Journal of the Endocrine Society, 2023, **7**, 1–10
https://doi.org/10.1210/jendso/bvad077
Advance access publication 7 June 2023
Clinical Research Article

Age at Menarche, age at Natural Menopause, and Risk of Lung and Colorectal Cancers: A Mendelian Randomization Study

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Abstract

Background: The roles of age at menarche and age at menopause in the etiology of lung and colorectal cancers are unclear.

Objective: We aimed to investigate potential causal associations between age at menarche, age at natural menopause, and risk of lung and colorectal cancers using a Mendelian randomization (MR) approach.

Methods: From the Trøndelag Health Study in Norway, we defined two cohorts of 35 477 and 17 118 women to study the effects of age at menarche and age at natural menopause, respectively. We ran univariable MR to evaluate the potential causal associations. We performed multivariable MR adjusting for genetic variants of adult body mass index (BMI) to estimate the direct effect of age at menarche.

Results: Genetically predicted 1-year increase in age at menarche was associated with a lower risk of lung cancer overall (hazard ratio [HR, 0.64; 95% CI, 0.48-0.86), lung adenocarcinoma (HR, 0.61; 95% CI, 0.38-0.99), and lung non-adenocarcinoma (HR, 0.66; 95% CI, 0.45-0.95). After adjusting for adult BMI using a multivariable MR model, the direct effect estimates reduced to HR 0.72 (95% CI, 0.54-0.95) for lung cancer overall, HR 0.67 (95% CI, 0.43-1.03) for lung adenocarcinoma, and HR 0.77 (95% CI, 0.54-1.09) for lung non-adenocarcinoma. Age at menarche was not associated with colorectal cancer. Moreover, genetically predicted age at natural menopause was not associated with lung and colorectal cancers.

Conclusion: Our MR study suggested that later age at menarche was causally associated with a decreased risk of lung cancer overall and its subtypes, and adult BMI might be a mediator.

Key Words: colorectal cancer, HUNT, lung cancer, menarche, Mendelian randomization, menopause

Abbreviations: AAM, age at menarche; AAMP, age at natural menopause; BMI, body mass index; GWAS, genome-wide association study; HR, hazard ratio; HUNT, Trøndelag Health Study; IVW, inverse-variance weighted; MR, Mendelian randomization; PC, principal component of ancestry; PGS, polygenic score; SNP, single-nucleotide polymorphism.

Lung and colorectal cancers are the two most common cancers in women after breast cancer [1]. In many countries, lung cancer morbidity and mortality have been decreasing among men but increasing among women [1]. Although a large part of the sex difference can be explained by changes in smoking habits, factors that are specific to women may play a role [2]. Tobacco smoking, the major risk factor for lung cancer, is strongly associated with small-cell lung cancer but less strongly with lung adenocarcinoma [3]. Besides, approximately 20% of European females with lung cancer have never smoked, and lung adenocarcinoma is the most common histologic type among these women [4]. Unlike lung cancer, no single risk factor accounts for most of the cases of colorectal cancer [1].

Several studies have suggested that female sex hormones and reproductive factors such as age at menarche (AAM) and age at natural menopause (AAMP) may play a role in lung and colorectal tumorigenesis [5-9]. Estrogen receptors α and β are expressed in both normal and cancerous lung and colonic cells [6, 10, 11]. AAM and AAMP have been used as surrogate markers for lifetime exposure to endogenous estrogens. They have been identified as risk factors for sex hormone-related malignancies such as breast cancer in a large meta-analysis study [12]. However, results from epidemiological studies investigating the relationships between these reproductive factors and risks of lung cancer and colorectal cancers are inconsistent [7, 13-15].

Mendelian randomization (MR) is a method that can be used to explore a potential causal relationship between an exposure and outcome by using genetic variants as instrumental variables for the exposure [16]. Because the genetic variants

Received: 9 March 2023. **Editorial Decision:** 1 June 2023. **Corrected and Typeset:** 3 July 2023

are randomly distributed at conception, confounding and reverse causation are less likely to occur in MR analyses than in conventional observational studies [17]. Some MR studies demonstrated causal effects of AAM and AAMP [18-20]. For instance, Day et al found that an increase in genetically predicted AAM was associated with a reduced risk of breast cancer [18]. Ruth et al found that each 1-year delay in AAMP increased the relative risks of hormone-sensitive cancers such as endometrial and breast cancer by up to 5% [19]. However, an MR study showed no evidence of causal relationship between AAM or AAMP and colorectal cancer risk, but the study had limited power to detect weak effects [20]. No MR studies to date have investigated the potential causal associations of AAM and AAMP on lung cancer overall and its histologic types.

In addition, adult body mass index (BMI) may be an important mediator in the association between AAM and risk of cancer. An MR study showed a causal effect of earlier AAM on higher adiposity in adulthood [21]. Other MR studies found that higher adult BMI was associated with increased risks for lung cancer overall, its histologic types, and colorectal cancer [22-24]. The possible mediating effect of adult BMI on the association between AAM and lung and colorectal cancers has not been studied well in previous literature. Multivariable MR could answer this research question by allowing the estimation of independent effects of several exposures (AAM and adult BMI in our case) on disease outcomes simultaneously [25].

Thus, the aims of the current study were to (1) investigate causal relationships between AAM, AAMP, and risks of lung cancer overall and its histologic types as well as colon and rectal cancers using univariable MR methods; and (2) explore the direct effect of AAM on such cancer types after taking account of adult BMI as a possible mediator using multivariable MR methods. We hypothesized that early AAM and late AAMP reflecting a lifetime exposure to higher levels of endogenous estrogen were associated with an increased risk of lung and colorectal cancers.

Methods

Study Population

The Trøndelag Health Study (HUNT) is a large population-based health study in Norway [26]. The study enrolled participants aged 20 years or older in four surveys: HUNT1 (1984-1986), HUNT2 (1995-1997), HUNT3 (2006-2008), and HUNT4 (2017-2019). All adults living in the area of northern Trøndelag, Norway, were invited to complete general questionnaires on health and lifestyle factors and to undergo clinical examinations [26, 27].

For the current study, we included a total of 41 944 women from the HUNT2 and HUNT3 surveys. Diagnoses of lung and colorectal cancers were obtained from the Cancer Registry of Norway up to December 31, 2018. We first excluded 5313 women who did not have information on genetic variants. We then defined two analysis cohorts to study the effects of AAM and AAMP, respectively. To investigate the effect of AAM, we included 35 477 women who had complete information on reported AAM and measured BMI in HUNT2 or HUNT3 in the first analysis cohort. To explore the effect of AAMP, we included 17 118 women who had experienced a natural (non-surgical) menopause in HUNT2 or HUNT3 in the second analysis cohort. A flow chart of the two analysis cohorts is given in Fig. 1.

The study has been approved by the Regional Committees for Medical and Health Research Ethics (REK South-East 2019/337). All participants signed informed written consent on participation in HUNT, with linkage to previous HUNT surveys and specific registries in accordance with the Declaration of Helsinki.

Age at Menarche, age at Natural Menopause, and BMI Assessments

Female reproductive factors such as AAM and AAMP were collected in the HUNT2 and HUNT3 questionnaires based on self-reporting. Postmenopausal women were defined as those who reported an age at menopause or a history of bilateral oophorectomy or hysterectomy. Menopause was considered as non-natural if both ovaries and/or uterus were surgically removed before or at the age of menopause, or if women only reported age at one of these surgeries but not at menopause. The remaining menopausal women were defined as natural menopause except those who did not report age at the two surgeries and at menopause. Participant's height and weight were measured by health professionals and used to calculate adult BMI (kg/m^2). AAM, AAMP, and adult BMI were retrieved either from HUNT2 or HUNT3. If women participated in both HUNT2 and HUNT3 surveys, we calculated the mean of the two values for AAM and adult BMI. For menopausal status, we kept information from HUNT3 unless they reported a natural menopause in HUNT2.

Genotyping and Defining Genetic Instruments

DNA was extracted from blood samples collected in HUNT2 or HUNT3 and stored at the HUNT Biobank. Genotyping was performed using Illumina HumanCoreExome arrays [28]. We used findings from recent genome-wide association studies (GWASs) of European ancestry for AAM, adult BMI, smoking, and AAMP to define genetic instruments in our analyses. Day et al identified 389 independent genetic variants for AAM [18]. Among them, 34 variants were not available in the HUNT Study, leaving 355 single-nucleotide polymorphisms (SNPs) as genetic instruments for AAM. Locke et al reported 97 SNPs for adult BMI; our genetic instruments for BMI comprised 38 SNPs specific to women [29]. Furberg et al identified three most important SNPs for smoking behavior [30]. Ruth et al identified 290 genetic variants for AAMP [19]. The genetic instruments for AAMP in the HUNT Study was based on 257 available SNPs.

The effect allele (*exposure*-increasing allele) was coded as 1 and the other allele as 0. Then each SNP had a value between 0 and 2. Polygenic scores (PGSs) were computed to increase the statistical power of the analyses [31]. We calculated externally weighted PGSs for each participant by multiplying the number of exposure-increasing alleles the participant carried by the variant's coefficient for exposure (beta-coefficient in the corresponding GWAS) and summing across all variants. Thus, a higher PGS*AAM* reflects a later AAM. Similarly, a higher PGS*AAMP* reflects a later AAMP and a higher PGS*BMI* a higher adult BMI. Because some SNPs related to AAM are also associated with adult BMI and therefore could have pleiotropic effects [18], we computed a restricted PGS*AAM* comprising 281 AAM-only SNPs after excluding 74 SNPs that were also associated with adult BMI at a P value <.05.

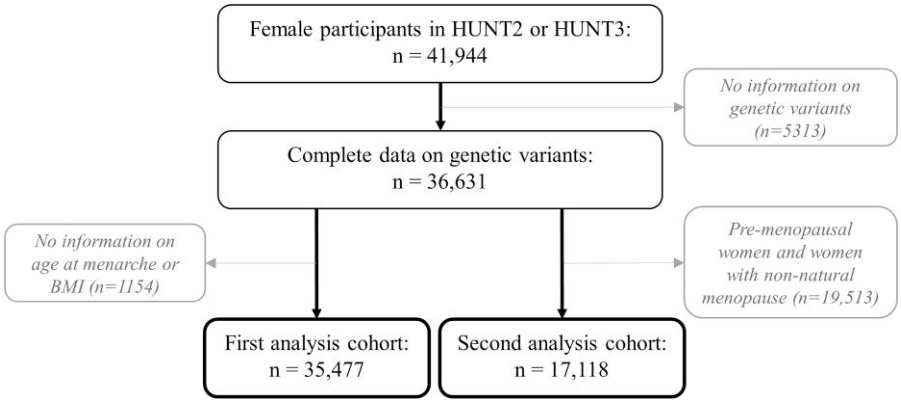

Figure 1. Flow chart of study population.

Supplementary Table S1 presents the characteristics of SNPs included in the analyses and in generating the full PGS*AAM*, restricted PGS*AAM*, PGS*BMI*, and PGS*AAMP* [32]. The effect allele frequency was consistent between the GWASs and the HUNT Study.

Other Variables

Based on information of smoking status and pack-years, participants were classified into detailed categories of smoking as: never, former (≤10, 10-20, >20 pack-years), and current (≤10, 10-20, >20 pack-years). Other variables were categorized as: passive smoking (never, ever), alcohol consumption (never, 1-4, ≥5 times/month), physical activity (inactive, low, moderate, high level), total sitting time daily (0-4, 5-7, ≥8 hours), family history of cancer (yes, no) based on "Is there any family member such as father, mother, siblings, children who reported cancer?", reported doctor-diagnosed chronic obstructive pulmonary disorder (yes, no) based on "Have you been diagnosed as having chronic bronchitis or emphysema by a doctor?", and a history of diabetes (yes, no) was based on the question: "Have you had, or do you have diabetes?" and/or nonfasting blood glucose level ≥11 mmol/L. If women participated in both HUNT2 and HUNT3 surveys, information for these covariates was retrieved from HUNT2 if available. Missing information on each variable was classified as an "unknown" category and included in the analyses. The categorization of variables in the current study were commonly used in previous HUNT publications [33, 34]. Batch for genotyping and 20 principal components of ancestry (PCs) were included in the association models.

Ascertainment of Lung and Colorectal Cancers

Participants' information was linked to the Cancer Registry of Norway (www.kreftregisteret.no) using an 11-digit personal identification number. Data from the Cancer Registry of Norway are considered reasonably accurate, close to complete, and timely [35]. The Tenth Revision of the International Statistical Classification of Diseases and Related Health Problems codes used for registration of lung, colon, and rectal cancers are C33-C34, C18, and C19-C20, respectively. Lung cancer histologic types were classified according to the International Classification of Disease of Oncology [36]. They were further categorized into two main subtypes: adenocarcinoma and non-adenocarcinoma including all other cell types based on possible difference in etiology [4] and the same classification in previous studies [9, 37] to increase statistical power.

Statistical Analyses

MR analysis makes three key assumptions as following, the instrumental variable (1) is strongly associated with the exposure (relevance assumption), (2) does not share a common cause with the outcome (independence assumption), and (3) only affects the outcome through the exposure (exclusion restriction assumption) [16]. The assumptions of multivariable MR are similar, but the genetic variants are associated with one or more of the exposures [25]. Here, the main MR analysis was performed using data from the HUNT2 and HUNT3 surveys. We first performed univariable MR analysis to assess the total effects of AAM on lung and colorectal cancers using the full PGS*AAM* based on 355 SNPs for AAM in the first analysis cohort of 35 477 women (Fig. 2a). We next conducted multivariable MR to assess the direct effects of AAM after taking account of the potential mediating effect of adult BMI, in which both AAM and adult BMI were considered as exposures (Fig. 2b). As sensitivity analyses, univariable MR analysis using the restricted PGS*AAM* based on the 281 AAM-only SNPs was performed to confirm the direct effects of AAM derived from the multivariable MR (Fig. 2c). Because smoking is the major risk factor for lung cancer, we further conducted a multivariable MR analysis for the association between AAM and lung cancer by additionally including the three major smoking SNPs. Finally, we performed univariable MR using the PGS*AAMP* based on the 257 SNPs for AAMP to evaluate the total effect of AAMP on lung and colorectal cancers in the second analysis cohort of 17 118 women who had a natural menopause (Fig. 2d).

Linear regression was applied to compute the F-statistic and R^2-value between the PGS*AAM* and self-reported AAM, between the PGS*BMI* and measured adult BMI, and between the PGS*AAMP* and self-reported AAMP. The PGS is considered as a valid instrumental variable if F-statistic >10 [16]. The associations between the PGS*AAM*, PGS*AAMP*, and other variables were estimated using linear regression for continuous variables and logistic regression for dichotomized categorical variables.

For univariable MR analyses, PGS and Wald method were used [38]. The models were adjusted for batch and 20 PCs. To compute multivariable MR estimates of AAM and adult BMI on risk of lung and colorectal cancers, we applied SNP-level

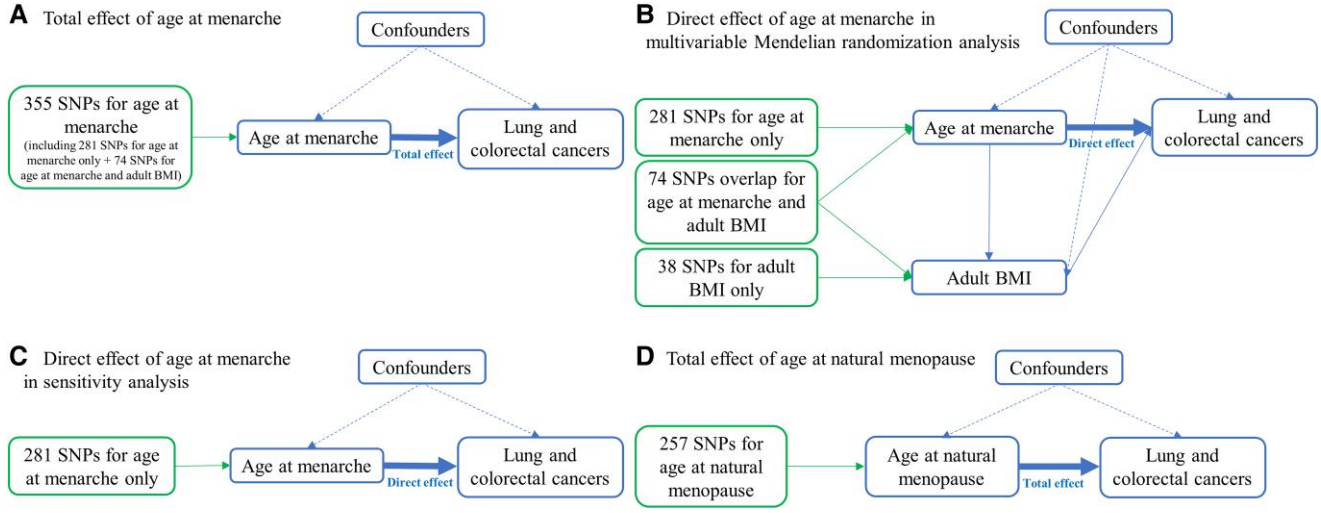

Figure 2. Schematic representation of univariable and multivariable Mendelian randomization analyses in the HUNT Study. (A) Total effect of age at menarche on risk of lung and colorectal cancers. (B) Direct effect of age at menarche on risk of lung and colorectal cancers in multivariable Mendelian randomization analysis. (C) Direct effect of age at menarche on risk of lung and colorectal cancers in sensitivity analysis. (D) Total effect of age at natural menopause on risk of lung and colorectal cancers.

analysis and the inverse-variance weighted (IVW) method [39, 40]. We generated beta coefficients and standard errors from linear regression of AAM and adult BMI on individual SNPs, and coefficients (ln(hazard ratio)) and standard errors from Cox regression of risk of lung cancer overall, its subtypes (adenocarcinoma and non-adenocarcinoma), colorectal, colon, and rectal cancers on individual SNPs. Adjustment for batch and 20 PCs was made for the SNP-AAM associations and for the SNP-outcome associations, we additionally adjusted for age and age squared for the SNP-BMI associations [29]. An IVW estimate of the causal effect combines the ratio estimates of each genetic variant in a random-effects meta-analysis model [41]. Sanderson-Windmeijer conditional F-statistic was computed to assess the strength of the instruments for AAM conditional on adult BMI and inversely [42]. We tested for heterogeneity between SNPs using Cochran's Q statistic [43]. If the *P* value for the Q statistic was lower than .05, it indicates the presence of heterogeneity and can imply the presence of pleiotropy. We then ran MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) to identify and correct for potential outliers ($P < .05$) [44]. Additional sensitivity analyses included a weighted median method that can give valid MR estimates even if up to 50% of the variants are invalid [45]. We also tested for pleiotropy using MR-Egger method to calculate intercepts and *P* values of the intercepts [46].

Finally, we ran two-sample MR analysis to assess the total effects of AAM and AAMP on lung cancer using summary statistics data from the GWASs by Day et al for AAM, by Ruth et al for AAMP, and by McKay et al for lung cancer (International Lung Cancer Consortium) [18, 19, 47]. The International Lung Cancer Consortium included the largest number of lung cancer cases and controls (n = 29 266 and n = 56 450, respectively). Data were available for lung cancer overall and lung adenocarcinoma from both sexes, but were not sex stratified. In presence of heterogeneity, we used Steiger filtering to remove variants that were strongly associated with the outcome rather than with the exposure [48]. Statistical analyses were performed in R version 4.1.3 and STATA/MP 17 (College Station, TX, USA).

Results

Characteristics of the Participants

Table 1 presents the characteristics of women included in the two analysis cohorts, whereas Supplementary Table S2 describes the characteristics of the excluded women [32]. In the first analysis cohort of 35 477 women, 456 had lung cancer including 171 lung adenocarcinoma, 690 had colon cancer, and 240 had rectal cancer. The mean age was 50.0 years, with 52.3% being ever smokers and 78.2% being ever passive smokers (Table 1). Women who were excluded (n = 6467) were older (mean age, 55.0 years) and had lower percentages of ever smokers, passive smokers, and alcohol consumers (Supplementary Table S2) [32]. In the second analysis cohort of 17 118 women who had a natural menopause, 328 women had lung cancer including 114 lung adenocarcinoma, 539 had colon cancer, and 177 had rectal cancer. Their mean age was 65.4 years, with 52.2% being ever smokers and 81.8% being ever passive smokers (Table 1). Women who were excluded (n = 1931) were also older (mean age, 69.3 years old) and had lower percentages of ever smokers, passive smokers, and alcohol consumers (Supplementary Table S2) [32].

Effect of AAM on Lung and Colorectal Cancers

The effect estimates of full PGS*AAM* represented the total effects of AAM. They showed that a 1-year increase in genetically predicted AAM was associated with a 36% decreased risk of lung cancer overall (hazard ratio [HR], 0.64; 95% CI, 0.48-0.86). Similar effects were observed for subtypes, with a 39% decreased risk for lung adenocarcinoma (HR, 0.61; 95% CI, 0.38-0.99) and a 34% decreased risk for lung non-adenocarcinoma (HR, 0.66; 95% CI, 0.45-0.95) (Table 2). After adjusting for genetically predicted adult BMI using the multivariable MR method, the direct effect estimates for each 1-year increase in AAM compared with the total effect estimates were slightly attenuated for lung cancer overall (HR, 0.72 vs. 0.64), lung adenocarcinoma (HR, 0.67 vs. 0.61), and lung non-adenocarcinoma (HR, 0.77 vs. 0.66) (Table 2 and Supplementary Table S3) [32]. This suggested

Table 1. Characteristics of women with complete information in HUNT2 and HUNT3

Variables	Women with complete information on genetic data, age at menarche, and adult BMI	Women with a natural menopause and complete information on genetic data
Number of subjects	35 477	17 118
Age (y)	50.1 ± 16.9	65.4 ± 11.2
BMI (kg/m²)	26.6 ± 4.7	27.1 ± 4.5
Number of lung cancer cases (%)	456 (1.3)	328 (1.9)
Lung adenocarcinoma cases (%)	171 (0.5)	114 (0.7)
Lung non-adenocarcinoma cases (%)	285 (0.8)	214 (1.2)
Number of colorectal cancer cases (%)	930 (2.6)	716 (4.2)
Colon cancer cases (%)	690 (1.9)	539 (3.2)
Rectal cancer cases (%)	240 (0.7)	177 (1.0)
Smoking status, % (never/ever/unknown)	46.2/52.3/1.5	46.1/52.2/1.8
Passive smoking, % (never/ever/unknown)	21.0/78.2/0.8	17.2/81.8/1.0
Alcohol consumption (times/month), % (never/1-4/≥5/unknown)	34.7/53.9/8.1/3.3	45.7/41.9/8.5/3.9
Physical activity, % (inactive[a]/active[b]/unknown)	21.5/50.0/28.4	25.4/41.0/33.7
Total sitting time daily (hours), % (0-4/5-7/≥8/unknown)	31.0/29.2/26.5/13.4	31.5/30.3/25.5/12.7
Family history of cancer, % (no/yes/unknown)	71.5/27.8/0.7	63.0/36.5/0.5
Reported COPD, % (no/yes)	98.1/1.9	97.5/2.5
History of diabetes, % (no/yes/unknown)	97.0/2.9/0.1	95.8/4.1/0.2

Data are given as mean ± SD for continuous variables. If women participated in both HUNT2 and HUNT3 surveys, data were retrieved from HUNT2 if available.
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; HUNT, Trøndelag Health Study.
[a]Inactive: women with no physical activity or ≤2 hours light activity only per week.
[b]Active: women with low, moderate, or high level of physical activity. Physical activity level classified as low (≥3 hours light activity only per week, or ≤2 hours light activity and <1 hour hard activity per week), moderate (≥3 hours light activity and <1 hour hard activity per week, or 1 to 2 hours hard activity per week regardless of light activity), or high (≥3 hours hard activity per week regardless of light activity).

Table 2. Association of age at menarche with risk of lung or colorectal cancer based on univariable MR analyses and multivariable MR analyses among women in HUNT2 and HUNT3 (n = 35 477)

		Total effect using the full PGS*AAM* in univariable MR[a]		Direct effect using multivariable MR[b]		Direct effect using the restricted PGS*AAM* in univariable MR[c]	
	Cases	HR[d] (95% CI)	*P* value	HR[e] (95% CI)	*P* value	HR[f] (95% CI)	*P* value
Lung cancer overall	456	0.64 (0.48–0.86)	.003	0.72 (0.54–0.95)	.02	0.68 (0.49–0.96)	.03
Lung adenocarcinoma	171	0.61 (0.38–0.99)	.04	0.67 (0.43–1.03)	.07	0.72 (0.42–1.25)	.25
Lung non-adenocarcinoma	285	0.66 (0.45–0.95)	.03	0.77 (0.54–1.09)	.14	0.66 (0.43–1.01)	.06
Colorectal cancer	930	1.03 (0.84–1.26)	.79	1.03 (0.84–1.27)	.74	1.00 (0.79–1.27)	.98
Colon cancer	690	1.04 (0.82–1.32)	.75	1.09 (0.86–1.37)	.49	1.03 (0.78–1.35)	.84
Rectal cancer	240	1.00 (0.67–1.50)	1.00	0.94 (0.65–1.36)	.74	0.93 (0.59–1.49)	.78

Abbreviations: AAM, age at menarche; BMI, body mass index; HR, hazard ratio; IVW, inverse-variance weighted; MR, Mendelian randomization; PCs, principal components of ancestry; PGS*AAM*, polygenic score for age at menarche; SNP, single-nucleotide polymorphism.
[a]Full PGS*AAM* based on 355 AAM SNPs was used to study the total effect of AAM in univariable MR [18].
[b]Full set of SNPs (355 AAM SNPs + 38 BMI SNPs) were used to study the direct effect of AAM controlling for adult BMI in multivariable MR [18, 29].
[c]Restricted PGS*AAM* based on 281 AAM-only SNPs after excluding 74 BMI-associated SNPs was used to study the direct effect of AAM in univariable MR [18].
[d]Hazard ratio adjusted for batch and 20 PCs, per 1-year increase in genetically predicted age at menarche.
[e]Summarized statistics were generated to run the IVW method. Each exposure was regressed on the full set of SNPs (355 AAM SNPs + 38 BMI SNPs), adjusted for batch and 20 PCs and additionally adjusted for age and age squared for BMI as the exposure. Cox regression was run for each outcome, adjusted for batch and 20 PCs.
[f]Hazard ratio adjusted for BMI, batch and 20 PCs, per 1-year increase in genetically predicted age at menarche.

that adult BMI might have partially mediated the association between AAM and lung cancer. Although reduced, the 1-year increase in AAM still had a direct effect on lung cancer overall with a 28% decreased risk (HR, 0.72; 95% CI, 0.54-0.95) (Table 2). In the sensitivity analyses using the restricted PGS*AAM* (the 281 AAM-only SNPs), results generally supported the direct effect estimates derived from the multivariable MR analyses with a 32% decreased risk for lung cancer overall (HR, 0.68; 95% CI, 0.49-0.96; Table 2). In the additional multivariable MR adjusting for genetically predicted smoking in addition to adult BMI, results were very similar to the direct effect estimates for AAM found in the

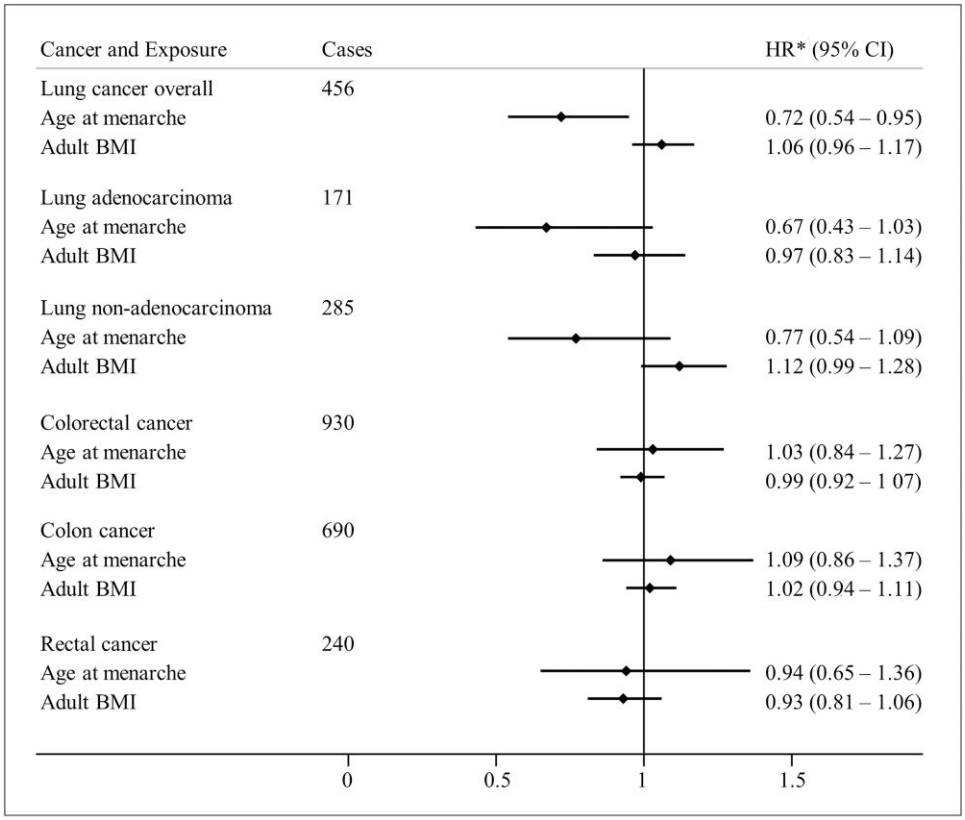

Figure 3. Illustration of the direct effects of age at menarche and adult BMI on lung and colorectal cancers among women in HUNT2 and HUNT3 using the multivariable MR method (n = 35 477).

original multivariable MR analysis for lung cancer overall and its subtypes (Supplementary Table S4) [32]. Meanwhile, 1-level increase in genetically predicted smoking was clearly associated with an increased risk of lung cancer. Genetic predisposition to later AAM was not associated with risks of colorectal, colon, and rectal cancers (Table 2). The direct effects of AAM and adult BMI on lung and colorectal cancer outcomes derived from the multivariable MR analyses are presented in Fig. 3. As shown in Supplementary Table S3 on multivariable MR analyses, results from the weighted median method showed similar associations. Cochran's Q tests for the multivariable IVW method did not suggest heterogeneity of the ratio estimates of the SNPs ($P > .05$) except for colorectal and colon cancers [32]. One outlier SNP (rs12401738) was detected by MR-PRESSO for the associations between AAM, adult BMI and colon cancer. This SNP was removed from the corresponding analyses. There was no evidence of horizontal pleiotropy because the corresponding intercepts from the MR-Egger method did not deviate markedly from 0 and the *P* value of the intercept test was above .05 (Supplementary Table S3) [32].

The full PGS*AAM* based on 355 SNPs had an F-statistic of 1879 and accounted for 5.0% of the variation of AAM in the first analysis cohort. The restricted PGS*AAM* based on the 281 AAM-only SNPs had an F-statistic of 1395 and accounted for 3.8% of the variation. The PGS for adult BMI had an F-statistic of 658 and accounted for 1.8% of the variation of BMI. The conditional F-statistics for both AAM (S-W F-statistic = 40.1) and BMI (S-W F-statistic = 22.2) were also larger than the rule-of thumb cutoff of 10, suggesting that our PGSs were good instruments. Supplementary Table S5 and S6 present the associations between the full and restricted PGSs for AAM and potential confounders [32]. Considering multiple testing burden using Bonferroni correction for *P* value, the full PGS*AAM* remained to be associated with adult BMI and physical activity ($P < .006$). Therefore, we re-ran the analyses additionally adjusting for physical activity when using the full PGS*AAM* and the results were similar (Supplementary Table S7) [32]. The restricted PGS*AAM* was associated with adult BMI even after the 74 BMI-related SNPs were excluded. Thus, we adjusted for the phenotype of adult BMI when the restricted PGS*AAM* was used.

Effect of AMMP on Lung and Colorectal Cancers

Genetic predisposition to later AAMP was not associated with lung cancer overall, its subtypes, colorectal, colon, and rectal cancers in the HUNT study (Table 3). The PGS for AAMP had an F-statistic of 763 and accounted for 4.3% of the variation of AAMP in the second analysis cohort. The PGS*AAMP* was not associated with any potential confounders after the Bonferroni correction for *P* values (Supplementary Table S8) [32].

Two-sample MR Analyses to Assess the Total Effects of AAM and AAMP on Lung Cancer

In the two-sample MR analyses shown in Supplementary Table S9, there was no clear association between genetically

Table 3. Association of age at menopause with risk of lung or colorectal cancer based on univariable MR analyses among women who had a natural menopause in HUNT2 and HUNT3 (n = 17 118)

	Cases	HR[a] (95% CI)	*P* value
Lung cancer overall	328	0.99 (0.90-1.09)	.79
Lung adenocarcinoma	114	1.05 (0.89-1.24)	.54
Lung non-adenocarcinoma	214	0.95 (0.84-1.07)	.43
Colorectal cancer	716	0.99 (0.93-1.06)	.73
Colon cancer	539	0.96 (0.89-1.04)	.35
Rectal cancer	177	1.06 (0.93-1.21)	.37

Abbreviations: HR, hazard ratio; MR, Mendelian randomization; PCs, principal components of ancestry.
[a]Hazard ratio adjusted for batch and 20 PCs, per 1-year increase in genetically predicted age at natural menopause.

predicted 1-year increase in AAM and lung cancer risk (HR, 0.97; 95% CI, 0.92-1.02 for lung cancer overall and HR, 1.00; 95% CI, 0.94-1.06 for lung adenocarcinoma) [32]. Although *P* values for the Q-statistic suggested heterogeneity in the data, Steiger filtering detected no or few SNPs and the results remained similar (results not shown). Genetically predicted 1-year increase in AAMP was not associated with lung cancer risk (Supplementary Table S9) [32].

Discussion

Main Findings

In this population-based MR study including 35 477 women, we found that 1-year increase in genetically predicted AAM was associated with more than 30% decreased risk of lung cancer overall, lung adenocarcinoma, and lung non-adenocarcinoma. The direct effect estimates were slightly attenuated after controlling for genetically predicted adult BMI in multivariable MR, suggesting that adult BMI may have partially mediated the associations. The remaining direct effect of AAM on lung cancer overall demonstrated in the multivariable MR and in the univariable MR using the 281 AAM-only SNPs might be explained by mechanisms other than BMI. There was no association between AAM and colorectal, colon, and rectal cancers. In the second analysis cohort of 17 118 menopausal women, we did not observe causal associations of AAMP with risk of lung and colorectal cancers.

Comparison With Previous Literature

In line with the findings of our study, a recent pooled analysis of 536 450 women drawn from prospective cohorts reported that a 1-year increase in AAM was associated with a significant reduction of lung cancer risk (HR, 0.98; 95% CI, 0.96-0.99) [49]. Moreover, in a HUNT study of Norwegian women, we found that early menarche (≤12 years) was associated with an increased incidence of lung adenocarcinoma (HR, 1.43; 95% CI, 1.02-2.03) [9]. In contrast, a recent meta-analysis combining different study designs by Yin et al observed no association [5]. Likewise, Yin et al reported that age at menopause was not associated with lung cancer overall [5], whereas Brinton et al observed that women with an early AAMP were at increased risk for lung cancer, although it might reflect residual confounding by smoking [13].

Results from traditional observational studies on the association between AAM and AAMP and colorectal cancer risk have also been inconclusive. In the pooled analysis, Fuhrman et al found that a 1-year increase in AAM was associated with a reduced colon cancer risk (HR, 0.97; 95% CI, 0.96-0.99) [49], whereas a meta-analysis of 22 case-control and cohort studies reported no association [50]. A large prospective cohort study observed a positive association between age at menopause and risk of colorectal cancer [8], whereas most other studies found no association [7, 9, 15].

Few MR studies have explored the potential causal associations between AAM and AAMP and lung and colorectal cancers risks [20]. Our findings are consistent with those of the MR study by Neumeyer et al, who did not find a causal relationship between AAM and AAMP and colorectal cancer risk [20]. This previous study was a two-sample MR study using the same genetic instruments for AAM and data from three large consortia of colorectal cancer (n = 12 944 for cases and n = 10 741 for controls in females). However, fewer SNPs (n = 54) were used as genetic instruments for AAMP compared with our study, and it was unclear if the analysis on effect of AAMP was performed only among postmenopausal women.

Strengths and Limitations

Our MR study is one of the first to investigate the potential causal associations of AAM and AAMP with the risk of lung cancer overall, lung cancer subtypes (adenocarcinoma and non-adenocarcinoma) and colorectal, colon, and rectal cancers. It is also one of the first to elucidate if adult BMI was a mediating factor for the association. The information on cancer cases from the Cancer Registry of Norway is accurate and complete [35]. The MR approach, if the assumptions are satisfied, can reduce bias due to reverse causation and confounding that are likely to occur in conventional observational studies. Based on the generally high F-statistics of the PGSs, weak instruments were less likely in this one-sample MR analysis of the HUNT population. The PGSs explained a large amount of variation of our exposures such as AAM, adult BMI, and AAMP. We were able to investigate the associations between the PGSs and potential confounders in the one-sample MR settings. Even if we cannot rule out unmeasured confounders, the PGSs were not associated with important confounders such as smoking. Our additional multivariable MR analysis including smoking SNPs further supported the direct effect estimates of AAM on lung cancer from the original multivariable MR analysis. Moreover, there was no evidence of strong pleiotropy based on the results of Cochran's Q and MR-Egger tests for the associations between AAM and lung cancer outcomes.

Our study had several limitations. First, because there was a decrease in the participation in the HUNT Study, selection bias cannot be excluded. Participants tended to be healthier than non-participants [51]. Second, AAM and AAMP were self-reported and were prone to misclassification. Nevertheless, menarche and menopause ages have been shown to be reported with good reliability [52, 53]. Third, the sample size and the number of lung and colorectal cancer cases in our study were generally small, especially in the analysis for AAMP that was performed among postmenopausal women. This might have made it difficult to detect small effects. Fourth, measurements of childhood BMI were not available in the HUNT Study. Childhood obesity may lead to earlier puberty onset [54, 55]. Meanwhile, higher childhood

body size showed a protective effect on risk of lung and colorectal cancers after accounting for adult body size in an MR study [56]. Thus, childhood BMI might be a negative confounder for the association between AAM and cancer risk [57]. Because of a lack of adjustment of this negative confounder in our study, the observed effect estimates of AAM on lung cancer risk may have been underestimated. Moreover, the MR-Egger tests did not show strong pleiotropic effects of the AAM SNPs. Fifth, in the two-sample MR analyses we used the accessible International Lung Cancer Consortium data that were not sex stratified. As we do not expect such associations among men, the obtained effect estimates in the two-sample MR settings may have been attenuated toward the null. Finally, most participants in the HUNT Study were of European ancestry, which might reduce the generalizability to other ethnic populations.

Conclusion

Overall, our Mendelian randomization study suggested that later age at menarche was causally associated with a decreased risk of lung cancer overall and its subtypes. These inverse associations might have been mediated by adult BMI, although age at menarche still showed a direct effect on lung cancer overall. Age at menopause was not associated with the risk of lung and colorectal cancers.

Acknowledgments

The Trøndelag Health Study (HUNT) is a collaboration between the HUNT Research Centre (Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology), Trøndelag County Council, Central Norway Regional Health Authority, and the Norwegian Institute of Public Health.

Funding

The project was supported by The Norwegian Cancer Society (project ID 182688-2016) and The Research Council of Norway "Gaveforsterkning." M.D. was supported by funding from the Norwegian Women's Public Health Association (N.K.S.: 40014) and top-up funding from the collaboration partner between St Olav hospital and NTNU, Norwegian University of Science and Technology. Y.Q.S. was supported by a researcher grant from The Liaison Committee for Education, Research and Innovation in Central Norway (project ID 2018/42794). L.J. was supported by funding from the collaboration partner between The Liaison Committee for Education, Research and Innovation in Central Norway and Central Norway Regional Health Authority (project ID 30320).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The corresponding author had access to all the data in the study and had final responsibility for the decision to submit for publication.

Author Contributions

M.D. conducted statistical analyses, interpreted results, and wrote the initial draft of the manuscript. Y.Q.S. and X.M.M. contributed to the study design and statistical analyses. X.M.M. was responsible for applying and acquiring data. L.J. contributed to the statistical analyses. All authors participated in the data interpretation, contributed to the manuscript writing with important intellectual content, and approved the final version of the manuscript.

Disclosures

The authors have nothing to disclose.

Data Availability

Data from the HUNT Study that is used in research projects will, when reasonably requested by others, be made available on request to the HUNT Data Access Committee (hunt@medisin.ntnu.no). The HUNT data access information describes the policy regarding data availability (https://www.ntnu.edu/hunt/data).

Ethics Approval and Consent to Participate

All participants gave their informed consent for participation in HUNT. The current study was approved by the Norwegian Regional Committees for Medical and Health Research Ethics (REK sør-øst 2019/337). The study was performed in accordance with the Declaration of Helsinki.

Disclaimer

Data from the Cancer Registry of Norway (CRN) have been used in this publication. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by CRN is intended nor should be inferred.

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