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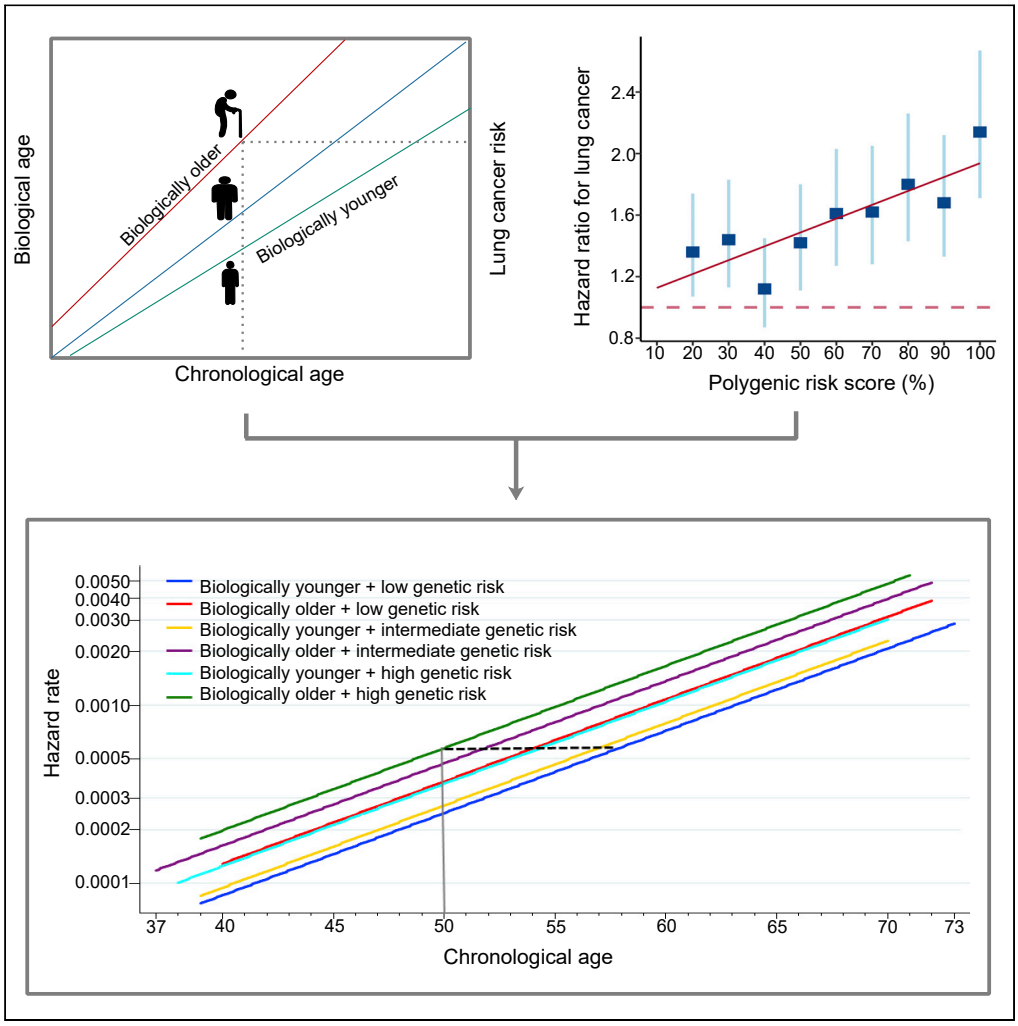

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Highlights

PhenoAgeAccel is an independent risk for lung cancer

Biological aging and genetics jointly contributed to the incidence of lung cancer

PhenoAgeAccel and PRS could facilitate the risk assessment of lung cancer

Ma et al., iScience 26, 106018
March 17, 2023
https://doi.org/10.1016/j.isci.2023.106018

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Association between biological aging and lung cancer risk: Cohort study and Mendelian randomization analysis

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SUMMARY

Chronological age only represents the passage of time, whereas biological age reflects the physiology states and the susceptibility to morbidity and mortality. The association between biological age and lung cancer risk remains controversial. Hence, we conducted a prospective analysis in the UK Biobank study and two-sample Mendelian randomization analysis to investigate this association. Biological aging was evaluated by PhenoAgeAccel, derived from routine clinical biomarkers. Independent of chronological age, PhenoAgeAccel was positively associated with the risk of overall and histological subtypes of lung cancer. There was a joint effect of PhenoAgeAccel and genetics in lung cancer incidence. In Mendelian randomization analysis, the genetically predicted PhenoAgeAccel was associated with the increased risk of overall lung cancer, small cell, and squamous cell carcinoma. Our findings suggest PhenoAgeAccel is an independent risk factor for lung cancer, which could be incorporated with polygenic risk score to identify high-risk individuals for lung cancer.

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide, with an estimated 2.2 million new cancer cases and 1.8 million deaths in 2020.[1] Apart from smoking, aging is one of the most important determinants of risk for lung cancer.[2] Although everyone ages, the rate at which aging occurs is heterogeneous, and between-person variations in the pace of aging mostly manifest as differences in biological aging and susceptibility to disease.[3,4] Hence, chronological age alone is not sufficient to reflect the state of biological aging.

A novel multi-system-based aging measure, namely Phenotypic age (PhenoAge), has been developed and widely validated to be a surrogate for biological aging in recent studies.[5,6] PhenoAge was derived from 9 multi-system clinical chemistry biomarkers. Phenotypic age acceleration (PhenoAgeAccel), which indicates the difference between biological age and chronological age, has been proven to be an effective biomarker to differentiate risk for several health outcomes. Previous studies have showed that biological aging measured by telomere length or DNA methylations was associated with the risk of lung cancer, but these findings remain arguable.[7–9] However, these molecular biomarkers are unlikely to be routinely used in large populations due to expensive and time-consuming testing. Hence, PhenoAgeAccel, derived from routinely collected clinical biomarkers, could provide a powerful tool to predict and monitor healthspan as well as age-related illnesses such as type 2 diabetes.[10–13] Since different indicator of biological aging capturing distinct underlying senescence mechanisms, the role of PhenoAgeAccel in the development of lung cancer needed to be further evaluated.

Genetics also plays an important role in the development of lung cancer. Polygenic risk score (PRS), which was derived from genome-wide associations studies (GWAS) by combining means of weighted sum of allele counts, has been proven to be effective tools to quantify individuals' genetic risk of lung cancer in recent studies.[14,15] The association between PhenoAgeAccel and lung cancer risk in different genetic risk groups, and the potential interactions, as well as joint effects were also needed to be further evaluated.

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https://doi.org/10.1016/j.isci.2023.106018

In this study, we first investigate the association between PhenoAgeAccel and lung cancer risk based on the UK Biobank cohort study, and then assess whether PhenoAgeAccel could provide an additional predictive value of lung cancer risk independent from chronological age. Furthermore, the joint and interactive effects between PhenoAgeAccel and PRS on the risk of lung cancer were also analyzed. To further account for residual confounding, reverse causation, or regression dilution,[16] we also used two-sample Mendelian randomization (MR) to investigate the causal association between PhenoAgeAccel and lung cancer risk.

RESULTS

Study characteristics

Of 385,074 participants in the UK Biobank cohort, 1,775 lung cancer incident events were diagnosed over a median follow-up of 7.2 years (interquartile range: 6.4–7.7). Although the average chronological ages of the two groups were almost similar (56.3 years for both biologically younger and biologically older group), an average of 7.9 years difference in clinically measured biological aging were observed between these two groups (Table 1).

Association between PhenoAgeAccel and lung cancer risk

Figure 1A shows that individuals with incident lung cancer had higher PhenoAgeAccel than those without lung cancer during follow-up. After adjusting for chronological age and other covariates, per 5-year increase in PhenoAgeAccel was associated with an increased risk of lung cancer by 31% (hazard ratio (HR) = 1.31, 95% confidence intervals (CI): 1.26–1.37, $p < 0.001$) (Table 2). There was a significantly gradient increase in lung cancer risk from decile 1 to 10 of PhenoAgeAccel with $P_{trend}<0.001$ (Figure 1B). Figure 1C shows that compared with biologically younger group, biologically older group was associated with a higher risk for lung cancer (HR = 1.62, 95%CI: 1.46–1.80, $p < 0.001$). Furthermore, compared with participants of low accelerated aging (the bottom quintile of PhenoAgeAccel), those of intermediate (quintiles 2–4) and high accelerated aging group (the top quintile) had higher risk of lung cancer, with HR of 1.42 (95%CI: 1.21–1.67, $p < 0.001$) and 2.24 (95%CI: 1.88–2.66, $p < 0.001$), respectively (Figure 1D). Subgroup analysis showed the significant dose-response trend between PhenoAgeAccel and lung cancer risk was consistent across different histological subtypes of lung cancer (Table S1). Table S2 shows that stronger associations were observed among men than women and among previous/current smokers than never smoking. In sensitivity analysis, these results were robust by the exclusion of individuals with incomplete covariates (Table S3) and those with lung cancer event within the first two years of follow-up (Table S4), as well as under competing risk of death (Table S5). Further adjusting for additional smoking-related information or other lifestyle factors, PhenoAgeAccel was still positively associated with lung cancer risk (Tables S6 and S7).

PhenoAgeAccel provides additional information in the prediction of lung cancer independent of age

We then evaluated the impact of PhenoAgeAccel on the prematurity of lung cancer incidence using the rate advancement period (RAP). Biologically older group was expected to advance their risk of lung cancer about 4.51 years (RAP 95% CI: 3.49–5.53) comparing with biologically younger group (Table 2 & Figure S2). For instance, a biologically older participant at 50.0-year-old almost had the same lung cancer risk as a biologically younger participant at 54.5-year-old. Subgroup analysis also showed the association between PhenoAgeAccel and lung cancer risk was consistent across different age groups (Table S2). These findings indicated that PhenoAgeAccel, independent of chronological age, could provide additional information on the association between biological aging and lung cancer risk. Furthermore, we observed that participants with higher PhenoAgeAccel levels had a higher 5-year absolute risk of lung cancer than their peers with lower PhenoAgeAccel levels (Figure 2A). Besides, adding PhenoAgeAccel to the basic model (chronological age and smoking status) could improve the predictive performance (area under the ROC curve (AUC) = 0.83 vs AUC = 0.82, $p < 0.001$; continuous net reclassification index = 10.5%, 95%CI: 2.1%–12.9%) (Figure 2B).

The joint impact of PhenoAgeAccel and genetic risk

The PRS was constructed and significantly associated with the risk of lung cancer in our dataset (Figure S3). Compared to participants with low genetic risk, the multivariable HR among participants with high genetic risk was 1.48 (95%CI, 1.32–1.66, $p < 0.001$) (Table S8). Besides, there was weak correlation ($r = 0.08$) between PRS and PhenoAgeAccel (Figure S4). When combining PhenoAgeAccel and genetic risk, we observed the

Table 1. Baseline characteristics of participants

	Overall (N=385,074)	Biologically younger (N=210,582)	Biologically older (N=174,492)
PhenoAgeAccel, years[a]	0.0 ± 5.1	−3.6 ± 2.5	4.3 ± 3.9
Chronological age, years	56.3 ± 8.1	56.3 ± 8.0	56.3 ± 8.3
Sex, n (%)			
Women	202,643 (52.6)	125,122 (59.4)	77,521 (44.4)
Men	182,431 (47.4)	85,460 (40.6)	96,971 (55.6)
Ethnicity, n (%)			
Non-White	17,325 (4.5)	8,528 (4.1)	8,797 (5.0)
White	362,370 (94.1)	199,225 (94.6)	163,145 (93.5)
Unknown	5,379 (1.4)	2,829 (1.3)	2,550 (1.5)
Education, n (%)			
No degree	255,080 (66.2)	133,541 (63.4)	121,539 (69.7)
Degree	125,409 (32.6)	74,850 (35.5)	50,559 (29.0)
Unknown	4,585 (1.2)	2,191 (1.1)	2,394 (1.3)
Townsend deprivation index	−1.3 ± 3.1	−1.6 ± 3.0	−1.0 ± 3.2
BMI, kg/m^2	27.4 ± 4.8	26.4 ± 4.0	28.7 ± 5.2
Smoking status, n (%)			
Never	210,874 (54.8)	123,124 (58.5)	87,750 (50.3)
Former	131,699 (34.2)	72,087 (34.2)	59,612 (34.2)
Current	40,592 (10.5)	14,494 (6.9)	26,098 (15.0)
Unknown	1,909 (0.5)	877 (0.4)	1,032 (0.6)
Family history of lung cancer, n (%)			
No	337,833 (87.7)	185,296 (88.0)	152,537 (87.4)
Yes	47,241 (12.3)	25,286 (12.0)	21,955 (12.6)
History of asthma, n (%)			
No	340,667 (88.5)	188,988 (89.8)	151,679 (86.9)
Yes	44,407 (11.5)	21,594 (10.2)	22,813 (13.1)
History of allergy and/or eczema, n (%)			
No	295,729 (76.8)	160,244 (76.1)	135,485 (77.7)
Yes	89,345 (23.2)	50,338 (23.9)	39,007 (22.3)
History of emphysema and/or bronchitis, n (%)			
No	378,847 (98.4)	208,391 (99.0)	170,456 (97.7)
Yes	6,227 (1.6)	2,191 (1.0)	4,036 (2.3)
Albumin, g/L	45.2 ± 2.5	45.8 ± 2.4	44.6 ± 2.5
Alkaline phosphatase, U/L	82.9 ± 22.5	79.4 ± 20.7	87.2 ± 23.8
Creatinine, umol/L	72.2 ± 14.2	68.8 ± 12.4	76.3 ± 15.2
Glucose, mmol/l	5.1 ± 1.0	4.9 ± 0.5	5.4 ± 1.2
C-reactive protein, mg/dL	0.3 ± 0.3	0.2 ± 0.2	0.4 ± 0.4
Lymphocyte percent, n (%)	28.9 ± 7.1	30.9 ± 6.8	26.6 ± 6.8
Mean cell volume, fL	91.1 ± 4.3	91.0 ± 3.8	91.3 ± 4.8
Red cell distribution width, n (%)	13.5 ± 0.9	13.1 ± 0.5	13.9 ± 0.9
White blood cell count, 1000 cells/ul	6.9 ± 1.7	6.3 ± 1.4	7.5 ± 1.8

Abbreviation: BMI, body mass index; PhenoAgeAccel, Phenotypic age acceleration.

Data are presented as mean ± SD for continuous variables and n (%) for categorical variables.

[a]PhenoAgeAccel represents the residual of PhenoAge regression on chronological age. The average PhenoAgeAccel in the biologically younger group was −3.6 ± 2.5 years, suggesting 3.6 years younger than chronological age. The mean PhenoAgeAccel in the biologically older group was 4.3 ± 3.9 years, indicating 4.3 years older than chronological age.

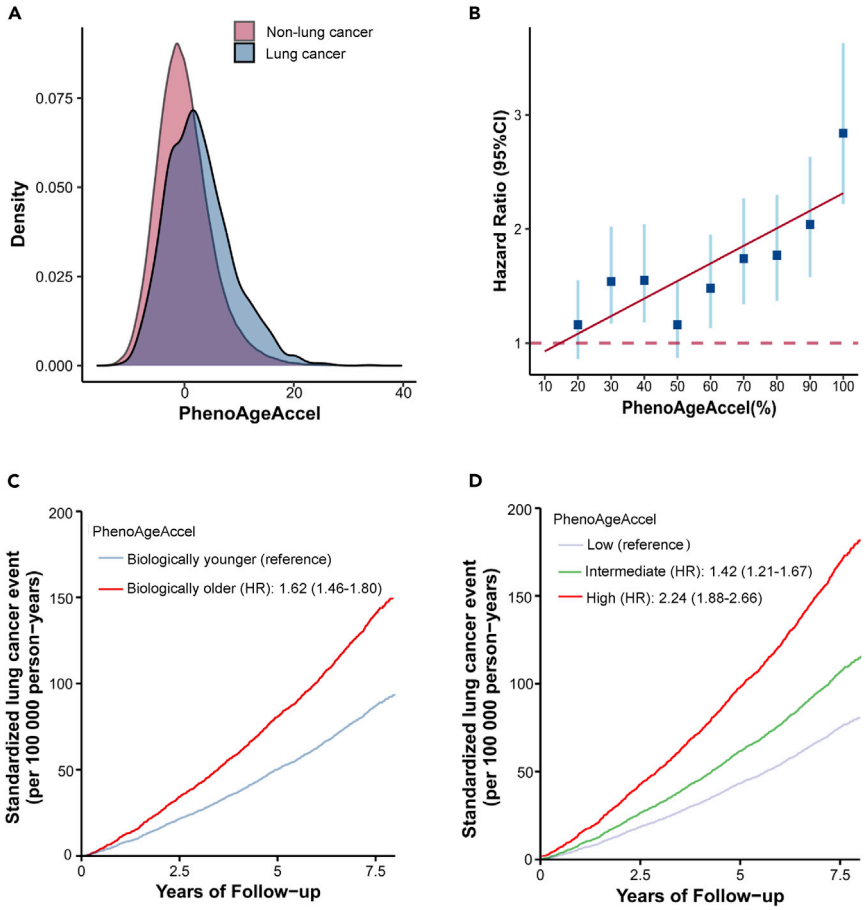

Figure 1. Distribution of PhenoAgeAccel and association between PhenoAgeAccel and lung cancer

(A) The distribution of PhenoAgeAccel across non-lung cancer and lung cancer; (B) Individuals were split equally into ten groups based on PhenoAgeAccel, and the HR was estimated for each group in comparison with the first group (P_{trend}<0.001); (C) (D)The standardized cumulative incidence of rate among participants with biologically older and younger, as well as individuals with low accelerated aging (the bottom quintile of PhenoAgeAccel), intermediate accelerated aging (quintiles 2–4), and high accelerated aging (the top quintile). Abbreviation: PhenoAgeAccel, Phenotypic age acceleration.

joint effect of PhenoAgeAccel and PRS on the development of lung cancer. Specifically, compared to participants with low genetic risk and biologically younger, those with high genetic risk and biologically older had the higher risk of lung cancer (HR = 2.42, 95% CI: 2.01–2.90, p < 0.001) with 8.16 years advancement in lung cancer occurrence (RAP 95% CI: 6.37–9.95) (Figure 3). A similar joint impact was noted in sensitivity analyses (Tables S9 and S10 and Figure S5), while there was no interaction between PhenoAgeAccel and PRS (Table S11).

Causal association between PhenoAgeAccel and lung cancer risk

To further confirm the association between PhenoAgeAccel and lung cancer risk, we used the genetic variants of PhenoAgeAccel as instruments to perform MR analysis. After removal of the outliers that was identified by Radial MR plots (Figure S6), neither heterogeneity (p = 0.204) or unbalanced pleiotropy (*P* for MR-PRESSO Global Test = 0.198) was observed (Table S12). Based on the remaining 82 genetic variants, we found that genetically predicted PhenoAgeAccel was associated with lung cancer risk (IVW method: OR = 1.02, 95% CI: 1.00–1.04, p = 0.041) (Table 3). In the subgroup analysis according to histopathological type, the MR analyses demonstrated that PhenoAgeAccel was causally associated with the increased risk of small-cell lung cancer (IVW: OR = 1.06, 95% CI: 1.01–1.12, p = 0.013) and squamous cell lung cancer (IVW: OR = 1.07, 95% CI: 1.04–1.10, p < 0.001). Leave-one-out sensitivity analysis also showed the similar results (Figure S7).

Table 2. Association between PhenoAgeAccel and lung cancer risk

	N (cases)	Person-Years	Model 1			Model 2		
			HR (95% CI)	*p* value	RAP (95% CI)	HR (95% CI)	*p* value	RAP (95% CI)
Per 5-year increase	385,074 (1,775)	2,718,602	1.49 (1.44–1.55)	<0.001	3.91 (3.43–4.39)	1.31 (1.26–1.37)	<0.001	2.53 (2.09–2.97)
Category[a]								
Biologically younger	210,582 (613)	1,497,199	Ref.		Ref.	Ref.		Ref.
Biologically older	174,492 (1,162)	1,221,402	2.14 (1.94–2.37)	<0.001	7.36 (6.24–8.48)	1.62 (1.46–1.80)	<0.001	4.51 (3.49–5.53)
Category[b]								
Low accelerated aging	77,015 (182)	550,275	Ref.		Ref.	Ref.		Ref.
Intermediate accelerated aging	231,044 (906)	1,633,407	1.68 (1.43–1.98)	<0.001	5.03 (3.43–6.63)	1.42 (1.21–1.67)	<0.001	3.30 (1.76–4.84)
High accelerated aging	77,015 (687)	534,920	3.51 (2.96–4.16)	<0.001	12.13 (10.26–14.00)	2.24 (1.88–2.66)	<0.001	7.51 (5.79–9.23)
P_{trend}			<0.001			<0.001		

Abbreviation: HR, hazard ratio; CI, confidence interval; RAP, rate advancement period; BMI, body mass index.

Model 1: Adjusting for chronological age, sex, ethnicity, center, education, Townsend deprivation index, and BMI.

Model 2: Additionally adjusted for smoking status, family history of lung cancer, history of asthma, history of allergy and/or eczema, and history of emphysema and/or bronchitis.

[a]Biologically older and younger respectively represent PhenoAgeAccel >0 and <0.

[b]Low accelerated aging refers to the bottom quintile of PhenoAgeAccel (quintile 1: < −4.15 years); intermediate accelerated aging refers to quintiles 2–4 (−4.15 to 3.69 years); high accelerated aging refers to the top quintile groups (quintile 5: 3.69 to 39.64 years).

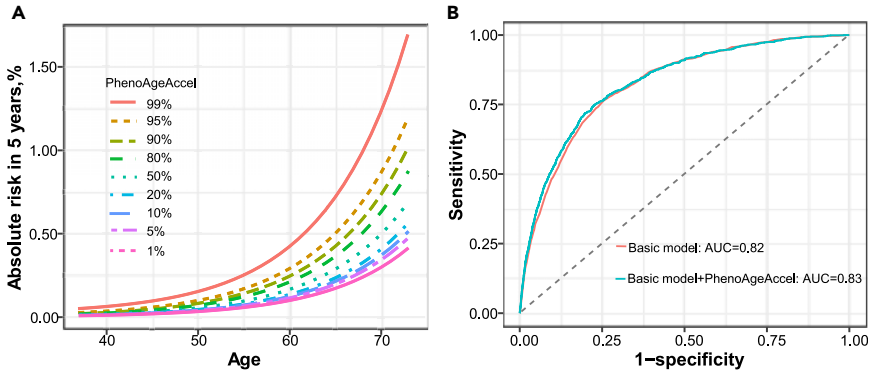

Figure 2. PhenoAgeAccel could provide additional information for lung cancer risk assessment

(A) Absolute risk estimates of lung cancer by different PhenoAgeAccel; (B) Receiver operating characteristic (ROC) curve and corresponding area under the ROC curve (AUC). Abbreviation: PhenoAgeAccel, Phenotypic age acceleration; ROC, receiver operating characteristic.

DISCUSSION

In this large-scale prospective cohort, our study found a positive association between PhenoAgeAccel and the risk of overall lung cancer and histological types. Compared with biologically younger group at the same chronological age, biologically older group had a higher 5-year absolute risk of lung cancer. PhenoAgeAccel could provide additional information on the association between biological aging and lung cancer risk independent of chronological age. Furthermore, there was a joint effect of PhenoAgeAccel and genetic risk in lung cancer incidence. Finally, two-sample Mendelian randomization analysis demonstrated that genetically predicted PhenoAgeAccel was associated with the increased risk of overall lung cancer, small-cell, and squamous cell carcinoma.

The associations between aging and lung cancer risk were hypothesized to be a result of increasing accumulation of unrepaired damage of exposure to carcinogens (e.g., nicotine),[17] the age-associated decline in the immune system,[2] and increased cellular senescence.[18] "Lung age" (derived from the first second of forced expiration, body height, and age) has been proved to be an indicator of pulmonary obstructive impairment and significantly associated with postoperative respiratory complications and survival in patients with lung cancer.[19] Furthermore, previous cohort studies also indicated biological age acceleration measured by DNA methylation or telomere length was associated with lung cancer risk.[7,20,21] However, to our knowledge, this is the first study to evaluate the association between clinically measured biological aging (PhenoAgeAccel) and lung cancer risk using observational studies and MR analysis simultaneously. Our observational analysis showed that people who are biologically older have a higher risk of lung cancer than those who are biologically younger although they are the same age. On the other hand, compared with other aging indicators, PhenoAgeAccel is easily attainable and cost-effective, so it will have a broad clinical application prospect in mass screenings for lung cancer.

The two-sample MR analysis further supported our observational analysis findings. There were causal association between PhenoAgeAccel and the risk of overall lung cancer, as well as small-cell and squamous cell carcinoma. This is partly consistent with previous MR analysis that reported telomere length measured biological aging is a potential causal risk for overall lung cancer and adenocarcinoma.[20] These findings differed from the MR analysis that found that genetically predicted epigenetic clock may be protective against lung cancer.[22] These inconsistent findings are due to the different indicator of biological aging capturing distinct underlying senescence mechanisms.[23] For instance, PhenoAgeAccel incorporates nine multi-system clinical chemistry biomarkers to predict all-cause mortality.[10] PhenoAgeAccel-related susceptibility loci were enriched in immune-related pathways,[6] which are closely associated with tumor development. Besides, previous study observed that slowly aging naked mole-rats are particularly resistant to cancer development, whereas, rapidly aging mice develop cancer within 2 years.[24] Overall, these findings would provide intuitive information for people on the potential benefit of delaying aging on lung cancer prevention and could improve public awareness of health hazards of biological aging. Individuals could slow down biological aging by quitting smoking, restricting caloric, exercising regularly, and other healthy lifestyle behaviors,[25–27] thereby preventing the development of age-related illnesses.

A

Subgroup	N (cases)	Person−Years	HR (95% CI)	*P* value	RAP (95% CI)
Low genetic risk					
Biologically younger	69,759 (160)	495,581	Reference		Reference
Biologically older	56,949 (310)	398,466	1.70 (1.40−2.06)	<0.001	4.88 (3.05−6.71)
Intermediate genetic risk					
Biologically younger	69,190 (194)	491,802	1.22 (0.99−1.50)	0.064	1.83 (−0.11−3.77)
Biologically older	57,637 (360)	403,444	1.88 (1.56−2.28)	<0.001	5.84 (4.04−7.64)
High genetic risk					
Biologically younger	69,245 (251)	493,716	1.55 (1.27−1.90)	<0.001	4.07 (2.21−5.93)
Biologically older	57,686 (477)	404,716	2.42 (2.01−2.90)	<0.001	8.16 (6.37−9.95)

B

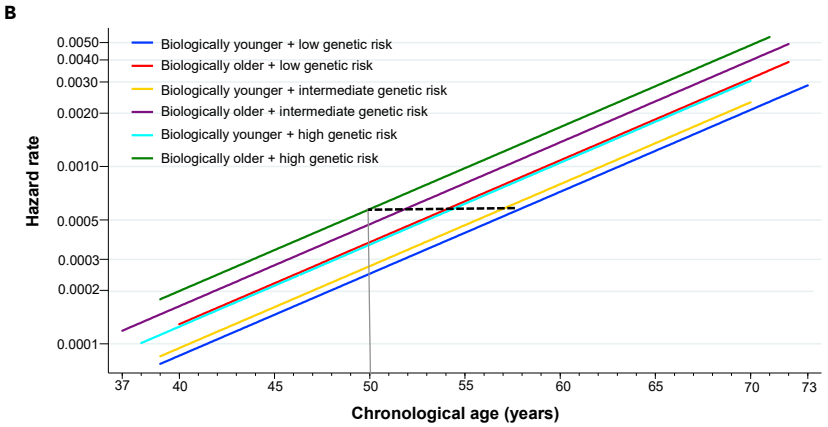

Figure 3. Hazard risk and rate advancement period of incident lung cancer according to biological aging and genetic categories

(A) The HR for lung cancer across each group was estimated via Cox regression model after adjusting for chronological age, sex, ethnicity, center, education, Townsend deprivation index, BMI, smoking status, family history of lung cancer, history of asthma, history of allergy and/or eczema, history of emphysema and/or bronchitis, the top 10 principal components of ancestry, and genotyping batch.

(B) The dashed line is the RAP, assuming a constant disease rate during the follow-up period. The y axis is on the natural log scale. Compared with individuals at biologically younger and low genetic risk group, the RAP of lung cancer occurrence in the other groups. Abbreviation: PhenoAgeAccel, Phenotypic age acceleration; BMI, body mass index; RAP, rate advancement period; ROC, receiver operating characteristic.

PRS, as an indicator of genetic risk, has been proved to effectively predict lung cancer risk.[14,28] Aging inequality was largely due to a comprehensive set of life course circumstances. For example, recent studies have confirmed that traumas and adversities in childhood and adulthood affect the risk of disease in later life, presumably via an acceleration of the aging process.[29,30] Our gene-environment interaction analysis indicated a joint effect of genetic susceptibility to lung cancer and biological aging. These results further support the opinion that the development of lung cancer is the result of the interplay between genetic and environmental risk factors and suggest that individuals at high genetic risk of lung cancer should pay more attention to their biological aging.

Genetic and other biological markers, which capture biological signals representing susceptibility, could aid early detection and secondary prevention for lung cancer by raising awareness and influencing positive behavioral change among high-risk individuals; and facilitating targeted screening and prevention strategies.[7,31] Of note, PhenoAgeAccel, based on routine clinical chemistry biomarkers, has been proved to be effective in differentiating risk for a variety of health outcomes within diverse subpopulation.[10] Here, we further confirmed the positive association between biological aging and lung cancer risk. PhenoAgeAccel might be served as one of the concrete intermediate measures for behavior intervention

Table 3. Mendelian randomization estimates for the effect of PhenoAgeAccel on overall lung cancer and histological types

Outcome	SNPs	IVW		Weighted median		MR-Egger	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Lung cancer	82	1.02 (1.00–1.04)	0.041	1.04 (0.98–1.11)	0.206	1.03 (1.00–1.06)	0.082
Small-cell carcinoma	88	1.06 (1.01–1.12)	0.013	1.16 (1.01–1.34)	0.045	1.07 (1.00–1.14)	0.063
Adenocarcinoma	80	1.01 (0.98–1.04)	0.494	0.98 (0.90–1.05)	0.530	1.02 (0.98–1.07)	0.258
Squamous cell carcinoma	83	1.07 (1.04–1.10)	<0.001	1.12 (1.02–1.22)	0.020	1.08 (1.03–1.13)	0.002

IVW, inverse-variance weighted regression (assumes there is no unbalanced horizontal pleiotropy); Weighted median allows there is up to 50% of the weights in the Mendelian randomization analysis come from invalid instruments; MR-Egger, Mendelian randomization Egger regression (allows that all the genetic variants come from invalid instruments).

instead of long-term outcome (i.e., the occurrence of lung cancer), and further clinical trials are needed to confirm this. Taken together, these findings suggest that PhenoAgeAccel and PRS could be used in discriminating subpopulations at high risk of lung cancer, who might benefit from a practically feasible precision intervention and lung cancer screening program.

Limitations of the study

However, there were some limitations in this study. Firstly, we could not explore the association between the change in biological aging and lung cancer risk, because the multiple biomarker measures were available on only a small fraction of participants.[32] Secondly, there was no external cohort validation in this study, which limited the generalizability of our findings. Hence, these findings should be verified in prospective studies with more diverse and larger populations.

Overall, this study using observational and Mendelian randomization analysis has demonstrated that PhenoAgeAccel (clinically measured biological aging) is associated with the increased risk of lung cancer. Independent of chronological age, PhenoAgeAccel could provide additional information for lung cancer risk assessment. Our findings show that PhenoAgeAccel is a potential biomarker for lung cancer risk. Moreover, biological aging and genetic risk jointly contributed to lung cancer incidence. Future research studies need to verify the combined application of biological aging and PRS in risk assessment for lung cancer.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106018.

ACKNOWLEDGMENTS

This study was conducted using the UK Biobank resource. We would like to thank all participants and the UK Biobank team for their contribution. This work was supported by National Natural Science Foundation of China (81803306, 9194330001, 81820108028, 81973123, 82273724, 82273714), Natural Science Foundation of Jiangsu Province (BK20180675), the Excellent Youth Foundation of Jiangsu Province (BK20220100), Research Unit of Prospective Cohort of Cardiovascular Diseases and Cancer, Chinese Academy of Medical Sciences (2019RU038), and the Science and Technology Service Network Initiative of Chinese Academy of Sciences (No.KFJ-STS-QYZD-2021-08-001).

AUTHOR CONTRIBUTIONS

H.S. and G.J. contributed to the conception and critical revision of the manuscript. Z.M., M.Z., and C.Z. performed data analysis, drafted, and revised the manuscript. H.W., M.J., Y.H., X.W., and J.Z. were responsible for data cleaning. Y.W., R.Y., J.D., L.X., H.M., and Z.H. provided research guidance and contributed to the interpretation of results. All of the authors reviewed or revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

Received: July 1, 2022
Revised: December 14, 2022
Accepted: January 16, 2023
Published: January 19, 2023

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
UK Biobank	https://www.ukbiobank.ac.uk	N/A
Software and algorithms		
R	https://www.r-project.org	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Hongbing Shen (hbshen@njmu.edu.cn).

Materials availability

The study did not generate any new materials.

Data and code availability

- The data used in this study are available at the UK Biobank repository, www.ukbiobank.ac. (Application Number 48700)
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Observational analysis participants

The analysis data were obtained from UK Biobank, a large population-based cohort study in the United Kingdom. Approximately 500,000 participants aged 40-70 were recruited during 2006–2010 in 22 assessment centers. Details of study design, recruitment, and procedures were previously described.[32] Briefly, this cohort collects a wide range of information involving physical assessments, self-reported health behavior, biological samples, and health-related conditions. Figure S1 shows the inclusion and exclusion criteria for the study participants. Finally, a total of 385,074 individuals were included in the present analysis of PhenoAgeAccel and lung cancer risk. We further excluded 4,608 participants without genetic data and then filtered those with sex mismatch or high missingness, or excess heterozygosity, leaving 380,466 available participants for the joint and interactive effect analysis between PRS and PhenoAgeAccel.

The UK Biobank study received approval from the Multi-center Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland, as well as informed consent from all participants.

Data sources for two-sample MR analysis

We used two-sample MR to evaluate the causal association between PhenoAgeAccel and lung cancer risk using GWAS summary statistics. The genetic instruments for PhenoAgeAccel were obtained from a large-scale GWAS including 107,460 individuals of European ancestry,[6] which identified 7,561 independent genetic variants associated with PhenoAgeAccel ($p < 5 \times 10^{-8}$). The GWAS summary statistics of lung cancer were derived from International Lung Cancer Consortium (ILCCO), which included 29,266 lung cancer cases and 56,450 controls from European ancestry.[33] We extracted the summary statistics for all these genetic variants, and then filtered out SNPs in linkage disequilibrium ($r^2 > 0.001$), palindromic SNPs and SNPs with large influence on the MR findings (i.e., outliers). The Cochran Q statistics was used to assess the

heterogeneity among the genetic variants. If there was evidence of heterogeneity, we used the Radial MR plot to detect the outliers.

METHOD DETAILS

PhenoAge and PhenoAgeAccel calculation

Clinical chemistry data were measured by 10 immunoassay analysers [6× DiaSorin Liaison XL & 4× Beckman Coulter DXI 800 and 4 clinical chemistry analysers (2× Beckman Coulter AU5800 & 2× Siemens Advia 1800)]. To correct the skewness of distributions of clinical chemistry biomarkers, we set the bottom 1% of values to the first percentiles and the top 1% to the 99th percentile.[6]

PhenoAge was calculated based on mortality scores from the Gompertz proportional hazard model on chronological age and nine multi-system clinical chemistry biomarkers to predict all-cause mortality.[5] And PhenoAge has been generalized and applied in UK Biobank study.[6,34] We calculated PhenoAgeAccel after subtracting the effect of chronological age by regression residual and PhenoAgeAccel indicated how much older (or younger) a person's PhenoAge is than chronological age. PhenoAgeAccel >0 and <0 was defined as biological age older and younger than chronological age, respectively.

PRS calculation

Based on the cross-ancestry genome-wide meta-analysis,[35] significant SNPs ($p < 5*10^{-8}$ for European ancestry populations) in linkage disequilibrium (LD; $r^2 > 0.1$), the SNP with the lowest p value was included in the PRS. Finally, we used 23 SNPs to calculate the lung cancer PRS based on the following equation:

$$\mathrm{PRS} = \sum_{j=1}^{M} \beta_j \times SNP_j$$

where M denotes the total number of SNPs, and β_j represents the per-allele log odds ratio (OR) for lung cancer associated with SNP_j, which is reported by the previous GWAS.[35]

In addition, we also used the same method to re-calculate PRS based on 18 SNPs from a lung cancer GWAS of European descent as a sensitivity analysis.[33] Previous findings have proved that the 18-PRS could effectively predict lung cancer risk in the UK biobank database.[14]

Outcome

Lung cancer events (ICD-10 code C33-34) were obtained through the National Health Service central cancer and death registries in England, Wales, and Scotland. The date of complete follow-up was October 31, 2015 for Scotland, as well as March 31, 2016 for England and Wales. Censoring referred to death, withdrawal from the study, or failure to suffer from lung cancer at the end of follow-up.

Covariates

The association between PhenoAge and lung cancer incidence might be affected by demographic characteristics, smoking, BMI, family and personal medical history, and socioeconomic factors.[36] Chronological age was computed by subtracting the date of birth from the baseline assessment. Besides, missing data in continued covariates were imputed with the sex-specific mean value of each variable. And missing values of categorical covariates were handled by assigning individual to an "unknown" category for each corresponding variable.

QUANTIFICATION AND STATISTICAL ANALYSIS

Observational analysis

Multivariable Cox regression model was used to calculate HR and CI of lung cancer risk associated with PhenoAgeAccel. We used Schoenfeld's residuals to test the proportional hazard assumption. RAP measures the baseline age difference at which exposed participants reaching the same rate (risk) of disease as unexposed participants.[37] RAP is the ratio of adjusted log (HR) for the exposure and the adjusted log (HR) for chronological age.[38] We compared HR for participants at biologically younger and biologically older group, as well as these of low accelerated aging (the bottom quintile of PhenoAgeAccel), intermediate accelerated aging (quintiles 2-4), and high accelerated aging (the top quintile) groups. To further

evaluate the influence of PhenoAgeAccel on lung cancer risk at the same chronological age, we calculated the 5-year absolute risk of lung cancer by different PhenoAgeAccel at the same chronological age. Furthermore, we also used receiver operating characteristic (ROC) curve analysis and the continuous NRI to assess whether PhenoAgeAccel could offer additional predictive value to lung cancer risk evaluation, independent of chronological age. Besides, the joint and interactive effects of biological aging (PhenoAgeAccel) and genetic risk in lung cancer development were also evaluated. We tested the additive interaction of PhenoAgeAccel and PRS by relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP),[39] and the multiplication interaction by likelihood ratio tests. The PRS was categorized as low, intermediate, and high genetic risk groups based on the tertiles distribution of PRS among non-lung cancer individuals.[40,41]

We further conducted subgroup analyses to assess the robustness of results and potential interaction using the likelihood ratio test. Meanwhile, we carried out several sensitivity analyses to evaluate the robustness of results: 1) restricting our analysis to individuals with complete covariates; 2) excluding patients with lung cancer in the first two years of follow-up; 3) further adjusting pack-years and a squared pack-years term; 4) further adjusting for other lifestyle factors (i.e., alcohol status, alcohol intake frequency, physical activity, and healthy diet score); 5) taking the competing risk of death into consideration; 6) reclassifying PRS based on quintile; 7) re-analyzing the joint effect of PRS and PhenoAgeAccel just based on white British population.

Two-sample MR analysis

In this study, two-sample MR analysis was mainly performed via inverse variance weighting (IVW). In addition, Mendelian randomization Egger regression (MR-Egger regression) was used to detect the potential unbalanced pleiotropy, Given the lower accuracy and statistical power of MR-Egger regression, Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) was also performed to test for pleiotropic biases.[42] Weighted median model was conducted to assess the robustness of the results if there was a proportion of invalid instruments.[43] In addition, the MR analyses were also performed for three subtypes (small cell, adenocarcinoma, and squamous cell carcinoma) of lung cancer, respectively. Finally, leave-one-out sensitivity analysis was performed to assess the robust of MR findings by omitting one SNP in turn. The MR analyses were conducted using the "*TwoSampleMR*" R package.

Two-sided P value of <0.05 was considered to be statistically significant. All Analyses Were Performed with R Software Version 3.6.0-.