Received: 2 November 2022 | Revised: 15 March 2023 | Accepted: 26 May 2023

DOI: 10.1002/cac2.12450

CANCER COMMUNICATIONS Open Access

LETTER TO THE EDITOR

Enhanced selection of people for lung cancer screening using *AHRR* (cg05575921) or *F2RL3* (cg03636183) methylation as biological markers of smoking exposure

Dear Editor,

Lung cancer (LC) is the leading cause of cancer mortality globally, accounting for more than 1.7 million deaths per year [1]. There is consensus that LC screening needs to target those at high risk who are most likely to benefit from screening in order to maximize benefits and minimize potential harms. Given the key role of smoking in determining LC risk [2], most of the low-dose computed tomography (LDCT) screening trials have defined heavy smoking as an eligibility criterion for LC screening [3]. Although LDCT screening has been effective in reducing LC mortality [4, 5], it is crucial to improve the accuracy of the selection criteria for LC screening to decrease morbidity and healthcare-associated costs [6]. Various LC risk prediction models [7] and molecular biomarkers [8] have been suggested as tools for enhanced risk stratification and selection of people for LC screening. Methylation of aryl hydrocarbon receptor repressor (*AHRR*, cg05575921) and coagulation factor II receptor-like 3 (*F2RL3*, cg03636183) in whole-blood DNA have been reported as promising biomarkers for predicting LC risk [9]. A recent study reported that adding *AHRR* (cg05575921) methylation to the LDCT criteria improved the specificity of LC risk prediction by excluding low-risk individuals [10]. However, the potential of these methylation markers to enhance lung cancer risk prediction beyond the best-established LC risk models is yet to be evaluated. These risk models are recommended and foreseen for selecting participants in LC screening programs currently being established or planned in many countries. In a cohort study of men and women aged 50-75 years from Germany (ESTHER study), we aimed to investigate to what extent determining the methylation status of *AHRR* (cg05575921) or *F2RL3* (cg03636183) in whole-blood DNA may enhance prediction of LC risk, individually and in combination with the meanwhile established LC risk prediction models, as compared to the heavy smoking criteria used in the LDCT screening trials.

The study population included 162 ever-smoking participants who were diagnosed with LC between 2001 and 2018 and 721 ever-smoking participants without LC diagnosis during 17 years of follow-up who were randomly selected from a cohort of 9,940 men and women aged 50-75 at recruitment in 2000-2002 (Supplementary Materials and Methods). An overview of the criteria for selecting heavy smokers from different LDCT trials and that of LC risk models is provided in Supplementary Tables S1 and S2, respectively. Population characteristics are shown in Supplementary Table S3. The performances of *AHRR* (cg05575921) methylation, *F2RL3* (cg03636183) methylation, the LC risk models and their combinations for predicting LC occurrence among ever-smoking participants during 17 years of follow-up are presented in Supplementary Table S4. The areas under the receiver operating characteristic curves (AUCs) of the LC risk models ranged from 0.654 to 0.746. *AHRR* (cg05575921) methylation outperformed all models with an AUC of 0.764 (95% CI = 0.727-0.800). Adding *AHRR* (cg05575921) methylation significantly improved the prediction ability of all LC risk models, with increases in AUCs ranging from 0.036 to 0.133 ($P < 0.05$ for all 10 LC risk models). Likewise, *F2RL3* (cg03636183) methylation outperformed all LC risk models (AUC = 0.768, 95% CI = 0.731-0.805), and adding *F2RL3* (cg03636183) methylation to LC risk models significantly improved the prediction ability of all LC risk models, with

List of abbreviations: *AHRR*, aryl hydrocarbon receptor repressor (cg05575921) methylation; AUC, area under the receiver operating characteristic curve; CI, confidence interval; DANTE, Detection and Screening of Early Lung Cancer with Novel Imaging Technology; DLCST, Danish Lung Cancer Screening Trial; *F2RL3*, coagulation factor II receptor-like 3 (cg03636183) methylation; ITALUNG, Italian Lung Cancer Computed Tomography Screening Trial; LC, lung cancer; LDCT, low dose computed tomography; LUSI, German Lung Cancer Screening Intervention trial; MILD, Multicentric Italian Lung Detection Trial; NELSON, *Nederlands Leuvens Longkanker Screenings Onderzoek* trial; NLST, United States National Lung Screening Trial.

TABLE 1 Missed lung cancer cases occurring during 17 years of follow-up using various trial criteria, DNA methylation markers, lung cancer risk models or combinations of lung cancer risk models and DNA methylation markers.

	Missed lung cancer cases according to trial criterion, DNA methylation marker and/or lung cancer risk models[§]							
	NLST		MILD, DANTE, ITALUNG, DLCST		NELSON, LUSI		DEPISCAN	
Predictor	***n***	**Percent difference [¶]**	***n***	**Percent difference [¶]**	***n***	**Percent difference [¶]**	***n***	**Percent difference [¶]**
Trial criterion	62		53		55		53	
AHRR	44	−29.0%*	29	−45.3%*	29	−47.3%*	25	−52.8%*
F2RL3	38	−38.7%*	23	−56.6%*	24	−56.4%*	22	−58.5%*
LCRAT	46	−25.8%	30	−43.4%*	31	−43.6%*	27	−49.1%*
LCDRAT	48	−22.6%	29	−45.3%*	30	−45.5%*	28	−47.2%*
Bach	49	−21.0%	35	−34.0%*	35	−36.4%*	30	−43.4%*
Pittsburgh Predictor	49	−21.0%	36	−32.1%*	37	−32.7%*	32	−39.6%*
LLPi	55	−11.3%	38	−28.3%	39	−29.1%	37	−30.2%*
LLPv3	55	−11.3%	40	−24.5%	39	−29.1%	37	−30.2%*
LLP	58	− 6.5%	49	−7.5%	49	−10.9%	42	−20.8%
Hoggart	59	− 4.8%	48	−9.4%	49	−10.9%	45	−15.1%
Spitz	67	+8.1%	59	+11.3%	59	+7.3%	55	+3.8%
$PLCO_{m2012}$	68	+9.7%	60	+13.2%	60	+9.1%	56	+5.7%
LLP+*AHRR*	33	−46.8%*	21	−60.4%*	23	−58.2%*	19	−64.2%*
Spitz+*AHRR*	34	−45.2%*	22	−58.5%*	23	−58.2%*	19	−64.2%*
LLPi+*AHRR*	38	−38.7%*	21	−60.4%*	22	−60.0%*	17	−67.9%*
LLPv3+*AHRR*	39	−37.1%*	22	−58.5%*	22	−60.0%*	19	−64.2%*
Pittsburgh+*AHRR*	39	−37.1%*	24	−54.7%*	25	−54.5%*	19	−64.2%*
Bach+*AHRR*	42	−32.3%*	25	−52.8%*	25	−54.5%*	20	−62.3%*
LCDRAT+*AHRR*	43	−30.6%*	24	−54.7%*	24	−56.4%*	20	−62.3%*
LCRAT+*AHRR*	44	−29.0%*	23	−56.6%*	24	−56.4%*	21	−60.4%*
Hoggart+*AHRR*	44	−29.0%*	26	−50.9%*	26	−52.7%*	22	−58.5%*
$PLCO_{m2012}$+*AHRR*	45	−27.4%*	28	−47.2%*	30	−45.5%*	24	−54.7%*
LCRAT+*F2RL3*	29	−53.2%*	23	−56.6%*	23	−58.2%*	19	−64.2%*
LCDRAT+*F2RL3*	30	−51.6%*	23	−56.6%*	23	−58.2%*	19	−64.2%*
LLP+*F2RL3*	31	−50.0%*	20	−62.3%*	20	−63.6%*	21	−60.4%*
LLPi+*F2RL3*	31	−50.0%*	20	−62.3%*	21	−61.8%*	17	−67.9%*
LLPv3+*F2RL3*	31	−50.0%*	21	−60.4%*	21	−61.8%*	18	−66.0%*
Pittsburgh+*F2RL3*	31	−50.0%*	21	−60.4%*	21	−61.8%*	19	−64.2%*
Bach+*F2RL3*	32	−48.4%*	23	−56.6%*	23	−58.1%*	20	−62.3%*
Hoggart+*F2RL3*	34	−45.2%*	21	−60.4%*	22	−60.0%*	17	−67.9%*
Spitz+*F2RL3*	34	−45.2%*	23	−56.6%*	23	−58.2%*	19	−64.2%*
$PLCO_{m2012}$+*F2RL3*	38	−38.7%*	24	−54.7%*	24	−56.4%*	19	−64.2%*

[§]To ensure comparability, cutoffs of DNA methylation markers, lung cancer risk models and their combinations were adjusted in such a way that the same numbers and proportions of controls remaining free of LC were classified as non-eligible as with the respective trial criteria.

[¶]Percent difference from the number of missed lung cancer cases using the respective LDCT trial criterion.

*P presented from the McNemar test is < 0.05 for assessing the differences in missed cases compared to the prediction by the respective LDCT trial criterion.

Abbreviations: ***AHRR***- aryl hydrocarbon receptor repressor (cg05575921) methylation; **DANTE**- Detection and Screening of Early Lung cancer with Novel Imaging Technology; **DLCST**- Danish Lung Cancer Screening Trial; ***F2RL3***- coagulation factor II receptor-like 3 (cg03636183) methylation; **ITALUNG**- Italian Lung Cancer Computed Tomography Screening Trial; **LC**- lung cancer; **LCRAT**- Lung Cancer Risk Assessment Tool; **LCDRAT**- Lung Cancer Death Risk Assessment Tool; **LDCT**- low-dose computed tomography; **LLP**, Liverpool Lung Project Risk Model; **LLPi**, Liverpool Lung Project Incidence Risk Model; **LLPv3**, Liverpool Lung Project Risk Model version 3; **LUSI**- German Lung Cancer Screening Intervention trial; **MILD**- Multicentric Italian Lung Detection Trial; **n**- number; **NELSON**- *Nederlands-Leuvens Longkanker Screenings Onderzoek* trial; **NLST**- United States National Lung Screening Trial; **$PLCO_{m2012}$**- Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial Model 2012; **95% CI**- 95% confidence interval.

increases in AUCs ranging from 0.041 to 0.129 ($P < 0.05$ for all 10 LC risk models). The parameters of the methylation markers and LC risk model combinations are provided in Supplementary Table S5. The predictive performance of derived scores by LC types and demographic subgroups is reported in Supplementary Tables S6 and S7, respectively. Despite increased random variation in type-specific analyses, results were rather consistent across LC types and demographic subgroups for both *AHRR* (cg05575921) and *F2RL3* (cg03636183) methylation. However, both markers were stronger predictors of LC risk among former smokers than among current smokers.

Comparisons of the performances of *AHRR* (cg05575921) methylation, *F2RL3* (cg03636183) methylation, LC risk models and their combinations with the four different heavy smoking-based criteria used in different LDCT trials for correctly predicting incident cases during 17 years of follow-up are presented in Table 1. As shown in Supplementary Table S1, when the four definitions of heavy smoking were applied to ever-smoking ESTHER study participants, 61.7% (NLST), 67.3% (MILD, DANTE, ITALUNG, DLCST), 66.6% (LUSI, NELSON) and 67.3% (DEPISCAN) of LC cases were correctly identified as eligible for screening and 66.9%, 58.4%, 58.8% and 54.5% participants remaining free of LC during follow-up were correctly identified as not eligible, respectively. At cutoffs classifying the same numbers and proportions of controls remaining free of LC as non-eligible as the trial criteria, *AHRR* (cg05575921) methylation missed between 29.0% (NLST criteria) and 52.8% (DEPISCAN criteria) less incident LC cases compared to the selection criteria of LDCT trials. These reductions of missed cases were higher than those achieved by any of the 10 LC risk models. The by far highest reductions of missed cases, ranging up to 46.8% (NLST criteria), 67.9% (DEPISCAN criteria) and 60.4% (other trials criteria), were achieved by combinations of AHRR (cg05575921) with the LC risk models (all $P < 0.05$). *F2RL3* (cg03636183) methylation missed between 38.7% (NLST criteria) and 58.5% (DEPISCAN criteria) less incident cases as compared to the selection criteria of LDCT trials, at cutoffs classifying the same numbers and proportions of controls remaining free of LC as non-eligible as the trial criteria. Again, the by far highest reductions of missed cases, ranging up to 53.2% (NLST criteria), 67.9% (DEPISCAN criteria), 63.6% (NELSON and LUSI criteria) and 62.3% (other trials criteria), were achieved by combinations of the *F2RL3* (cg03636183) methylation marker with the LC risk models (all $P < 0.05$). As shown in Supplementary Table S8, at cutoffs classifying the same numbers and proportions of controls remaining free of LC as non-eligible as the trial criteria, those selected by the LC risk models alone were more often current smokers as compared to the combinations of methylation marker with the LC risk models. However, there were no major differences in the sex and age distribution of those selected for screening by the methylation markers compared to those selected by the LDCT trial selection criteria or the risk models (Supplementary Table S9).

Selecting smokers most likely to benefit from screening is one of the major challenges for implementing LDCT screening for LC. We showed that *AHRR* (cg05575921) and *F2RL3* (cg03636183) methylation markers outperformed the eligibility criteria employed in the LDCT trials, as well as the best performing LC risk models. When combined with *F2RL3* (cg03636183) methylation, up to 67.9% less LC cases were missed at comparable levels of specificity reached by the LDCT trial selection criteria. As preparations for implementing LDCT screening in high-risk population are on the way in multiple countries, we provide timely empirical evidence that measurement of *AHRR* (cg05575921) and/or *F2RL3* (cg03636183) methylation in whole-blood DNA, alone or in combination with the best established LC risk models, could substantially enhance selection of people at high risk of LC who would be more likely to benefit from LDCT screening. Such a personalized, risk-adapted approach could make LDCT screening more effective and efficient. In particular, the present study demonstrated that the proportion of missed LC cases could be substantially reduced. Further research, ideally based on DNA methylation measurements in large prospective cohort studies from different countries and in participants of randomized controlled LC screening trials, should replicate and extend our findings, aim for further optimization of risk stratification, and provide in-depth analyses of the cost-effectiveness of screening strategies based on enhanced risk stratification.

DECLARATIONS

AUTHOR CONTRIBUTIONS

HB conceived and supervised the study. BS and BH were responsible for coordinating the follow-up and work-up of the data of the ESTHER study. MB analyzed the data, interpreted the results and drafted the manuscript. MB and HB critically revised the manuscript for important intellectual content. All authors reviewed and approved the final version for submission.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the microarray unit of the Genomics and Proteomics Core Facility of the German Cancer Research Center (DKFZ) for carrying out the DNA methylation analyses using the Illumina Human Methylation arrays.

Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors have no competing financial interest to disclose.

FUNDING INFORMATION

The ESTHER study was supported by grants from the Baden-Württemberg State Ministry of Science, Research and Arts (Stuttgart, Germany); the Federal Ministry of Education and Research (Berlin, Germany): Grant IDs-01ET0717 and 01GY1320A; the Federal Ministry of Family Affairs, Senior Citizens, Women and Youth (Berlin, Germany); and the Saarland State Ministry of Social Affairs, Health, Women and Family (Saarbrücken, Germany). The sponsors had no role in the study design, data collection, analysis and interpretation of data and in the preparation, review, or approval of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committees of the University of Heidelberg (58/2000) and of the state medical board of Saarland, Germany. The study was conducted in adherence with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, and written informed consent was collected from all participants. The study does not report data on any individual participant.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

Data of the ESTHER study, due to restrictions of informed consent, are not publicly available. However, anonymized data may be obtained for use, covered by participants' informed consent, based on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.