



Association of milk intake with hay fever, asthma, and lung function: a Mendelian randomization analysis

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Abstract

Background Previous observational studies have indicated a protective effect of drinking milk on asthma and allergy. In Mendelian Randomization, one or more genetic variants are used as unbiased markers of exposure to examine causal effects. We examined the causal effect of milk intake on hay fever, asthma, forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) by using the lactase rs4988235 genotype associated with milk intake.

Methods We performed a Mendelian Randomization study including 363,961 participants from the UK Biobank.

Results Observational analyses showed that self-reported milk-drinkers vs. non-milk drinkers had an increased risk of hay fever: odds ratio (OR) = 1.36 (95% CI 1.32, 1.40, $p < 0.001$), asthma: OR = 1.33 (95% CI 1.38, 1.29, $p < 0.001$), yet a higher FEV1: $\beta = 0.022$ (SE = 0.004, $p < 0.001$) and FVC: $\beta = 0.026$ (SE = 0.005, $p < 0.001$). In contrast, genetically determined milk-drinking vs. not drinking milk was associated with a lower risk of hay fever: OR = 0.791 (95% CI 0.636, 0.982, $p = 0.033$), and asthma: OR = 0.587 (95% CI 0.442, 0.779, $p = 0.001$), and lower FEV1: $\beta = -0.154$ (standard error, SE = 0.034, $p < 0.001$) liter, and FVC: $\beta = -0.223$ (SE = 0.034, $p < 0.001$) liter in univariable MR analyses. These results were supported by multivariable Mendelian randomization analyses although not statistically significant.

Conclusions As opposed to observational results, genetic association findings indicate that drinking milk has a protective effect on hay fever and asthma but may also have a negative effect on lung function. The results should be confirmed in other studies before any recommendations can be made.

Keywords Allergic disease · Asthma · Hay fever · Milk · Lung function

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Introduction

The increase in allergic disease and asthma in the past decades has been attributed to changes in lifestyle and/or environmental factors [1]. There has been a decline in the consumption of milk in this period [1] and thus there is a great interest in determining the impact of drinking or avoiding milk, including raw farm milk, breastfeeding, and intake of milk during pregnancy, on the development of allergic disease and asthma [2–17]. Previous observational studies have suggested a possible protective effect of drinking milk on asthma [12, 14]. However, the apparent protection of milk against asthma may result from avoidance of milk among parents of asthmatic children, rather than an actual prophylactic effect [13]. Furthermore, milk consumption has been suspected to increase mucus and asthma symptoms [18].

Lactose is the primary carbohydrate in milk. It is metabolized by the lactase enzyme on the enterocytes. Most Northern Europeans can digest lactose their whole life, but in some individuals, lactase activity is reduced after they are accustomed to normal food. The single nucleotide polymorphism *LCT*-13910 C/T rs4988235 genotype exhibits complete correlation with lactase persistence in Northern Europeans. It is located 13,910 base pairs upstream of the lactase gene on chromosome 2q21-22 within intron 13 of the adjacent *MCM6* gene. The C and T alleles are the lactase non-persistent and lactase-persistent alleles, respectively. The rs4988235 genotype is autosomal recessively inherited. Homozygotes for the C allele cannot digest lactose, whereas heterozygotes and homozygotes for the T allele are able to digest lactose. Milk consumption may cause lactase non-persistent individuals symptoms of lactose intolerance, e.g., diarrhea and flatulence. They tend to consume less milk compared with lactase persistent individuals [19].

Causal inference from conventional epidemiologic studies between milk intake and allergic respiratory disease is difficult due to the potential confounding and reverse causation. Mendelian randomization examines causality by using one or more genetic variants, typically single nucleotide polymorphism (SNP), as instruments for exposures. It is based on the random allocation of genes from parents to offspring, which will not be associated with the confounding factors inherent in conventional observational studies. The validity of the instrument is dependent on three assumptions, referred to as ‘relevance’, ‘independence’, and ‘exclusion’. Here we used the rs4988235 genotype as an instrument for milk intake to test the causality of the association of milk consumption with hay fever, asthma, and lung function (illustrated in Fig. 1), in a Mendelian randomization meta-analysis of 363,961 participants from the UK Biobank study.

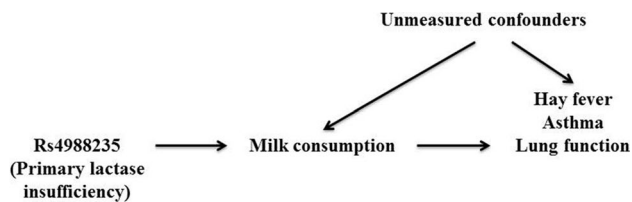


Fig. 1 Directed acyclic graph of an instrumental variable. The instrument (the rs4988235 genotype) must be associated with the exposure (milk consumption), it must be independent of unmeasured confounders, and it must be independent of the outcome (hay fever/asthma/lung function) given the exposure (milk consumption) and the unmeasured confounders

Methods

Study population

The UK Biobank is a large prospective study with more than 500,000 participants (409,694 of genetically European ancestry) from across the United Kingdom and aged 40–69 years at recruitment/initial assessment in 2006–2010 [20]. We included 363,961 participants with data on the rs4988235 genotype (including 83,976 cases of hay fever and 40,364 cases of asthma) of genetically determined European ancestry.

Genotypes

The lactase SNP was directly genotyped and on the opposite strand as the *LCT*-13910 C/T rs4988235 genotype which means that the G and A alleles are the lactase non-persistent and lactase-persistent alleles, respectively. Approximately 450,000 of the participants have been genotyped using the UK Biobank Axiom array from Affymetrix. We had data on the rs4988235 genotype from this array. Minor allele frequency and Hardy Weinberg Equilibrium *p*-value for the rs4988235 were 0.25 and 0.600, respectively. We analyzed the genetic variant in two ways (1) an additive model with a per allele milk-increasing effect, (2) major homozygotes vs. heterozygotes and minor homozygotes combined.

In additional analyses, we used the asthma-associated SNPs (1) rs36045143, rs1837253, rs12413578, rs10876864, rs1059513, rs301805, rs1342326, rs744910, rs3894194, and rs2284033 in a simple genetic risk score for asthma [21, 22], (2) the rs10838738, rs10938397, rs10968576, rs11847697, rs12444979, rs13107325, rs1424233, rs1514175, rs1555543, rs17782313, rs1805081, rs206936, rs2112347, rs2241423, rs2287019, rs2568958, rs29941, rs3810291, rs4929949, rs543874, rs713586, rs7647305, rs9939609, rs10146997, rs1121980, and rs7138803 associated with body mass index (BMI) in a weighted genetic risk score [23], (3) and the rs16969968 associated with smoking [24].

Milk intake

We defined milk drinking status according to the answer to the question: "What type of milk do you mainly use?" ($N = 363,961$). If the participants answered "Full cream", "Semi-skimmed", or "Skimmed", they were categorized as milk-drinkers ($N_{\text{milk-drinkers}} = 335,107$). Otherwise (including the answers "Soya", "Other type of milk", "Never/rarely have milk", "Do not know", or "Prefer not to answer"), they were categorized as no-milk drinkers ($N_{\text{no-milk drinkers}} = 28,854$).

In additional analyses, we restricted the no-milk drinkers to those answering “Never/rarely have milk”.

The amount of milk consumed (in glasses) was defined among those who answered the following question at the first assessment: “How many glasses/carton/ 250 ml of milk (excluding milkshakes) did you consume yesterday?”. The options were half a glass, one, two, three, four, five or six glasses of milk (N of responders to this question = 2298).

Hay fever and asthma

Hay fever was defined as a positive answer to the question: “Has a doctor ever told you that you have had any of the following conditions? Hay fever, allergic rhinitis, or eczema” (N = 363,961, N_{cases} = 83,976). Asthma was defined as a positive answer to the question: “Has a doctor ever told you that you have had any of the following conditions? Asthma” (N = 363,961, N_{cases} = 40,364).

Due to the non-specific nature of the hay fever variable (i.e. including eczema), we assessed two other definitions of hay fever in additional analyses using information from the initial assessment visit 2006–2010: (1) We defined hay fever status according to self-reported use of medication for hay fever. From the complete list of > 6000 recorded types of medication, we manually excluded the medication that < 100 persons were taking or which we knew was not used in the treatment of hay fever. For each of the remaining medications, we assessed the general indication and excluded those not related to or too non-specific to treatment of hay fever. We ended up with 31 entries of medication as previously published [23]. Persons who reported taking one or more of the medications on the list were defined as having hay fever according to this definition, and persons who did not were defined as not having hay fever. According to this definition, 7110 participants out of 363,961 were defined as having hay fever. (2) We defined hay fever status according to doctor diagnosis. The participants stated in the UK Biobank touch screen questionnaire whether they had been told by a doctor that they have other serious illnesses or disabilities. If they answered “Yes” they were later asked by an interviewer: “In the touch screen you selected that you have been told by a doctor that you have other serious illnesses or disabilities, could you now tell me what they are?” If the participant answered “hay fever” we defined the participant as having hay fever. Otherwise, we defined the participant as not having hay fever. According to this definition, 14,135 participants out of 363,961 were defined as having hay fever.

Lung function

Forced expiratory volume in 1-s (FEV1) and forced vital capacity (FVC) were measured at baseline by spirometry using a Vitalograph Pneumotrac 6800 (Vitalograph, UK)

operated via a PC for data capture and in order to visualize flow graphs from each test. The spirometer was calibrated in the beginning of each day by the Spirotrac software supplied with the Pneumotrac 6800. FEV1 and FVC were measured in 327,803 of the included individuals and expressed in liters.

Other covariates

All the 363,961 individuals included had data on age, sex, and 40 genetic principal components. Other covariates had the following number of missing values: smoking status categorized as never, former or current smokers (N_{missing} = 1427), smoking quantity was expressed in cigarettes per day, alcohol status categorized as drinkers and non-drinkers (N_{missing} = 258), and body mass index (BMI) expressed in kg/m² (N_{missing} = 1247).

Statistical analyses

Statistical analyses were performed with SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA), STATA, version 12 (StataCorp, College Station, TX, USA), and the statistical software R, version 3.3.3 (<http://www.r-project.org/>). The *p*-values are two-tailed, and statistical significance was defined as *p* < 0.05. Participants with missing value in one or more variables were excluded in the regression models. The univariable genetic association analyses of the milk-associated SNP and milk intake status, hay fever, asthma and lung function were assessed using linear and logistic regression and performed unadjusted; adjusted for age, gender, and genetic principal components (PCs); and further adjusted for smoking, alcohol, and BMI (Tables 1, 2). The conventional observational analyses of the associations of milk intake status with hay fever, asthma and lung function were assessed by logistic and linear regression analyses and were performed unadjusted; adjusted for age, and gender; and further adjusted for smoking status, alcohol status and BMI (Tables 3 and 4). Third, for the model adjusted for age, gender, and genetic PCs, instrumental variable (IV) analyses were performed by the “MendelianRandomization” package in R using the inverse variance-weighted method (“mr_ivw”), similar to the ratio method for a single IV. This model is considered the most correct: it is most likely to account for factors unaccounted for by the genetic instruments (such as the principal components) and least likely to infer additional bias by breaking the ‘randomization’ (by adjusting for, e.g., BMI that could infer bias if associated with an unmeasured confounder).

In additional analyses, we performed multivariable MR analyses incl. genetically predicted milk consumption, smoking, and BMI in a single model using the ‘ivreg2’ and ‘bootstrap’ in Stata, adjusted for age, gender, and the first

Table 1 Associations of genetically determined levels of milk intake with lung function (N=325,737)

	Unadjusted			Adjusted for age, sex, and PCs			Further adjusted for smoking, alcohol and BMI		
	Beta	SE	p	Beta	SE	p	Beta	SE	p
<i>FEV1, liter</i>									
Lactase genotype									
GG (non-persistence)	0 (ref)			0 (ref)			0 (ref)		
GA (persistence)	-0.017	0.006	0.004	-0.011	0.004	0.008	-0.009	0.004	0.023
AA (persistence)	0.017	0.003	<0.001	0.010	0.002	<0.001	0.009	0.002	<0.001
AA vs. GA/GG	0.016	0.003	<0.001	0.010	0.002	<0.001	0.008	0.002	<0.001
Per milk-increasing allele	-0.014	0.002	<0.001	-0.009	0.002	<0.001	-0.008	0.002	<0.001
Genotype × milk*			0.201			0.536			0.775
Drinking vs. not drinking milk (genetically determined)			-0.154	0.034	<0.001				
Per glass of genetically determined higher milk intake**			-0.138	0.031	<0.001				
<i>FVC, liter</i>									
Lactase genotype									
GG (non-persistence)	0 (ref)			0 (ref)			0 (ref)		
GA (persistence)	-0.027	0.008	<0.001	-0.020	0.006	<0.001	-0.017	0.006	0.003
AA (persistence)	-0.044	0.008	<0.001	-0.031	0.006	<0.001	-0.026	0.006	<0.001
AA vs. GA/GG	0.020	0.004	<0.001	0.014	0.003	<0.001	0.011	0.003	<0.001
Per milk-increasing allele	-0.019	0.003	<0.001	-0.013	0.002	<0.001	-0.011	0.002	<0.001
Genotype × milk*			0.247			0.623			0.933
Drinking vs. not drinking milk (genetically determined)			-0.223	0.034	<0.001				
Per glass of genetically determined higher milk intake**			-0.200	0.031	<0.001				

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; principal component, SE, standard error. *rs4988235 genotype (per allele) × drinking milk (no/yes) interaction test. **SNP-milk intake estimates, N=2298. SNP-outcome estimates: N=325,737

10 PCs. We also examined the association between a genetic risk score for asthma and drinking milk, adjusted for age, gender, and the first 10 PCs.

Results

Descriptive statistics of the participants are shown in Table 5. The rs4988235 genotype was significantly associated with smoking status, BMI, and drinking milk in crude analyses. When restricting the no-milk drinkers to those answering “Never/rarely have milk”, the results were similar.

Observational analyses

Self-reported milk-drinkers vs. non-milk drinkers had an OR = 1.36 (95% CI: 1.32, 1.40, $p < 0.001$) and OR = 1.33 (95% CI: 1.29, 1.38, $p < 0.001$) of hay fever and asthma, respectively (Table 3, adjusted for age, sex, smoking and alcohol status, and BMI). Milk intake quantity was not significantly associated with hay fever or asthma in the smaller sample with available data on quantity of milk intake ($n = 2298$).

Self-reported milk-drinkers had higher FEV1: $\beta = 0.022$ (SE = 0.004, $p < 0.001$) and FVC: $\beta = 0.026$ (SE = 0.005, $p < 0.001$) than non-milk drinkers (Table 4, adjusted for age, sex, smoking and alcohol status, and BMI). Milk intake quantity (per glass of milk yesterday) was significantly associated with higher FVC: $\beta = 0.057$ (SE = 0.021, $p = 0.008$) but not with FEV1: $\beta = 0.027$ (SE = 0.017, $p = 0.122$).

Mendelian randomization analyses

The rs4988235 variant was associated with 1.06-fold increased odds of drinking milk per each lactose persistence allele (95% CI: 1.04, 1.08, SE = 0.011, $p < 0.001$, N = 363,961) (adjusted for age, sex and 40 PCs). Among milk drinkers, each lactose persistence allele increased milk intake by 0.065 L (95% CI: 0.017, 0.113, SE = 0.025, $p = 0.008$, N = 2298) (adjusted for age, sex and 40 PCs).

Genetically determined higher milk intake was associated with a lower risk of hay fever: OR = 0.986 (95% CI: 0.974, 0.999, $p = 0.036$) per milk-increasing allele (Table 2, adjusted for age, sex and PCs). Genetically determined drinking vs. not drinking milk was associated with a lower risk of hay fever: OR = 0.791 (95% CI: 0.636, 0.982,

Table 2 Associations of genetically determined milk intake with hay fever and asthma (N = 361,302)

	Unadjusted				Adjusted for age, sex, and PCs				+Smoking, alcohol, and BMI					
	OR	LCI	UCI	p	OR	SE****	LCI	UCI	p	OR	LCI	UCI	p	
Hay fever														
Lactase genotype														
GG (non-persistence)	1 (ref)				1 (ref)					1 (ref)				
GA (persistence)	0.989	0.956	1.022	0.514	1.001		0.968	1.036	0.939	1.000	0.967	1.035	0.967	
AA (persistence)	0.956	0.925	0.988	0.007	0.983		0.951	1.016	0.304	0.983	0.951	1.016	0.305	
AA vs. GA/GG	0.965	0.951	0.981	<0.001	0.982		0.966	0.997	0.022	0.982	0.967	0.998	0.026	
Per milk-increasing allele	0.972	0.960	0.985	<0.001	0.986	0.006	0.974	0.999	0.036	0.987	0.974	0.999	0.041	
Genotype X milk*				0.047					0.044				0.048	
Drinking vs. not drinking milk (genetically determined)														
Per glass of genetically determined higher milk intake*****														
Hay fever, no-milk drinkers**														
Lactase genotype														
GG (non-persistence)	1 (ref)				1 (ref)					1 (ref)				
GA (persistence)	1.030	0.929	1.145	0.568	1.033		0.930	1.149	0.535	1.039	0.934	1.155	0.479	
AA (persistence)	1.033	0.933	1.143	0.529	1.062		0.958	1.177	0.253	1.069	0.963	1.185	0.210	
AA vs. GA/GG	1.007	0.957	1.060	0.785	1.032		0.980	1.088	0.229	1.035	0.982	1.090	0.203	
Per milk-increasing allele	1.010	0.970	1.051	0.635	1.029	0.022	0.987	1.072	0.177	1.031	0.989	1.075	0.148	
Hay fever, milk drinkers***														
Lactase genotype														
GG (non-persistence)	1 (ref)				1 (ref)					1 (ref)				
GA (persistence)	0.989	0.955	1.025	0.549	1.002		0.967	1.038	0.911	1.001	0.966	1.037	0.965	
AA (persistence)	0.954	0.921	0.987	0.007	0.980		0.946	1.014	0.246	0.979	0.945	1.014	0.232	
AA vs. GA/GG	0.963	0.947	0.979	<0.001	0.978		0.962	0.994	0.008	0.978	0.962	0.995	0.009	
Per milk-increasing allele	0.970	0.957	0.983	<0.001	0.984	0.007	0.970	0.997	0.016	0.984	0.970	0.997	0.017	
Per glass of genetically determined higher milk intake*****														
Asthma														
Lactase genotype														
GG (non-persistence)	1 (ref)				1 (ref)					1 (ref)				
GA (persistence)	0.976	0.933	1.021	0.286	0.976		0.934	1.021	0.295	0.971	0.928	1.05	0.198	
AA (persistence)	0.944	0.904	0.986	0.009	0.944		0.904	0.987	0.010	0.937	0.896	0.979	0.004	
AA vs. GA/GG	0.964	0.944	0.984	<0.001	0.964		0.944	0.984	<0.001	0.960	0.941	0.981	<0.001	
Per milk-increasing allele	0.969	0.953	0.986	<0.001	0.969	0.008	0.953	0.986	<0.001	0.966	0.950	0.982	<0.001	
Genotype x milk*				0.615					0.594				0.495	
Drinking vs. not drinking milk (genetically determined)														
Per glass of genetically determined higher milk intake*****														

Table 2 (continued)

	Unadjusted			Adjusted for age, sex, and PCs					+ Smoking, alcohol, and BMI				
	OR	LCI	UCI	<i>p</i>	OR	SE****	LCI	UCI	<i>p</i>	OR	LCI	UCI	<i>p</i>
Per glass of genetically determined higher milk intake*****					0.620		0.481	0.799	0.001				

'Genetically determined' refers to (1) an additive model with a per allele (A) milk-increasing effect, and (2) major homozygotes (AA) vs. heterozygotes (GA) and minor homozygotes combined (GG). AA: N = 205,376, GA: N = 133,909, GG: N = 22,017

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; OR, odds ratio; LCI, lower confidence interval; UCI, upper confidence interval; PC, principal component; SE, standard error. *rs4988235 genotype $\times$ drinking milk (no/yes) interaction test. **N = 28,854. ***N = 335,107. ****Used in IV-analyses. *****SNP-milk intake estimates, N = 2298. SNP-outcome estimates: N_{hay fever} = 361,302. N_{hay fever, milk drinkers} = 335,107. N_{asthma} = 361,302

$p=0.033$). The odds ratio of hay fever was OR = 0.810 (95% CI: 0.667, 0.983, $p=0.003$) per glass of genetically determined higher milk intake.

There was a statistically significant interaction between genotype and milk intake status. In milk-drinkers, the associations were strengthened with an odds ratio of hay fever: OR = 0.984 (95% CI: 0.970, 0.997, $p=0.016$) per milk-increasing allele. In non-milk drinkers the association was absent with an odds ratio of hay fever: OR = 1.029 (95% CI: 0.987, 1.072) per milk-increasing allele (Table 2, adjusted for age, sex, and PCs).

Genetically determined higher milk intake was associated with a lower risk of asthma: OR = 0.969 (95% CI: 0.953, 0.986, $p<0.001$) per milk-increasing allele (Table 2, adjusted for age, sex and PCs). Genetically determined drinking vs. not drinking milk was associated with a lower risk of asthma: OR = 0.587 (95% CI: 0.442, 0.779, $p=0.001$). The odds ratio of asthma was OR = 0.620 (95% CI: 0.481, 0.799, $p=0.001$) per glass of genetically determined higher milk intake.

Genetically determined higher milk intake was associated with lower FEV1: $\beta = -0.009$ (SE = 0.002, $p<0.001$) liter per milk-increasing allele (Table 1, adjusted for age, sex, and PCs). Genetically determined drinking vs. not drinking milk was associated with lower FEV1: $\beta = -0.154$ (SE = 0.034, $p<0.001$) liter. FEV1 was $\beta = -0.138$ (SE = 0.031, $p<0.001$) liter per glass of genetically determined higher milk intake.

Genetically determined higher milk intake was also associated with lower FVC: $\beta = -0.013$ (SE = 0.002, $p<0.001$) liter per milk-increasing allele (Table 1, adjusted for age, sex, and PCs). Genetically determined drinking vs. not drinking milk was associated with lower FVC: $\beta = -0.223$ (SE = 0.034, $p<0.001$) liter. FVC was $\beta = -0.200$ (SE = 0.031, $p<0.001$) liter per glass of genetically determined higher milk intake.

The primary definition of hay fever showed among milk-drinkers an OR = 0.984 (95% CI: 0.970, 0.997, $p=0.016$, N = 335,107, N_{cases} = 75,664) per milk increasing allele. Using self-reported hay fever medication as alternative definition of hay fever yielded an OR = 0.949 (95% CI: 0.911, 0.988, $p=0.011$, N = 335,107, N_{cases} = 6415) per milk increasing allele. Using self-reported hay fever classified as serious illness as alternative definitions of hay fever indicated an OR = 0.997 (95% CI: 0.968, 1.026, $p=0.820$, N = 335,107, N_{cases} = 12,688) per milk increasing allele.

The weighted genetic BMI-score was significantly associated with BMI with an estimate = 0.92 (95% CI: 0.85, 0.99, $p<0.001$, N = 84,946) kg/m² higher BMI per BMI-increasing allele. The smoking associated SNP was associated with an estimate = 0.96 (95% CI: 0.61, 1.32, $p<0.001$, N = 5006) cigarettes per day per smoking-increasing allele among current smokers and an estimate = 0.11 (95% CI: 0.07, 0.15,

Table 3 Associations of self-reported milk intake with hay fever and asthma

	N	Unadjusted				Adjusted for age and sex				+ Smoking, alcohol and BMI			
		OR	LCI	UCI	<i>p</i>	OR	LCI	UCI	<i>p</i>	OR	LCI	UCI	<i>p</i>
<i>Hay fever</i>													
Non-milk drinkers		1 (ref)				1 (ref)				1 (ref)			
Milk-drinkers	363,961	1.387	1.350	1.425	<0.001	1.358	1.322	1.395	<0.001	1.363	1.326	1.401	<0.001
Per glass of milk yesterday*	2298	0.968	0.852	1.101	0.629	0.972	0.853	1.107	0.679	0.978	0.858	1.116	0.749
<i>Asthma</i>													
Non-milk drinkers		1 (ref)				1 (ref)				1 (ref)			
Milk-drinkers	363,961	1.326	1.281	1.374	<0.001	1.303	1.258	1.349	<0.001	1.334	1.287	1.382	<0.001
Per glass of milk yesterday*	2298	1.095	0.902	1.328	0.355	1.099	0.903	1.337	0.345	1.148	0.940	1.401	0.175

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; OR, odds ratio; LCI, lower confidence interval; UCI, upper confidence interval. *Among those who reported to have a milk intake yesterday

Table 4 Associations of self-reported milk intake with lung function

	N	Unadjusted			Adjusted for age and sex			+ Smoking, alcohol and BMI		
		Beta	SE	<i>p</i>	Beta	SE	<i>p</i>	Beta	SE	<i>p</i>
<i>FEV1</i>										
Non-milk drinkers		0 (ref)			0 (ref)			0 (ref)		
Milk-drinkers	327,803	0.109	0.005	<0.001	0.016	0.004	<0.001	0.022	0.004	<0.001
Per glass of milk yesterday*	2068	0.135	0.024	<0.001	0.019	0.017	0.283	0.027	0.017	0.122
<i>FVC</i>										
Non-milk drinkers		0 (ref)			0 (ref)			0 (ref)		
Milk-drinkers	327,803	0.139	0.007	<0.001	0.011	0.005	0.023	0.026	0.005	<0.001
Per glass of milk yesterday*	2068	0.184	0.031	<0.001	0.040	0.022	0.063	0.057	0.021	0.008

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; SE, standard error. *Among those who reported to have a milk intake yesterday

$p < 0.001$, $N = 81,853$) cigarettes per day per smoking-increasing allele among never, former, and current smokers.

Multivariable MR analyses of genetically determined milk drinking, BMI and smoking quantity and adjusted for age, sex, and 10 principal components, showed non-significant inverse effects of genetically determined milk drinking on asthma with $OR = 0.66$ (95% CI: 0.17, 2.64, $p = 0.555$, $N = 81,564$, $F_{\text{milk}} = 4.7$, $F_{\text{BMI}} = 235$, $F_{\text{smoking}} = 11.1$) for having asthma, $OR = 0.72$ (95% CI: 0.17, 3.00, $N = 81,564$, $p = 0.650$, $F_{\text{milk}} = 4.7$, $F_{\text{BMI}} = 235$, $F_{\text{smoking}} = 11.1$) for having hay fever, FEV1 with estimate = -0.70 (95% CI: -24.3 , 22.9 , $p = 0.954$, $N = 73,525$, $F_{\text{milk}} = 4.1$, $F_{\text{BMI}} = 220$, $F_{\text{smoking}} = 5.5$) liter higher FEV1, and estimate = -1.3 (95% CI: -38.8 , 36.1 , $N = 73,525$, $p = 0.944$, $F_{\text{milk}} = 4.1$, $F_{\text{BMI}} = 220$, $F_{\text{smoking}} = 5.5$) liter higher FVC per milk-increasing allele, respectively. When restricting the no-milk drinkers to those answering “Never/rarely have milk”, the results were similar.

The genetic risk score for asthma was significantly associated with a higher odds ratio of asthma: $OR = 1.08$ (95% CI: 1.08, 1.09, $p < 0.001$, $N = 353,576$) per asthma-increasing

allele, adjusted for age, sex, and 10 principal components. The genetic risk-score for asthma was positively associated with drinking milk with $OR = 1.006$ (95% CI: 1.000, 1.012, $p = 0.0498$, $N = 353,576$) of drinking milk per asthma-increasing allele, adjusted for age, sex, and 10 principal components. Genetically determined higher risk of asthma was not associated with amount of milk intake among milk drinkers: estimate = 0.008 (95% CI: -0.007 , 0.023 , $p = 0.315$, $N = 2219$) glasses of milk per asthma-increasing allele, adjusted for age, sex, and 10 principal components.

Discussion

While observational studies showed higher risk of asthma and hay fever among milk drinkers and higher lung function, univariable Mendelian randomization analyses showed lower risk of hay fever and asthma, but lower FEV1 and FVC, for genetically determined higher milk intake and for genetically determined milk-drinkers compared to non-milk drinkers. These results were supported by multivariable

Table 5 Characteristics of the UK Biobank study population (N = 363,961)

Characteristics	rs4988235 genotype						p-value (Kruskal Wallis [*] or Chi ² test)							
	GG			GA			AA			Genotype	Hay fever	Asthma	FEV1	FVC
	N	% or mean (SD)	(SD)	N	% or mean (SD)	(SD)	N	% or mean (SD)	(SD)					
All	22,088	6.1		134,908	37.1		206,965	56.9		NA	NA	NA	NA	NA
<i>Sex</i>														
Women	11,993	54.3		73,602	54.6		112,959	54.6		0.7251	<0.0001	<0.0001	<0.0001	<0.0001
Men	10,095	45.7		61,306	45.4		94,006	45.4						
Age, years	22,088	56.9 (8.0)		134,908	56.9 (8.0)		206,965	56.9 (8.0)		0.8053	<0.0001	<0.0001	NA	NA
<i>Smoking status</i>														
Never	12,210	55.5		74,345	55.3		113,124	54.9		0.0013	<0.0001	<0.0001	0.0006	<0.0001
Former	7884	35.8		47,941	35.7		73,838	35.8						
Current	1908	8.7		12,106	9.0		19,178	9.3						
<i>Drinking alcohol</i>														
No	660	3.0		4244	3.1		6397	3.1		0.3865	<0.0001	<0.0001	<0.0001	<0.0001
Yes	21,417	97.0		130,567	96.9		200,418	96.9						
Alcohol intake, drinks/week	15,990	8.5 (8.8)		97,021	8.5 (8.8)		148,872	8.6 (8.6)		0.2590	<0.0001	0.0051	NA	NA
<i>BMI group</i>														
< 18.5 kg/m ²	139	0.6		711	0.5		976	0.5		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
18.5–25 kg/m ²	7566	34.4		44,793	33.3		67,054	32.5						
> 25–30 kg/m ²	9342	42.4		57,465	42.7		88,492	42.9						
> 30 kg/m ²	4972	22.6		31,481	23.4		49,723	24.1						
BMI, kg/m ²	22,019	27.2 (4.7)		134,450	27.3 (4.7)		206,245	27.4 (4.7)		<0.0001	<0.0001	<0.0001	NA	NA
<i>Drinking milk</i>														
No	2074	9.4		10,723	7.9		16,057	7.8		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Yes	20,014	90.6		124,185	92.1		190,908	92.2						
Milk yesterday, glasses*	137	0.97 (0.47)		880	1.11 (0.69)		1281	1.15 (0.75)		0.1371	0.6246	0.7065	NA	NA
Fizzy drinks yesterday, glasses	216	1.25 (0.82)		1263	1.17 (0.74)		1740	1.21 (0.74)		0.1135	0.0731	0.0793	NA	NA
Orange juice yesterday, glasses	647	0.98 (0.52)		3741	0.97 (0.51)		5437	0.96 (0.49)		0.7292	0.1040	0.0084	NA	NA
Energy intake yesterday**, kJ	2381	8900 (3212)		13,921	8863 (3186)		20,180	8835 (3080)		0.7856	0.0001	0.2708	NA	NA

Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; SD, standard deviation. * Among those who reported to have a milk intake yesterday. ** Calculated

Mendelian randomization analyses although these analyses lacked power and were not statistically significant.

Unlike our observational results, most previous traditional observational studies on the association of milk consumption on allergies and asthma show a protective effect of milk on hay fever and asthma. Mai and colleagues found that asthma was significantly associated with infrequent milk consumption among girls, but not boys [12]. Another study found that milk consumption did not protect against childhood asthma and concluded that previously observed protection of milk against asthma could be due to parents avoiding milk for their asthmatic children [13]. A third study found that the prevalence of recent asthma at age three was lower in children who at age two consumed full cream milk daily than in those who did not. Likewise, the prevalence of recent wheeze was lower in children who consumed milk products daily compared to those who did not [14]. Even intake of milk during pregnancy has been subject to investigating regarding a possible protective effect on asthma and allergy in the offspring. Thus, Bunyavanich and colleagues found that a higher maternal intake of, for example, milk in early pregnancy was associated with lower risk of allergy and asthma in mid-childhood [11].

Observational studies of drinking milk and lung volume are few. Thiara describes how consumption of milk has been believed to increase mucus secretion and worsen asthma [18]. Haas and colleagues found that milk lipids may disturb gas exchange in 11 asthmatic patients, but not in 10 healthy controls [15]. Woods and colleagues concluded that dairy products are unlikely to be a bronchoconstrictor in most asthmatic patients [16]. Yusoff and colleagues found an 8 weeks period of egg- and milk-free diet to reduce allergic symptoms and improve lung function in asthmatic children [17]. However, the study was only single-blinded and not randomized; the parents were given the option to choose for their children either the intervention group avoiding eggs and milk, or the control group consuming their ordinary diet. The available studies are quite small and inconclusive.

Although a genetic marker of milk consumption is less likely to be associated with common confounding factors and is unaffected by reverse causation, violation of the main principles of Mendelian randomization may hamper causal inference. According to the relevance assumption, the instrument must be associated with the exposure, i.e. the rs4988235 genotype needs to be associated with milk consumption. The risk of violating this assumption is reduced by using a genotype biologically associated with the digestion of lactose. In accordance with previous studies, we found that the rs4988235 was strongly associated with milk intake status supporting that rs4988235 is well-suited as a genetic instrument of milk intake. The exclusion assumption says that the rs4988235 genotype needs to be independent of the hay fever/asthma/lung function given the milk consumption

and unmeasured confounders, i.e. the rs4988235 genotype can only affect hay fever/asthma/lung function through milk consumption. This assumption is strengthened by the fact that the genotype codes for a lactose digesting enzyme rather than a complex trait such as BMI. To avoid confounding by population stratification, we adjusted for population structure using 40 principal components. The use of a single SNP prevents the use of pleiotropy robust methods such as MR Egger and MRMix [25]. However, our study included multivariable MR-analyses to account for the possible pleiotropic effect of the lactase SNP through smoking and BMI. In addition, we examined the association between a genetic asthma-score and drinking milk to investigate any reverse causation, e.g., if asthmatics stop drinking milk after they develop asthma.

A limitation of the study is the use of self-reported hay fever and asthma rather than clinical diagnoses or measurements. Non-differential misclassification of a binary outcome is likely to attenuate associations towards the null. We also defined milk intake status from self-report. Milk intake quantity was assessed in a very small number of individuals which makes the quantity measures in liter somewhat unreliable. The primary hay fever variable included eczema. Therefore, we assessed another two hay fever variables, one of which yielded a similar results and the other a non-significant result. The main weaknesses of the additional hay fever variables were the fact that one was based on self-reported medication rather than doctor-diagnosed hay fever, and the other only counted hay fever cases that were classified as a serious illness by the participants. It is likely that those drinking (more) milk have other dietary habits as well, e.g., substituting fizzy drinks or juice, or having a different total energy intake. However, the rs4988235 genotype was not associated with any of these. Although different dietary habits might still be an issue in the observational analyses, it is not likely to be in the Mendelian Randomization analyses.

In conclusion, as opposed to our traditional observational results that found higher risk of asthma and hay fever among milk drinkers and higher lung function but are likely to suffer from confounding and reverse causation, our genetic results indicate that drinking milk infers a lower risk of hay fever and asthma, but lower FEV1 and FVC. The results should be confirmed in other studies before any recommendations can be made.

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Declarations

Conflict of interest None.

Ethics Each participant has given informed consent. An independent Ethics and Governance Council oversees adherence to the Ethics and Governance Framework [20].

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