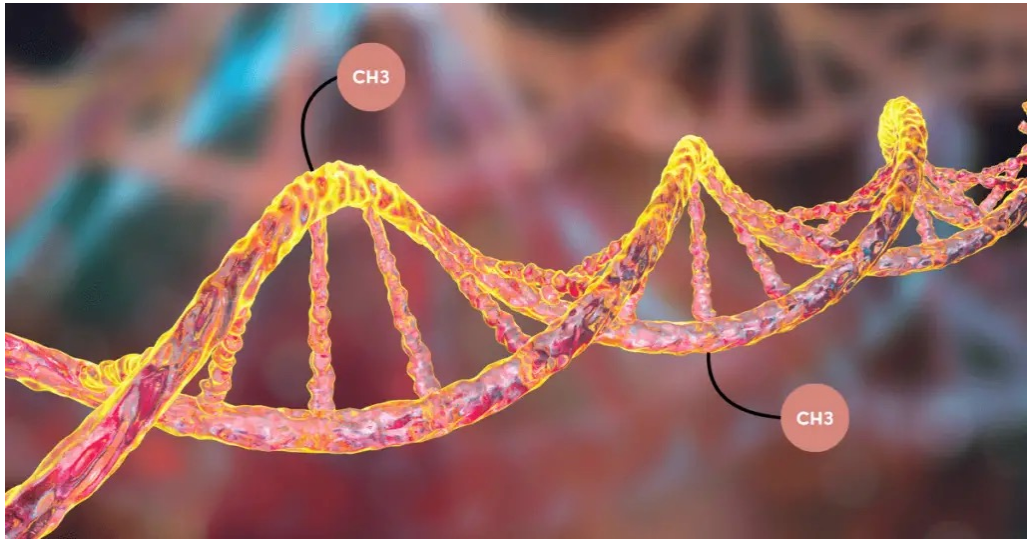
TEXAS A&M UNIVERSITY CORPUS CHRISTI
COLLEGE OF NURSING & HEALTH SCIENCES

The Association of Childhood Allergic Diseases with Prenatal Pollen Exposure Through At-Birth DNA Methylation



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Outline

- Background
- Purpose
- Study Design
- Methods
- Results
- Discussion
- Conclusions

Background

- Childhood allergic diseases, such as asthma and allergic rhinitis, are increasing in prevalence on a global scale.
- The impacts of childhood allergic diseases are profound, affecting families, parents, and schoolchildren.
- There are limited epidemiological data on environmental risk factors in early life for asthma and allergy in children.

Background - Continued

- Pollen is a recognized environmental risk factor for asthma and allergy in childhood.
- Some studies associated pollen season of birth with risk of asthma and allergic rhinitis.
- Other studies indicated a high risk of allergic sensitization or asthma hospitalization from high pollen exposure during pregnancy.
- None of these studies have explored the role of DNA methylation (DNAm) on the association of pollen exposure in early life and incidence of childhood allergic diseases.

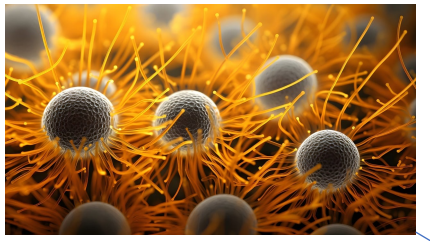
Purpose

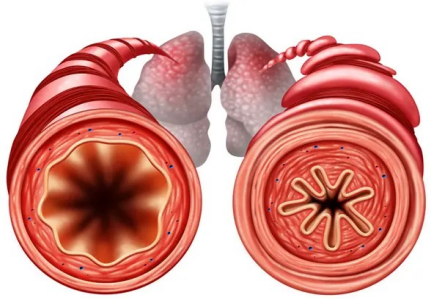
- The purpose of the study was to examine the potential connection of at-birth DNAm between prenatal pollen exposure and childhood asthma and rhinitis in a longitudinal birth cohort of the U.K.

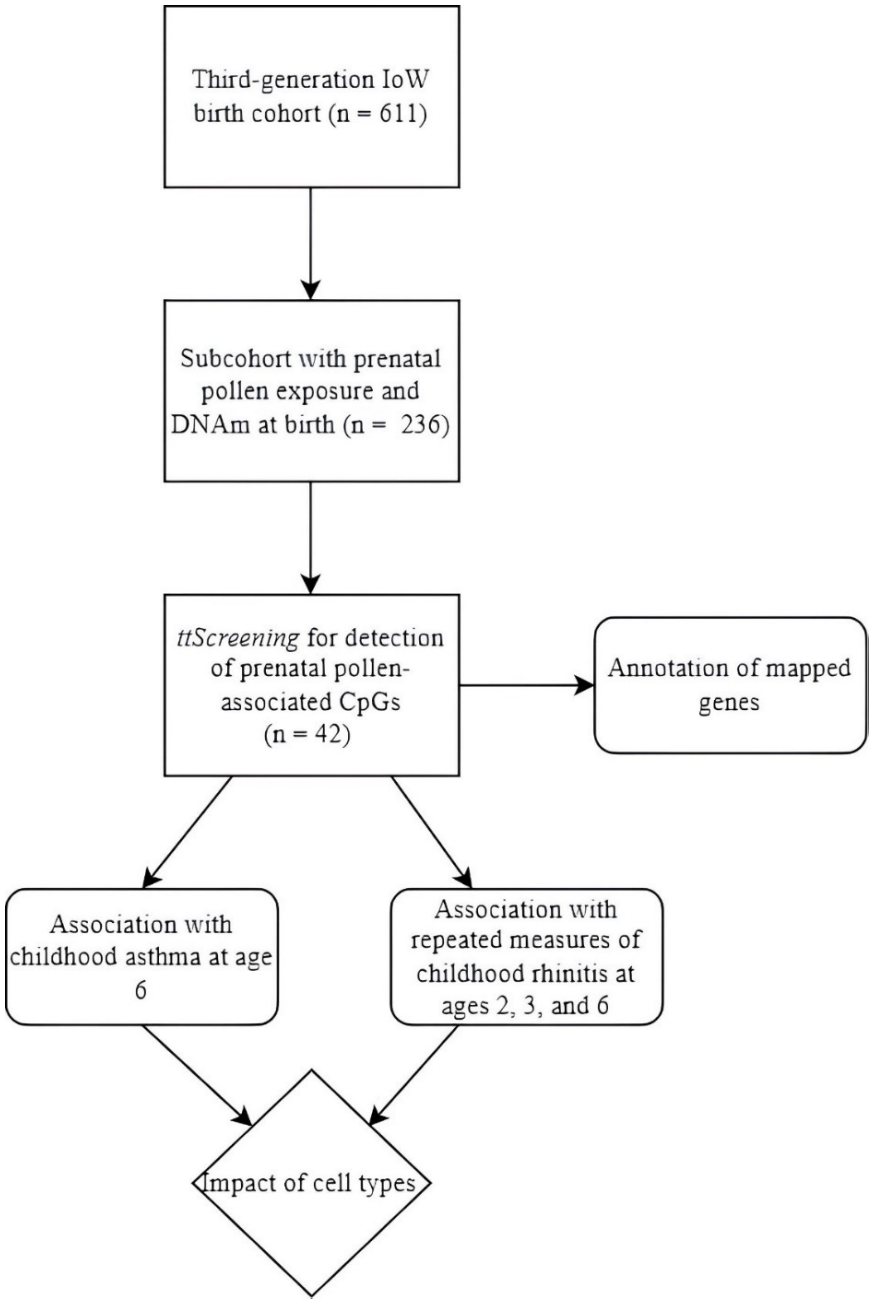
Isle of Wight, U.K.

Study Design

- A time-lagged design was utilized in the study:
  - Pollen exposure (pregnancy)
  - DNAm (at birth)
  - Asthma and allergic rhinitis (age 6 years)






Third-generation IoW birth cohort (n = 611)
Subcohort with prenatal pollen exposure and DNAm at birth (n = 236)
ttScreening for detection of prenatal pollen-associated CpGs (n = 42)
Annotation of mapped genes
Association with childhood asthma at age 6
Association with repeated measures of childhood rhinitis at ages 2, 3, and 6
Impact of cell types

Methods – Isle of Wight birth cohort (IOWBC)

- The Isle of Wight birth cohort (IOWBC) is a longitudinal cohort spanning three generations (F0, F1, and F2) on the Isle of Wight, U.K.
- This study was based off the third-generation (F2), children born between 2010 and 2022.
- F2 children are followed at ages 3 and 6 months and 1, 2, 3, and 6 years.
- Standardized questionnaires were used to collection information on demographics, clinical outcomes, and related risk factors.

Methods – Variables

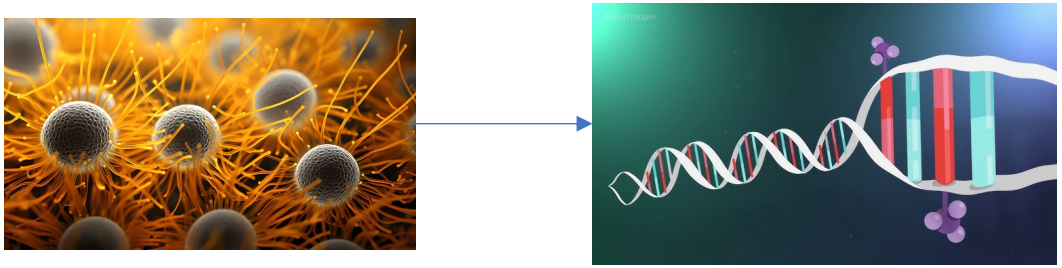
- Exposure
  - Sum of pollen counts during pregnancy
- Health outcomes
  - Childhood asthma status at age 6 years
  - Rhinitis status at ages 2, 3, and 6 years
- Mediator
  - DNAm expressed as M-values in cord blood or blood spots on Guthrie cards
- Covariates
  - Maternal risk factors (i.e., smoking, asthma status or wheeze, rhinitis), socioeconomic status (SES), passive smoke exposure in first year of life, and sex of offspring

Methods – Screening

- Screening of cytosine-phosphate-guanine (CpG) sites
  - Using the R package, *ttScreening*, a total of 551,710 sites were screened at the genome scale to detect potentially informative CpGs associated with prenatal pollen exposure.
  - No covariates were included in this step.

Methods – First Arm of Study

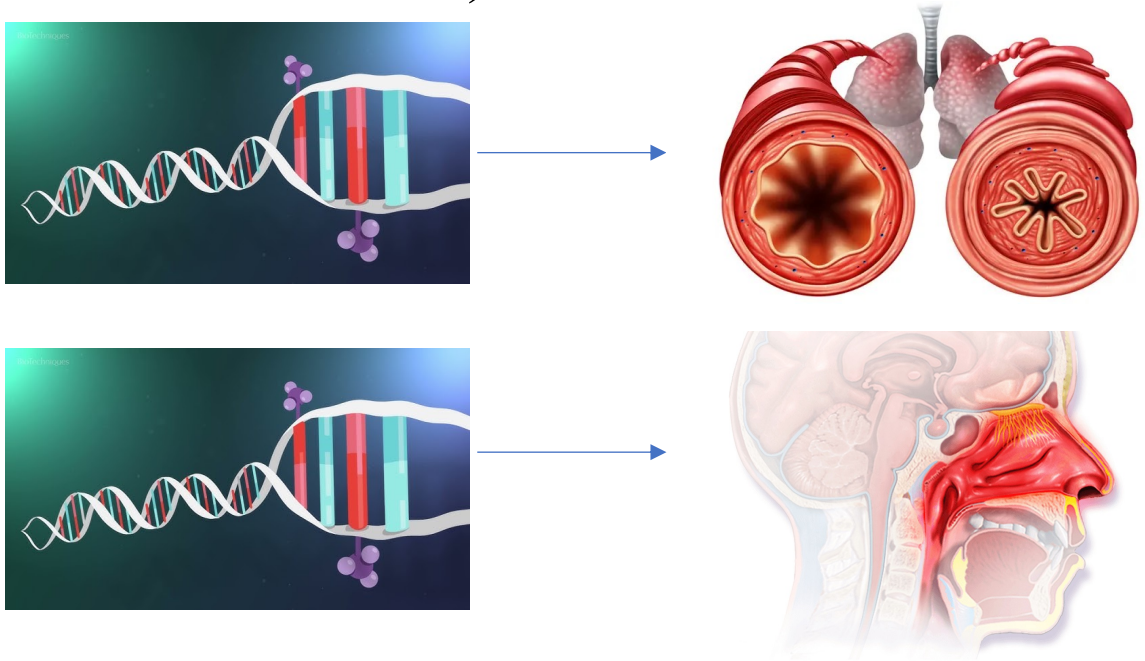
- Association of prenatal pollen exposure and DNAm at birth



- Linear regressions using the SAS procedure PROC GLM were applied with DNAm at birth as the dependent variable.

Methods – Second Arm of Study

- Association of prenatal-pollen exposure-related DNAm at birth and childhood allergic diseases (asthma and rhinitis)



Methods – Second Arm of Study

- Logistic regression using the SAS procedure PROC LOGISTIC were applied with childhood asthma at age 6 years as the dependent variable with no asthma as the reference group.
- Generalized linear regressions with repeated measures using the SAS procedure PROC GENMOD were applied with rhinitis at each age (2, 3, and 6 years) with no rhinitis as the reference group.
- Regression coefficients or odds ratios (OR) were estimated for each independent variable and 95% confidence intervals were calculated.

Summation of Pollen Exposure

- For study subjects with DNAm at birth, the average level of prenatal pollen exposure was 12489.36 grains/m^3 compared to 10780.29 grains/m^3 among the complete cohort (**$p < 0.05$**).

Variables	Descriptor	Complete cohort n (%)	Subcohort n (%)	p-value*
Risk Factors				
Maternal smoking	Yes	178 (31.79)	75 (32.75)	0.7548
	No	382 (68.21)	154 (67.25)	
Maternal asthma or wheeze	Yes	79 (16.12)	33 (16.02)	0.9687
	No	411 (83.88)	173 (83.98)	
Maternal rhinitis	Yes	217 (44.11)	97 (42.73)	0.6757
	No	275 (55.89)	130 (57.27)	
SES	1 (lowest)	77 (19.25)	30 (12.93)	0.0507
	2	97 (24.25)	67 (28.88)	
	3	114 (28.50)	72 (31.03)	
	4	61 (15.25)	40 (17.24)	
	5 (highest)	51 (12.75)	23 (9.91)	
Sex	Male	336 (56.00)	122 (51.69)	0.1827
	Female	264 (44.00)	114 (48.31)	
Passive smoke in the first year	Yes	58 (16.38)	24 (14.37)	0.4830
	No	296 (83.62)	143 (85.63)	
Health Outcomes				
Childhood asthma status at age 6	Yes	44 (7.20)	32 (13.56)	**0.0002**
	No	567 (92.80)	204 (86.44)	
Childhood rhinitis				
Age 2	Yes	42 (14.63)	24 (15.79)	0.6859
	No	245 (85.37)	128 (84.21)	
Age 3	Yes	40 (14.76)	24 (14.72)	0.9896
	No	231 (85.24)	139 (85.28)	
Age 6	Yes	38 (20.99)	24 (19.51)	0.6873
	No	143 (79.01)	99 (80.49)	

Results – Screening

- The screening process identified a total of 42 CpGs potentially associated with pollen exposure in pregnancy, based on data in the IOWBC.
- Based on selection frequency of each CpG site, the topmost CpG site was cg01375976.
  - Located at TSS1500 of gene *ZNF3*
  - Biological functions?
- How did we maximize the informativity of the 42 screened CpGs?

	Estimate/p-value	Gene	Gene Location	Biological Functions
cg01375976	0.0392 (<0.0001)	*ZNF3*	TSS1500	Negative regulation of transcription Nucleic acid binding Identical protein binding
cg22609722	-0.0425 (<0.0001)	*LTBP4*	Body	Regulator of transforming growth factor beta Calcium ion binding Glycosaminoglycan binding
cg07046197	-0.0294 (0.0002)	*CDC27*	TSS1500	Controls progression through mitosis Protein phosphatase binding
cg10571824	0.0442 (0.0003)	*MAD1L1*	Body	Cell cycle control and tumor suppression
cg11676546	0.0493 (0.0003)	*CHD7*	Exon31	Chromatin binding Helicase activity
cg26525457	0.0433 (0.0005)	*CYTH1*	Body	Lipid binding Guanyl-nucleotide exchange factor activity Membrane trafficking
cg15790214*	-0.0307 (0.0006)	*HCG11*	Body	Differentiation Proliferation
cg15794640	-0.0224 (0.0007)	*MAN2C1*	TSS1500	Oligosaccharide catabolism Carbohydrate binding and alpha-mannosidase activity
cg04365180	-0.0196 (0.0008)	*MAD1L1*	Body	Cell cycle control and tumor suppression
cg09356014	-0.0253 (0.0008)	*SH3PXD2A*	Body	Superoxide metabolism Phosphatidylinositol binding

Results – Association of prenatal pollen-associated CpGs with childhood asthma

- Of the 41 prenatal pollen-associated CpGs, DNAm at cg12318501 (*ZNF99*) and cg00929606 (*ADM2*), showed statistically significant associations with decreased odds of asthma, adjusting for covariates and cell types.
  - cg12318501: OR = 0.23, 95% CI 0.07-0.081, **p = 0.022**
  - cg00929606: OR = 0.11, 95% CI 0.02-0.53, **p = 0.006**

Results – Association of prenatal pollen-associated CpGs with childhood rhinitis

- Of the 41 prenatal pollen-associated CpGs, DNAm at cg15790214 *(HCG11*) significant associated with a decreased odds of rhinitis (OR = 0.22, 95% CI 0.07-0.72, **p = 0.01**).

Discussion

- Two prenatal pollen-associated CpGs, cg12318501 and cg00929606, were associated with decreased odds of asthma incidence at age 6 years.
- One prenatal pollen associated CpG, cg15790214, was associated with decreased odds of repeated measures of rhinitis.
- Strengths
  - First epigenetic study on pollen and childhood allergic diseases
  - Screening of CpGs for biomarker detection
  - Longitudinal cohort
  - Time-lagged modeling
- Limitations
  - Methylation sites sourced from two distinct platforms, Illumina Infinium Human Methylation450 BeadChip and Illumina Infinium MethylationEPIC BeadChip
  - DNAm measured in blood instead of nasal epithelia
  - Lack of replication

Conclusions

- DNAm in newborns may play an important role on the connection between pollen exposure in pregnancy and childhood allergic diseases.
- These findings could help in understanding the molecular pathways and processes of asthma and rhinitis in addition to improved prognosis of such diseases.

Acknowledgments

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