
MR-PRESSO		0.96 (0.93,1.00)	0.058	
HLA-DR on HLA-DR+ NK	17			
Inverse variance weighted		0.98 (0.96,1.00)	0.041	0.111
MR Egger		0.97 (0.95,1.00)	0.064	
Weighted median		0.98 (0.96,1.01)	0.19	
Weighted mode		0.99 (0.96,1.01)	0.244	
MR-PRESSO		0.98 (0.96,1.00)	0.057	

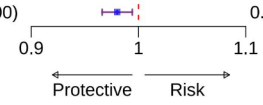


Fig. 3 A forest plot illustrating the genetic causal effect of the selected natural killer cell traits on the risk of pneumonia and bacterial pneumonia. The results were based on meta-analyses combining data from the UK Biobank and FinnGen. The primary analysis employed the IVW method with an instrument inclusion threshold of 5×10^{-6}. Abbreviations: IV, instrument variable; OR, odds ratio; CI, confidence interval; FDR, false discovery rate; NK, natural killer; MR, Mendelian randomization; MR-PRESSO Mendelian Randomization Pleiotropy RESidual Sum and Outlier

Table 2 Primary sensitivity analysis for the genetic causal effects of natural killer cell related traits and the risk of infection

		Pleiotropy			Heterogeneity						Directionality	
Outcome	**Exposure**	**Egger intercept**	**Egger se**	**Egger pval**	**IVW Q**	**IVW Qdf**	**IVW Qpval**	**Egger Q**	**Egger Qdf**	**Egger Qpval**	**Correct causal direction**	**Steiger pval**
Pneumonia	HLA-DR + NK Absolute Count	1.00E-02	4.75E-03	0.08	9.86	7	0.20	5.42	6	0.49	TRUE	3.16E-195
	HLA-DR + NK/NK	1.03E-03	3.39E-03	0.77	12.11	16	0.74	12.02	15	0.68	TRUE	9.36E-249
	HLA-DR + NK/CD3- lymphocyte	6.52E-03	4.37E-03	0.16	21.01	14	0.10	17.94	13	0.16	TRUE	3.63E-248
	HLA-DR on HLA-DR + NK	3.11E-05	3.57E-03	0.99	19.54	16	0.24	19.54	15	0.19	TRUE	4.86E-212
Bacterial pneumonia	HLA-DR + NK Absolute Count	5.10E-03	8.28E-03	0.56	8.49	7	0.29	7.98	6	0.24	TRUE	4.22E-195
	HLA-DR + NK/NK	4.29E-03	5.15E-03	0.42	15.15	16	0.51	14.46	15	0.49	TRUE	4.49E-249

The results presented here were based on meta-analyses combining data from the UK Biobank and the FinnGen project. The primary analysis employed the IVW method with an instrument inclusion threshold of 5×10^{-6}

Abbreviations: NK, natual killer; IVW, inverse variance weighted; se, standard error; Q, Cochran's Q test; pval, *P* value; df, degree of freedom

HLA-DR$^+$ NK/CD3 lymphocyte ratio was inversely associated with risk (OR=0.97, 95% CI=0.95–0.98, P_{FDR} = 0.019), and an increased HLA-DR$^+$ NK/NK ratio was identified as a protective factor (OR=0.98, 95% CI=0.97–0.99, P_{FDR} = 0.032). Interestingly, no significant associations were found between NK cell-related traits and other infection phenotypes, including cystitis and intestinal infection, suggesting the potential specificity of the protective role of NK cells against certain types of infection.

However, it is important to note that sensitivity analyses for all four exposure-outcome pairs in this secondary analysis yielded inconsistent results, as detailed in Supplementary file 3: Table S3D.

Steiger directionality test

The Steiger directionality test provided further evidence to support the hypothesis that the immunophenotypes of interest were genetically causally related to bacterial pneumonia or pneumonia risk. No evidence of reverse genetic causality was identified (see Supplementary file 3: Table S2E), which reinforces the directionality of the observed associations.

Discussion

Our comprehensive MR analysis provides compelling evidence for a genetically causal relationship between specific NK cell-related traits and the risk of pneumonia, particularly bacterial pneumonia. The primary analysis, with stringent criteria for instrumental variable selection, reveals four significant associations between NK cell-related traits and pneumonia risk. Notably, increased HLA-DR$^+$ NK absolute counts, increased HLA-DR$^+$ NK/NK ratios, increased HLA-DR$^+$ NK/CD3$^-$ lymphocyte ratios, and increased HLA-DR expression on HLA-DR$^+$ NK cells are all associated with reduced pneumonia risk. Similarly, an elevated HLA-DR$^+$ NK/NK ratio is associated with a reduced likelihood of bacterial pneumonia. Our analysis does not reveal significant associations between NK cell-related traits and other common bacterial infection, such as cellulitis, cystitis, or intestinal infection in the primary analysis. The robustness of our findings is supported by consistent results across multiple MR methods and sensitivity analyses. The absence of significant horizontal pleiotropy and heterogeneity in most cases, along with the results of the Steiger directionality test, further strengthens the validity of our genetic causal inferences. However, the discrepancies observed in our secondary analysis with less stringent criteria highlight the importance of rigorous instrumental variable selection in MR studies. While this analysis suggests potential associations with cellulitis, the inconsistent sensitivity analysis results call for cautious interpretation.

Recent advances in the field of NK cell biology have revealed a complex landscape of heterogeneous subpopulations with diverse functional characteristics. The traditional classification methods, which are primarily based on the expression of surface markers, have identified CD56 and CD16 as the key markers for subsets of NK cells. The $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{bright}$ subsets, which collectively constitute up to 10% and at least 90% of the total NK cell population, respectively, exhibit distinct phenotypic and functional profiles. The former is predominantly associated with cytokine production, whereas the latter demonstrates potent cytotoxic activity. Further research has led to the expansion of this classification, with the identification of four additional subpopulations: the additional subpopulations identified are $CD56^{bright}CD16^{dim}$, $CD56^{dim}CD16^{-}$, $CD56^{-}CD16^{bright}$, and $CD56^{dim}CD16^{dim}$ [46, 47]. This expanded classification highlights the complex heterogeneity within the NK cell population. However, the advent of sophisticated single-cell analytical techniques has further refined our understanding, revealing a landscape of NK cells that is more nuanced and complex than previously recognized. The functional diversity of NK cells is regulated by a complex interplay between inhibitory and activating receptors. Inhibitory receptors, including killer cell immunoglobulin-like receptors, CD94/NKG2A, ILT2, and TIGIT, interact with activating receptors such as NKG2D and the natural cytotoxicity receptors NKp46, NKp30, and NKp44 [48]. This intricate interplay of receptor-mediated signaling modulates NK cell responses, enabling them to exhibit natural cytotoxicity against abnormal cells, perform antibody-dependent cellular cytotoxicity, and produce various cytokines and growth factors [48, 49]. The application of advanced single-cell technologies has been pivotal in elucidating the true extent of NK cell heterogeneity. These methodologies have revealed a spectrum of NK cell states and subpopulations that were previously indiscernible, challenging traditional binary classifications and suggesting a more fluid developmental process [50]. Importantly, that the current study did not establish a genetic causal relationship between $CD16^{-}CD56^{+}$ expression on NK cells and infections. This finding is in accordance with the evolving perspective on NK cell biology, which emphasizes the complexity of NK cell subsets and their functional plasticity, which extends beyond the limitations of simple surface marker-based classifications.

Although peripheral blood has been the primary source for NK cell studies owing to its accessibility, emerging evidence suggests that tissue-specific microenvironments play a crucial role in shaping NK cell phenotypes. Dogra et al. proposed a model in which the characteristics of NK cells are closely linked to their anatomical location, irrespective of age and sex [51–54]. These findings emphasize the importance of considering tissue-specific factors when studying NK cell biology and function. However, a recent study focusing on the lung, a major site of NK cell distribution, demonstrated that the proportion of NK cells in lung tissue is comparable to, or slightly greater than, that in peripheral blood [55]. This distribution differs from that observed in NK cell populations in other tissues, such as the liver and other lymphoid organs [52, 56, 57]. In light of these findings, it seems reasonable to conclude that our study's focus on peripheral blood NK cells provides a suitable proxy for the lung NK cell status, particularly in the context of pneumonia and bacterial pneumonia. This approach is likely to yield more significant results for pulmonary infections than for infections at other anatomical sites.

HLA-DR expression in NK cells was identified as a marker of cellular activation. $HLA\text{-}DR^{+}$ NK cells were identified as a subset associated with proliferation and activation status. In healthy humans, the proportion of HLA-DR $^{+}$ NK cells among all NK cells in peripheral blood and immunocompetent organs, including the spleen and liver, is typically between 0% and 37.7%. However, these cells are present in notably greater amounts in lymph nodes and adenoid [58–60]. $HLA\text{-}DR^{+}$ NK cells play pivotal roles in a number of different aspects of immunity. In vitro, certain cytokines, including interleukin-18, interleukin-21, interleukin-2 and interleukin-15, have been demonstrated to induce the expression of HLA-DR on NK cells [61–65]. $HLA\text{-}DR^{+}$ NK cells expand through interactions with dendritic cells, macrophages, and neutrophils [66–69]. NK cells can acquire MHC II (including HLA-DR) through membrane contact with dendritic cells [68, 69]. These findings suggest potential mechanisms by which the immune system can rapidly activate NK cells during infections and orchestrate a coordinated defense against infections. Similarly, $HLA\text{-}DR^{+}$ NK cells have been linked to elevated IFN-γ production, as observed in $CD16^{-}CD56^{+}$ NK cells [60, 62, 64, 69]. Furthermore, IFN-γ has been demonstrated to induce high HLA-DR expression in NK cells. Furthermore, $HLA\text{-}DR^{+}$ $CD11c^{+}$ NK cells appear to be capable of ingesting and subsequently presenting particular antigens to $CD4^{+}$ and $CD8^{+}$ $CD11c^{+}$ T cells, thereby triggering their activation and proliferation [59, 70, 71]. This finding suggests potential involvement in antigen presentation, acting as a conduit between innate and adaptive immunity. An elevated level of circulating $HLA\text{-}DR^{+}$ NK cells has been observed in certain pathological conditions, including viral and bacterial infections [70, 72–76]. A high proportion of circulating $HLA\text{-}DR^{+}$ cells are detected in patients infected with human immunodeficiency virus, hepatitis C virus, human cytomegalovirus and dengue virus [66, 71, 73, 77–79]. These cells were found to play a protective role in the immune response,

demonstrating a correlation with the first line of defense, IFN-γ production, cytotoxicity and antigen presentation. It is hypothesized that enhancing the activity of HLA-DR$^+$ NK cells could result in more favorable outcomes in cases of viral and bacterial infections. It is further postulated that a genetic predisposition to generate a greater proportion of HLA-DR$^+$ NK cells in response to infections could confer enhanced protection against bacterial pneumonia or pneumonia.

The present study was characterized by several strengths and weaknesses. To guarantee the dependability of our study, we implemented a series of comprehensive measures, including enrollment in the most recently published, high-quality GWAS on the immune-cell spectrum. The other GWAS database on NK cells, which includes 46 subtypes, was not included in the present study since it only included females and the sample size was relatively small. Notably, in addition to the limitations of the MR methodology, which was previously reviewed [80], this study was also subject to several other limitations. (1) MR analysis based on populations of European ancestry would limit the generalizability of the findings. (2) The discrepancies observed in our secondary analysis with less stringent criteria highlighted the importance of rigorous instrumental variable selection in MR studies. Although this analysis indicated the possibility of an association with cellulitis, the inconsistent results of the sensitivity analysis precluded a definitive interpretation. (3) The utilization of the ICD for diagnosis enhances diagnostic accuracy, which might lead to several notable limitations. It was possible that milder infection or those managed in outpatient settings without a formal diagnosis might be underrepresented in the dataset. The study did not differentiate between infection on the basis of their causative agents, which precluded analysis of potential variations in NK cell responses to different pathogens. The observation that each subject in the study experienced only a few infections throughout their lifetime suggested that our dataset predominantly captured severe infections or instances of multiple infections. It was important to consider these limitations when attempting to generalize the findings to broader infectious disease contexts. (4) Transient factors, including age and lifestyle, might influence the NK cell-related traits captured at a particular moment. It was therefore possible that these factors did not accurately represent the lifelong immune properties dictated by the gene in question. Further investigations using large clinical cohorts are needed to elucidate the precise roles and mechanisms of NK cells during the onset and progression of certain infections. Such insights could inform the development of NK cell-based immunotherapies for specific patients. It would be advantageous for future studies to adopt a combined approach, integrating genetic, immunological and pharmacological perspectives, to gain a deeper understanding of the dualistic functions of NK cells in the context of infection pathogenesis.

Conclusions

In conclusion, our findings revealed a negative correlation between a high absolute HLA-DR$^+$ NK count and the risk of developing bacterial pneumonia and all types of pneumonia. Moreover, the ratio of HLA-DR$^+$ NK to CD3$^-$ lymphocytes was identified as a factor associated with a reduced risk of bacterial pneumonia and all forms of pneumonia. These findings constitute a novel discovery in the field of research. While the intricate roles of NK cells in infections continue to unfold, this study has shed light on specific HLA-DR$^+$ NK cell subsets that might be crucial in determining the risk of infection.

Abbreviations

NK	Natural killer
IFN-γ	Interferon-γ
MR	Mendelian randomization
RCT	Randomized controlled trial
GWAS	Genome-wide association study
HLA	Human leukocyte antigen
SNP	Singlesingle-nucleotide polymorphism
SAIGE	Scalable and Accurate Implementation of GEneralized mixed model
ICD	International Classification of Diseases
IVW	Inverseinverse-variance weighted
MR-PRESSO	Mendelian Randomization Pleiotropy RESidual Sum and Outlier
OR	Odds ratio
FDR	False discovery rate
CI	Confidence interval

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

All authors have approved the submitted final version and are to be personally accountable for their contributions. YXL wrote the paper taking into account all the coauthors' comments and suggestions and was responsible for the final content, conducting the literature search, and analyzing the data. SZ, XQW, and JSW advised on the analyses and drafted the paper. LH designed the study, advised on the analyses and visualization, and supervised the study. All authors revised the paper, interpreted the results, and read and approved the final manuscripts.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable. Only publicly available summary statistics were used.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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