Does DNA methylation mediate the association of age at puberty with FVC or FEV_1?

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Supplementary materials

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S. 1. Sample sizes at each follow-up for the study variables.

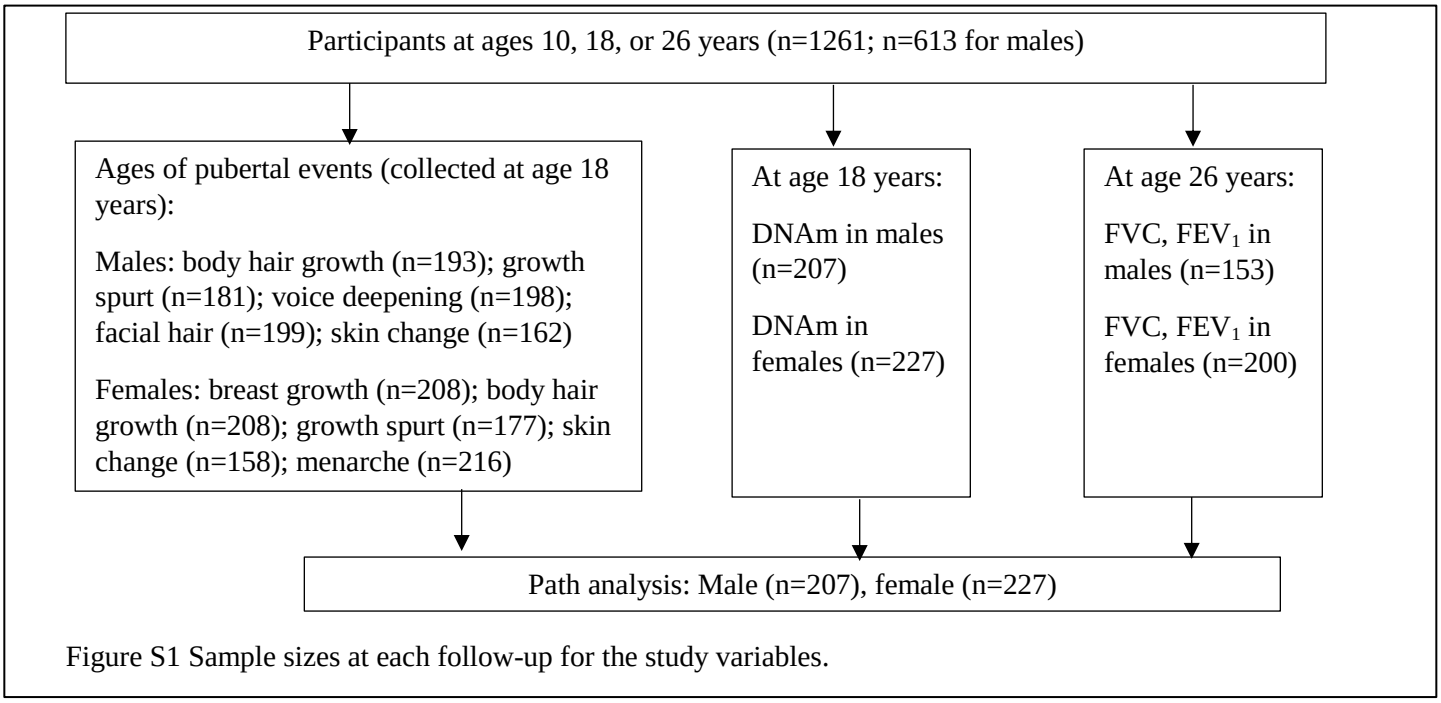

Figure S1 Sample sizes at each follow-up for the study variables.

S. 2. Methods for potential confounders, test of DNAm, cell types, flow chart of screening CpGs using forward and backward screening, and replication cohort ALSPAC

Potential confounders

Factors related to growth, demographic features, and environmental exposures might confound the association of age at pubertal events with height growth and lung function. Status of low birth weight (<2,500 grams), maternal smoking status during pregnancy, asthma status at age 10 years, body mass index (BMI) at age 10 years, socioeconomic status (SES), atopy at age 10 years, and personal smoking status at age 18 years were included in the analyses as potential confounders. Birth weight and maternal smoking during pregnancy were obtained at birth of the child. Asthma was defined as having 'physician diagnosed asthma' and either 'wheezing or whistling in the chest in the last 12 months' and/or 'current treatment for asthma', using the ISAAC questionnaire [1]. SES was ascertained by 'low', 'medium' and' high' according to assessment of parental occupation, level of household income and number of rooms in

the house [2]. Atopy was defined by having a skin prick testing (SPT) to at least one allergen test with mean wheal diameter ≥ 3 mm greater than the negative saline control [3].

DNA methylation (DNAm)

In the Isle of Wight birth cohort, peripheral blood samples were collected at ages 18 years. DNA was extracted from whole blood using a standard salting out procedure [4]. DNA concentration was estimated by fluorometric quantitation. For each sample, one microgram DNA was bisulfite-treated for cytosine to thymine conversion using the EZ 96-DNA methylation kit (Zymo Research, Irvine, CA, USA), following the manufacturer's protocol. The HumanMethylation450 BeadChip interrogates >484,000 CpGs and MethylationEPIC BeadChip has > 850,000 CpGs both associated with over 24,000 genes. Arrays were processed using a standard protocol with multiple identical control samples assigned to each bisulfite conversion batch to assess assay variability. DNA samples were randomly distributed on the arrays aiming to eliminate batch effects. DNAm was calculated by a ratio of methylated (M) over the sum of methylated and unmethylated (U) probes (β=M/[c+M+U]), where c is used as a constant to prevent zero in the denominator[5], denoted as β values for each CpG site. In our study, β values were logit transformed with base 2 (denoted as M values), and M values were used in all the analyses.

DNAm quality control and pre-processing

Probes not reaching a detection p-value of 10^{-16} in at least 95% of samples were excluded. Quantile normalization was applied to DNA methylation intensities as suggested in the CPACOR pipeline [6]. Autosomal probes were then extracted and converted to β values. ComBat was used to logit transformed β values to remove batch effects [7]. CpG sites with probe-SNPs within ten base pairs of the CpG site and with minor allele frequency (MAF) greater than 0.007 in the IOWBC (i.e., ~ ≥ 10 out of 1,456 subjects expected to have the minor allele in the cohort) were excluded.

Cell-type proportions

DNAm is influenced by heterogeneity of cell type compositions in peripheral blood, which may lead to biased inferences. Proportions of six cell types, CD4+ T cells, natural killer cells, neutrophil, B cells, monocytes, and eosinophils, were inferred using the R package *minfi* [8, 9] and included in the analyses as confounders.

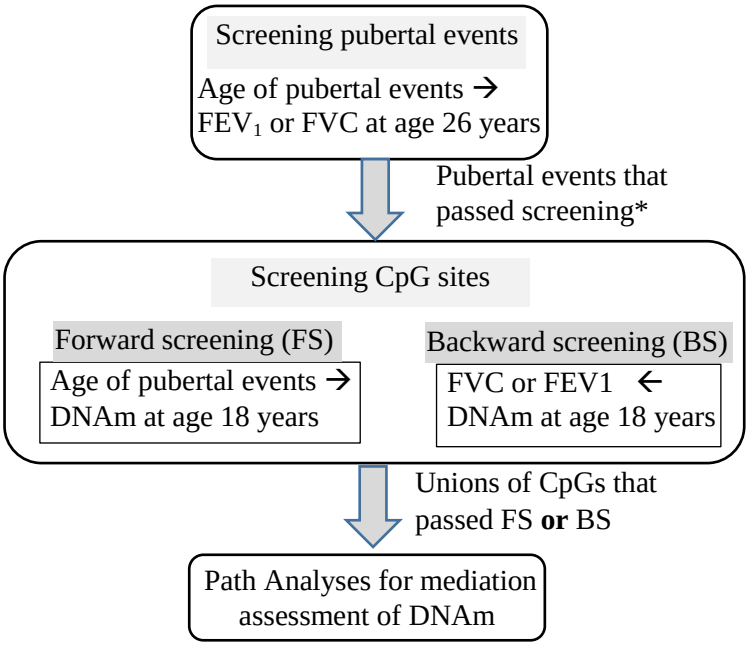

FIGURE S2. Flow chart of screening CpGs using forward and backward screening.
* If none of the pubertal events were associated with a lung function parameter, then that lung function parameter was excluded from subsequent analyses.

Replication cohort ALSPAC

Pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992 were invited to take part in the study. The initial number of pregnancies enrolled is 14,541 (for these at least one questionnaire has been returned or a "Children in Focus" clinic had been attended by 19th July 1999). Of these initial pregnancies, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join

the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14,541 pregnancies mentioned above. The number of new pregnancies not in the initial sample (known as Phase I enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 24 is 913 (456, 262 and 195 recruited during Phases II, III and IV respectively), resulting in an additional 913 children being enrolled. The phases of enrolment are described in more detail in the cohort profile paper and its update (see footnote 4 below). The total sample size for analyses using any data collected after the age of seven is therefore 15,454 pregnancies, resulting in 15,589 foetuses. Of these 14,901 were alive at 1 year of age. A 10% sample of the ALSPAC cohort, known as the Children in Focus (CiF) group, attended clinics at the University of Bristol at various time intervals between 4 to 61 months of age. The CiF group were chosen at random from the last 6 months of ALSPAC births (1432 families attended at least one clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool: http://www.bristol.ac.uk/alspac/researchers/our-data/.

S. 3. Identification of pubertal events, lung function parameters, and CpG sites to be included in path analyses (Figure 2 and 3 in the main text).

To identify whether age of onset of pubertal events and lung function parameters were included in path analyses, we examined the association of ages of these pubertal events with lung function parameters FVC and FEV_1 at age 26 years in the IOWBC. Statistical significance is set at 0.05.

In females, at age 26 years lung function parameters, early ages of onset of body hair growth and menarche onset showed statistically significant associations with lower FVC and FEV_1 (Table S1). For males, ages of body hair growth, growth spurt, voice deepening, and appearance of facial hair growth

were associated with FVC at age 26 years. No other statistically significant associations were identified at these two ages. For males, FEV_1 was not included in path analyses, since it was not associated the age of any pubertal events.

Table S1 The association of age of pubertal events and lung function measures at age 26 years. Potential confounders adjusted were listed in Figure 2 in the main text, including: asthma status at age 10 years, height at age 10 years, BMI at age 10 years, SES at age 10 years, low birth weight status, maternal smoking during pregnancy and atopy status at age 10 years.

Lung function	Female			Male		
	Pubertal events	Estimate[#]	p-value	Pubertal events	Estimate	p-value
FVC	Breast growth	0.041	0.062	**Body hair growth**	**0.104**	**0.015**
	Body hair growth	**0.051**	**0.032**	**Growth spurt**	**0.086**	**0.019**
	Growth spurt	0.040	0.059	**Voice deepening**	**0.110**	**0.021**
	Skin changes	0.031	0.211	**Facial hair growth**	**0.112**	**0.028**
	Menarche	**0.076**	**0.001**	Skin changes	0.057	0.257
FEV_1	Breast growth	0.019	0.271	Body hair growth	0.056	0.140
	Body hair growth	**0.042**	**0.027**	Growth spurt	0.041	0.217
	Growth spurt	0.025	0.127	Voice deepening	0.050	0.247
	Skin changes	0.030	0.135	Facial hair growth	0.032	0.484
	Menarche	**0.066**	**<0.001**	Skin changes	0.037	0.411
	Menarche	0.001	0.629	Skin changes	-0.001	0.809

[#]: Coeff. Estimate: Regression coefficient estimates, and the unit presented in litres (L). It represents expected lung function change for one year increase in pubertal age.

Next, we screened CpGs from two directions, which comprised of puberty-related CpGs (forward screening) and lung function-related CpGs (backward screening). (1) Forward screening was used to identify candidate CpGs such that DNAm at those CpGs was associated with ages of pubertal events. The pubertal events that passed the initial screening on their association with FVC or FEV_1 were included in this forward screening. For females, at 198 and 134 CpGs (in total, 321 unique CpGs with 11 overlappings), DNAm was associated with age at menarche and body hair growth, respectively (Figure 3). For males, at 152, 105, 131, and 109 CpGs (in total, 489 unique CpGs with 8 overlappings), DNAm

was associated with age at body hair growth, growth spurt, voice deepening, and facial hair growth, respectively (Figure 3). There were no common CpGs between males and females. CpGs (in total 321 unique CpGs for females and 489 unique CpGs for males) that survived this forward screening were included in subsequent path analyses. (2) In the backward screening of CpGs, for females, lung function parameters FVC and FEV_1 were used. For females, at 102 and 174 CpGs (in total, 259 unique CpGs with 17 overlappings), DNAm was associated with FVC and FEV_1 at age 26, respectively (Figure 3). For males, since in the initial screening, no pubertal events were associated with FEV_1, this lung function parameter was not used to screen CpG sites. In total, 62 CpGs were associated with FVC at age 26 (Figure 3). As in the forward screening, no CpGs overlapped between males and females. CpGs that survived this backward screening were treated as candidate CpGs and included in subsequent path analyses (Figure 3).

FIGURE S3 Mediation analysis on relationship of age at menarche with lung function parameter FVC.

The indirect coefficient (IEC) of 0.182 for age at menarche means that, with other factors kept constant, one year delay in the onset of menarche was associated with a 0.182 Liter increase in FVC at age 26 years. The path of indirect effect was menarche → DNAm at age 18 years → lung function FVC at age 26 years (the estimated effect was (-0.216)*(-0.203)=0.044). The direct effect coefficient (DE) for age at menarche on FVC at age 26 is -0.138 after controlling for other factors. The total effect coefficient for age at menarche on FVC at age 26 combined the indirect and direct effect (0.044+0.138=0.182).

S. 4. DNAm at age 18 years mediated the association of age of pubertal onset with lung function parameters in young adulthood.

Further assessment of the IOWBC discovered CpGs in ALSPAC

In total, 21 CpGs for females and 20 CpGs for males discovered in IOWBC were assessed in ALSPAC. Lung function measurement at age 24 years and DNAm at age 15 or 17 years in ALSPAC were analyzed.

In females, of the 21 unique CpGs, indirect effects at 10 CpGs (Table 2A and 2B) were consistent with those observed in IOWBC, but none of them showed statistically significant mediation effects. A more striking observation was the low ratio of indirect effect over direct effect at these 10 CpGs; all ratios were <0.07, substantially lower than those observed in the IOWBC. In males, ages of body hair growth and voice deepening were available in ALSPAC. Of the 11 IOWBC-discovered CpGs for these two pubertal events, consistent mediation effects were observed at 6 CpGs but were statistically insignificant (Table 2A and 2B). Overall, both direct and indirect effects of these two pubertal events were negligible in ALSPAC.

We also examined whether cell-type composition played a role in the above statistically insignificant findings potentially due to multicollinearity between certain cell types and other variables in the model [29]. In particular, we removed cell type proportions of the six cells from mediation analysis models in both cohorts. For the indirect effects discovered in IOWBC, all the directions were kept unchanged (Table S3A and S3B). In ALSPAC, the direction of indirect effects changed at only 2 of the 32 CpGs tested in ALSPAC (cg27626110 and cg25601319) (Table S3A and S3B). All the results in both cohorts indicated that cell types did not play any significant role in terms of the assessment of mediating effects (Tables S2A, S2B, S3A, and S3B). In summary, the IOWBC-discovered CpGs were not supported by data in ALSPAC regarding their role as a mediator, and such findings were not affected by cell type compositions.

Table S2A Association of age of pubertal events and lung function at age 26 through DNAm at CpGs survived forward screening at age 18 years in IOWBC. Potential confounders adjusted were listed in Figure 2 in the main text, including: asthma status at age 10 years, height at age 10 years, BMI at age 10 years, SES at age 10 years, low birth weight status, maternal smoking during pregnancy, atopy status at age 10 years, cell types, and smoking at age 18 years.

Pubertal events(sex)	Lung function at age 26	CpGs[@]	Gene	Region	Total effect[$] (IOWBC)		Direct effect[$] (IOWBC)		Indirect effect[$] (IOWBC)		Total effect (ALSAPC)		Direct effect (ALSAPC)		Indirect effect (ALSAPC)	
					Est[#]	p-val[#]	Est	p-val	Est	p-val	Est	p-val	Est	p-val	Est	p-val
Menarche (female)	FVC	**cg15711028**	*EIF4EBP2*	Body	0.19	0.01	0.15	0.04	0.04	**0.04**	0.21	8.00×10^{-4}	0.21	9.33×10^{-4}	2.68×10^{-3}	0.51
		cg08680129	*PRX*	TSS1500	0.18	0.01	0.14	0.06	0.04	**0.03**	0.22	5.62×10^{-4}	0.20	1.28×10^{-3}	1.41×10^{-2}	0.15
		cg05325369	*FBXO46*	5'UTR	0.17	0.02	0.13	0.07	0.04	**0.04**	0.21	8.49×10^{-4}	0.21	8.37×10^{-4}	-2.85×10^{-4}	0.84
		cg09131512	*IER3*	TSS1500	0.17	0.02	0.21	5.60×10^{-3}	-0.04	**0.04**	0.22	5.55×10^{-4}	0.21	6.82×10^{-4}	4.56×10^{-3}	0.52
		cg12033187		intergenic	0.16	0.03	0.13	0.09	0.04	**0.05**	0.21	1.17×10^{-3}	0.21	1.14×10^{-3}	-3.33×10^{-4}	0.90
		cg01315063	*C17orf96*	1stExon	0.19	0.01	0.13	0.07	0.06	**0.02**	0.21	7.98×10^{-4}	0.21	8.82×10^{-4}	1.74×10^{-3}	0.59
Menarche (female)	FEV1	cg03341697	*FUT4*	5'UTR;1stExon	0.23	1.09×10^{-3}	0.20	0.01	0.04	**0.05**	0.23	4.06×10^{-4}	0.23	3.89×10^{-4}	-7.40×10^{-4}	0.74
		cg17394530		intergenic	0.23	9.22×10^{-4}	0.28	5.54×10^{-5}	-0.05	**0.02**	0.23	3.61×10^{-4}	0.23	3.44×10^{-4}	-4.66×10^{-4}	0.90
		cg08894381		intergenic	0.23	1.01×10^{-3}	0.28	1.08×10^{-4}	-0.04	**0.04**	0.23	4.21×10^{-4}	0.23	4.40×10^{-4}	6.95×10^{-4}	0.77
		cg08680129	*PRX*	TSS1500	0.24	5.92×10^{-4}	0.20	0.01	0.05	**0.02**	0.23	3.01×10^{-4}	0.22	5.81×10^{-4}	1.02×10^{-2}	0.25
		cg09131512	*IER3*	TSS1500	0.23	1.58×10^{-3}	0.27	1.17×10^{-4}	-0.05	**0.03**	0.23	2.90×10^{-4}	0.23	3.40×10^{-4}	2.91×10^{-3}	0.54
		cg01315063	*C17orf96*	1stExon	0.24	5.85×10^{-4}	0.20	0.01	0.04	**0.05**	0.23	3.81×10^{-4}	0.23	4.40×10^{-4}	2.58×10^{-3}	0.54
		cg10219771		intergenic	0.24	4.91×10^{-4}	0.20	0.01	0.05	**0.03**	0.23	3.97×10^{-4}	0.23	4.03×10^{-4}	1.84×10^{-4}	0.95
Body hair growth (female)	FEV1	cg05414613	*SLC1A7*	Body	0.14	0.10	0.18	0.03	-0.05	**0.03**	0.19	2.35×10^{-3}	0.19	2.44×10^{-3}	8.90×10^{-4}	0.72
		cg16332936	*TBX1*	Body	0.14	0.11	0.09	0.30	0.05	**0.04**	0.18	3.10×10^{-3}	0.18	4.29×10^{-3}	7.04×10^{-3}	0.34
		cg04827020	*THBS1*	Body	0.17	0.04	0.12	0.16	0.05	**0.03**	0.18	3.30×10^{-3}	0.17	5.91×10^{-3}	1.13×10^{-2}	0.17
		cg23328390	*C6orf134*	TSS200	0.15	0.08	0.19	0.03	-0.04	**0.05**	0.19	2.77×10^{-3}	0.18	2.90×10^{-3}	6.87×10^{-4}	0.76
Body hair growth (male)	FVC	cg19979646	*SUGT1*	TSS200	0.11	0.19	0.18	0.04	-0.07	**0.03**	-1.73×10^{-3}	0.98	-0.01	0.93	4.17×10^{-3}	0.53
		cg13636404	*ZNF655*	TSS1500	0.14	0.10	0.08	0.37	0.06	**0.02**	9.97×10^{-4}	0.99	-4.05×10^{-4}	1.00	1.40×10^{-3}	0.71

		cg04130455		intergenic	0.15	0.08	0.23	0.01	-0.08	**0.01**	3.86×10^{-3}	0.95	0.01	0.93	-1.78×10^{-3}	0.83
		cg05679686	*CORO1C*	Body	0.15	0.09	0.10	0.25	0.05	**0.04**	1.39×10^{-2}	0.84	0.02	0.74	-8.56×10^{-3}	0.32
		cg21276535	*MAP2K1*	Body	0.16	0.05	0.11	0.20	0.06	**0.02**	4.81×10^{-3}	0.94	-2.28×10^{-4}	1.00	5.04×10^{-3}	0.36
		cg09616494	*DTHD1*	Body	0.13	0.15	0.08	0.38	0.05	**0.04**	1.66×10^{-3}	0.98	3.00×10^{-3}	0.96	-1.34×10^{-3}	0.69
		cg27626110	*SFXN1*	5'UTR	0.11	0.21	0.17	0.05	-0.06	**0.04**	-7.32×10^{-4}	0.99	-5.63×10^{-4}	0.99	-1.70×10^{-4}	0.96
Growth spurt* (male)	FVC	cg18278528	*SYNPO*	5'UTR;TSS1500	0.17	0.06	0.11	0.28	0.07	**0.03**						
		cg16965936	*PRDM15*	Body	0.18	0.04	0.24	0.01	-0.05	**0.03**						
		cg24011637	*FOSL2*	TSS1500	0.19	0.04	0.13	0.19	0.06	**0.04**						
Voice deepening (male)	FVC	cg06405341	*AXIN2*	Body	0.18	0.03	0.22	0.01	-0.04	**0.04**	-0.01	0.86	-0.01	0.84	1.50×10^{-3}	0.70
		cg01482375	*LATS1*	5'UTR	0.17	0.04	0.12	0.16	0.05	**0.04**	-0.02	0.77	-0.03	0.68	7.88×10^{-3}	0.28
Facial hair* (male)	FVC	cg01464985	*ZNF512*	TSS1500	0.15	0.07	0.22	0.01	-0.06	**0.01**						
		cg26065534	*ATP8A2*	Body	0.15	0.08	0.11	0.23	0.05	**0.04**						
		cg01808969	*FOXK1*	Body	0.16	0.07	0.22	0.02	-0.06	**0.03**						
		cg17959850	*EMB*	Body	0.16	0.06	0.10	0.26	0.06	**0.03**						

□: Age at body hair growth is identified by age at attainment of Tanner stage>2 in ALSAPC cohort. #: Est: Regression coefficient estimates, and the unit presented in litres(L). It represents the expected lung function change for one year increase in pubertal age. *: Growth spurt and facial hair in males are not available in ALSPAC. $: Total effect is the sum of direct and indirect effects of age of pubertal events on lung function at age 26 years. Indirect effect is the effect of age of pubertal events on lung function via height growth and its related CpGs. @: CpGs name in bold font had direction-consistent mediation effects in IOWBC and ALSPAC.

Table S2B Association of age of pubertal events and lung function at age 26 through DNAm at CpGs survived backward screening at age 18 years in IOWBC. Potential confounders adjusted were listed in Figure 2 in the main text, including: asthma status at age 10 years, height at age 10 years, BMI at age 10 years, SES at age 10 years, low birth weight status, maternal smoking during pregnancy, atopy status at age 10 years, cell types, and smoking at age 18 years.

Pubertal events(sex)	Lung function at age 26	CpGs[@]	Gene	Region	Total effect[$] (IOWBC)		Direct effect[$] (IOWBC)		Indirect effect[$] (IOWBC)		Total effect (ALSAPC)		Direct effect (ALSAPC)		Indirect effect (ALSAPC)	
					Est[#]	p-val[#]	Est	p-val	Est	p-val	Est	p-val	Est	p-val	Est	p-val
Menarche (female)	FEV1	cg25601319	*ARID1A*	TSS1500	0.22	1.93×10^{-3}	0.18	0.01	0.04	**0.03**	0.23	4.16×10^{-4}	0.23	4.07×10^{-4}	-2.25×10^{-4}	0.91
		cg12614633	*USP48*	TSS1500	0.23	1.37×10^{-3}	0.18	0.01	0.05	**0.03**	0.23	3.34×10^{-4}	0.23	3.23×10^{-4}	-8.50×10^{-4}	0.79
		cg05060880		intergenic	0.21	3.72×10^{-3}	0.16	0.02	0.05	**0.04**	0.23	3.44×10^{-4}	0.23	3.00×10^{-4}	-1.79×10^{-3}	0.70
Body hair growth (female)	FVC	cg00498211	*SLC1A4*	Body;5'UTR	0.17	0.04	0.12	0.16	0.05	**0.01**	0.14	0.02	0.15	0.02	-2.90×10^{-3}	0.60
		cg24432048	*LOC100133612*	Body	0.14	0.10	0.10	0.25	0.04	**0.05**	0.14	0.02	0.15	0.02	-2.71×10^{-3}	0.55
		cg04988476	*FGF12*	5'UTR	0.14	0.09	0.10	0.22	0.04	**0.05**	0.14	0.02	0.14	0.02	1.55×10^{-3}	0.64
Body hair growth (female)	FEV1	cg12614633	*USP48*	TSS1500	0.13	0.12	0.06	0.48	0.07	**0.01**	0.19	2.34×10^{-3}	0.19	2.32×10^{-3}	-8.80×10^{-5}	0.93
		cg25601319	*ARID1A*	TSS1500	0.12	0.15	0.08	0.36	0.04	**0.03**	0.19	2.52×10^{-3}	0.19	2.50×10^{-3}	5.74×10^{-6}	1.00
		cg04988476	*FGF12*	5'UTR	0.13	0.11	0.09	0.28	0.04	**0.04**	0.19	2.23×10^{-3}	0.19	2.52×10^{-3}	2.39×10^{-3}	0.54
		cg07143296	*PCNXL3*	TSS200	0.13	0.12	0.10	0.25	0.04	**0.05**	0.18	2.97×10^{-3}	0.18	3.52×10^{-3}	3.86×10^{-3}	0.52
Body hair growth (male)	FVC	**cg06998210**	*CA7*	Body;TSS1500	0.13	0.13	0.09	0.32	0.05	**0.05**	0.005	0.94	4.31×10^{-3}	0.95	1.03×10^{-3}	0.79
Growth spurt[*] (male)	FVC	cg01907761	*SPINK5L3*	TSS1500	0.20	0.03	0.15	0.10	0.05	**0.05**						
Voice deepening (male)	FVC	cg21300403	*BOLL*	Body;TSS200	0.15	0.08	0.10	0.21	0.04	**0.05**	-0.01	0.86	-0.01	0.90	-2.77×10^{-3}	0.58
Facial hair[*] (male)	FVC	cg14834881	*FAM190A*	Body	0.15	0.08	0.10	0.26	0.05	**0.03**						

□:Age at body hair growth is identified by age at attainment of Tanner stage>2 in ALSAPC cohort. [#]: Est: Regression coefficient estimates, and the unit presented in litres(L). It represents the expected lung function change for one year increase in pubertal age. *: Growth spurt and facial hair in males are not available in ALSPAC. $: Total effect is the sum of direct and indirect effects of age of pubertal events on lung function at age 26 years. Indirect effect is the effect of age of pubertal events on lung function via height growth and its related CpGs. [@]: CpGs name in bold font had direction-consistent mediation effects in IOWBC and ALSPAC.

Table S3A Association of age of pubertal events and lung function at age 26 through DNAm at CpGs survived forward screening at age 18 years in IOWBC without cell types in model. Potential confounders adjusted were listed in Figure 2 in the main text, including: asthma status at age 10 years, height at age 10 years, BMI at age 10 years, SES at age 10 years, low birth weight status, maternal smoking during pregnancy, atopy status at age 10 years, and smoking at age 18 years.

Pubertal events(sex)	Lung function at age 26	CpGs[@]	Gene	Region	Total effect[$] (IOWBC)		Direct effect[$] (IOWBC)		Indirect effect[$] (IOWBC)		Total effect (ALSAPC)		Direct effect (ALSAPC)		Indirect effect (ALSAPC)	
					Est[#]	p-val[#]	Est	p-val	Est	p-val	Est	p-val	Est	p-val	Est	p-val
Menarche (female)	FVC	**cg15711028**	*EIF4EBP2*	Body	0.19	0.01	0.15	0.03	0.04	**0.05**	0.21	4.41×10^{-4}	0.21	5.64×10^{-4}	3.96×10^{-3}	0.45
		cg08680129	*PRX*	TSS1500	0.18	0.01	0.14	0.05	0.04	**0.04**	0.22	3.47×10^{-4}	0.21	7.74×10^{-4}	1.31×10^{-2}	0.18
		cg05325369	*FBXO46*	5'UTR	0.17	0.02	0.14	0.06	0.04	**0.05**	0.21	5.31×10^{-4}	0.21	5.10×10^{-4}	-4.60×10^{-4}	0.81
		cg09131512	*IER3*	TSS1500	0.17	0.02	0.21	3.10×10^{-3}	-0.04	**0.04**	0.22	2.93×10^{-4}	0.21	4.07×10^{-4}	6.78×10^{-3}	0.39
		cg12033187		intergenic	0.17	0.02	0.13	0.08	0.04	**0.05**	0.21	8.26×10^{-3}	0.21	7.82×10^{-3}	-4.60×10^{-4}	0.86
		cg01315063	*C17orf96*	1stExon	0.19	0.01	0.14	0.06	0.06	**0.02**	0.21	4.70×10^{-4}	0.21	5.58×10^{-4}	2.65×10^{-3}	0.54
Menarche (female)	FEV1	cg03341697	*FUT4*	5'UTR;1stExon	0.24	8.18×10^{-4}	0.20	0.01	0.04	**0.04**	0.23	2.34×10^{-4}	0.23	2.17×10^{-4}	-3.60×10^{-4}	0.85
		cg17394530		intergenic	0.24	7.69×10^{-4}	0.28	5.60×10^{-3}	-0.04	**0.04**	0.23	2.27×10^{-4}	0.23	1.93×10^{-4}	-2.16×10^{-3}	0.60
		cg08894381		intergenic	0.24	9.4×10^{-4}	0.28	1.06×10^{-4}	-0.04	**0.04**	0.23	2.53×10^{-4}	0.23	2.65×10^{-4}	4.27×10^{-4}	0.80
		cg08680129	*PRX*	TSS1500	0.24	7.42×10^{-4}	0.20	5.67×10^{-3}	0.04	**0.03**	0.23	1.82×10^{-4}	0.22	3.41×10^{-4}	9.51×10^{-3}	0.29

		cg09131512	*IER3*	TSS1500	0.23	1.67×10^{-3}	0.27	1.16×10^{-4}	-0.05	**0.03**	0.24	1.53×10^{-4}	0.23	1.91×10^{-4}	4.36×10^{-3}	0.45
		cg01315063	*C17orf96*	1stExon	0.25	5.13×10^{-4}	0.20	0.01	0.04	**0.05**	0.23	2.06×10^{-4}	0.23	2.67×10^{-4}	4.12×10^{-3}	0.45
		cg10219771		intergenic	0.24	5.62×10^{-4}	0.20	0.01	0.05	**0.03**	0.23	2.33×10^{-4}	0.23	2.46×10^{-4}	1.88×10^{-4}	0.94
Body hair growth (female)	FEV1	cg05414613	*SLC1A7*	Body	0.15	0.08	0.19	0.02	-0.04	**0.05**	0.19	2.35×10^{-3}	0.19	2.33×10^{-3}	6.30×10^{-5}	0.98
		cg16332936	*TBX1*	Body	0.14	0.09	0.10	0.25	0.04	**0.04**	0.18	3.15×10^{-3}	0.18	4.37×10^{-3}	6.39×10^{-3}	0.38
		cg04827020	*THBS1*	Body	0.18	0.03	0.12	0.14	0.05	**0.03**	0.18	3.40×10^{-3}	0.17	6.26×10^{-3}	1.09×10^{-2}	0.21
		cg23328390	*C6orf134*	TSS200	0.16	0.06	0.20	0.02	-0.04	**0.05**	0.19	2.62×10^{-3}	0.18	2.79×10^{-3}	8.02×10^{-4}	0.77
Body hair growth□ (male)	FVC	cg19979646	*SUGT1*	TSS200	0.11	0.21	0.18	0.04	-0.07	**0.02**	-1.73×10^{-3}	0.98	-0.01	0.94	3.81×10^{-3}	0.64
		cg13636404	*ZNF655*	TSS1500	0.14	0.12	0.07	0.41	0.06	**0.02**	5.49×10^{-4}	0.99	3.04×10^{-4}	1.00	2.45×10^{-4}	0.95
		cg04130455		intergenic	0.15	0.08	0.23	0.01	-0.07	**0.01**	3.40×10^{-3}	0.96	0.01	0.93	-2.52×10^{-3}	0.76
		cg05679686	*CORO1C*	Body	0.15	0.10	0.10	0.26	0.05	**0.04**	1.48×10^{-2}	0.83	0.02	0.76	-6.63×10^{-3}	0.43
		cg21276535	*MAP2K1*	Body	0.16	0.05	0.11	0.20	0.06	**0.02**	2.81×10^{-3}	0.97	9.05×10^{-5}	1.00	2.72×10^{-3}	0.64
		cg09616494	*DTHD1*	Body	0.12	0.16	0.08	0.41	0.05	**0.05**	1.85×10^{-3}	0.98	3.49×10^{-3}	0.96	-1.64×10^{-3}	0.66
		cg27626110	*SFXN1*	5'UTR	0.11	0.22	0.17	0.05	-0.06	**0.03**	1.74×10^{-4}	1.00	-4.20×10^{-4}	1.00	5.92×10^{-4}	0.86
Growth spurt* (male)	FVC	cg18278528	*SYNPO*	5'UTR;TSS1500	0.18	0.06	0.12	0.20	0.06	**0.03**						
		cg16965936	*PRDM15*	Body	0.19	0.04	0.24	0.01	-0.05	**0.05**						
		cg24011637	*FOSL2*	TSS1500	0.18	0.05	0.12	0.20	0.06	**0.04**						
Voice deepening (male)	FVC	cg06405341	*AXIN2*	Body	0.17	0.04	0.23	0.01	-0.05	**0.04**	-0.01	0.84	-0.01	0.83	7.10×10^{-4}	0.85
		cg01482375	*LATS1*	5'UTR	0.17	0.04	0.12	0.16	0.05	**0.04**	-0.02	0.78	-0.03	0.67	1.06×10^{-2}	0.26
Facial hair* (male)	FVC	cg01464985	*ZNF512*	TSS1500	0.16	0.05	0.22	0.01	-0.05	**0.04**						
		cg26065534	*ATP8A2*	Body	0.15	0.10	0.10	0.26	0.04	**0.05**						
		cg01808969	*FOXK1*	Body	0.16	0.07	0.21	0.02	-0.05	**0.04**						
		cg17959850	*EMB*	Body	0.15	0.08	0.09	0.29	0.06	**0.03**						

□: Age at body hair growth is identified by age at attainment of Tanner stage>2 in ALSAPC cohort. #: Est: Regression coefficient estimates, and the unit presented in litres(L). It represents the expected lung function change for one year increase in pubertal age. *: Growth spurt and facial hair in males are not available in ALSPAC. $: Total effect is the sum of direct and indirect effects of age of pubertal events on lung function at age 26 years. Indirect effect is the effect of age of pubertal events on lung function via height growth and its related CpGs. @: CpGs name in bold font had direction-consistent mediation effects in IOWBC and ALSPAC.

Table S3B Association of age of pubertal events and lung function at age 26 through DNAm at CpGs survived backward screening at age 18 years in IOWBC without cell types in model. Potential confounders adjusted were listed in Figure 2 in the main text, including: asthma status at age 10 years, height at age 10 years, BMI at age 10 years, SES at age 10 years, low birth weight status, maternal smoking during pregnancy, atopy status at age 10 years, and smoking at age 18 years.

Pubertal events(sex)	Lung function at age 26	CpGs[@]	Gene	Region	Total effect[$] (IOWBC)		Direct effect[$] (IOWBC)		Indirect effect[$] (IOWBC)		Total effect (ALSAPC)		Direct effect (ALSAPC)		Indirect effect (ALSAPC)	
					Est[#]	p-val[#]	Est	p-val	Est	p-val	Est	p-val	Est	p-val	Est	p-val
Menarche (female)	FEV1	**cg25601319**	*ARID1A*	TSS1500	0.22	2.46×10^{-3}	0.18	0.01	0.04	**0.04**	0.23	2.23×10^{-4}	0.23	2.04×10^{-4}	1.31×10^{-3}	0.68
		cg12614633	*USP48*	TSS1500	0.23	1.26×10^{-3}	0.18	0.01	0.05	**0.03**	0.23	1.94×10^{-4}	0.23	1.84×10^{-4}	-1.08×10^{-3}	0.80
		cg05060880		intergenic	0.21	3.66×10^{-3}	0.16	0.02	0.05	**0.04**	0.23	1.90×10^{-4}	0.23	1.74×10^{-4}	-7.60×10^{-4}	0.88
Body hair growth (female)	FVC	cg00498211	*SLC1A4*	Body;5'UTR	0.16	0.05	0.13	0.12	0.04	**0.06**	0.14	0.02	0.15	0.01	-5.75×10^{-3}	0.38
		cg24432048	*LOC100133612*	Body	0.15	0.08	0.11	0.21	0.04	**0.04**	0.14	0.02	0.15	0.02	-2.14×10^{-3}	0.67
		cg04988476	*FGF12*	5'UTR	0.15	0.06	0.11	0.18	0.04	**0.04**	0.14	0.02	0.14	0.02	1.04×10^{-3}	0.69
Body hair growth (female)	FEV1	cg12614633	*USP48*	TSS1500	0.13	0.11	0.07	0.43	0.07	**0.01**	0.19	2.38×10^{-3}	0.19	2.37×10^{-3}	-1.03×10^{-4}	0.92
		cg25601319	*ARID1A*	TSS1500	0.13	0.14	0.08	0.33	0.04	**0.04**	0.19	2.46×10^{-3}	0.19	2.54×10^{-3}	8.19×10^{-4}	0.79
		cg04988476	*FGF12*	5'UTR	0.15	0.08	0.10	0.23	0.04	**0.03**	0.19	2.27×10^{-3}	0.19	2.44×10^{-3}	1.61×10^{-4}	0.63
		cg07143296	*PCNXL3*	TSS200	0.14	0.10	0.10	0.22	0.03	**0.05**	0.18	2.87×10^{-3}	0.18	3.52×10^{-3}	4.22×10^{-3}	0.50
Body hair growth (male)	FVC	**cg06998210**	*CA7*	Body;TSS1500	0.13	0.15	0.08	0.34	0.04	**0.06**	0.005	0.94	4.50×10^{-3}	0.95	5.96×10^{-4}	0.87
Growth spurt[*] (male)	FVC	cg01907761	*SPINK5L3*	TSS1500	0.19	0.03	0.15	0.10	0.04	**0.07**						
Voice deepening (male)	FVC	cg21300403	*BOLL*	Body;TSS200	0.15	0.07	0.11	0.21	0.05	**0.04**	-0.01	0.85	-0.01	0.87	-2.39×10^{-3}	0.63
Facial hair[*] (male)	FVC	cg14834881	*FAM190A*	Body	0.14	0.11	0.09	0.28	0.05	**0.05**						

□:Age at body hair growth is identified by age at attainment of Tanner stage>2 in ALSAPC cohort. [#]: Est: Regression coefficient estimates, and the unit presented in litres(L). It represents the expected lung function change for one year increase in pubertal age. *: Growth spurt and facial hair in males are not available in ALSPAC. $: Total effect is the sum of direct and indirect effects of age of pubertal events on lung function at age 26 years. Indirect effect is the effect of age of pubertal events on lung function via height growth and its related CpGs. [@]: CpGs name in bold font had direction-consistent mediation effects in IOWBC and ALSPAC.

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