Transient early wheeze and lung function in early childhood associated with chronic obstructive pulmonary disease genes

Marjan Kerkhof, MD, PhD,[a,b] H. Marike Boezen, PhD,[a,b] Raquel Granell, PhD,[c] Alet H. Wijga, PhD,[d] Bert Brunekreef, PhD,[e,f] Henriëtte A. Smit, PhD,[f] Johan C. de Jongste, MD, PhD,[g] Carel Thijs, PhD,[h] Monique Mommers, PhD,[h] John Penders, PhD,[h] John Henderson, MD,[c] Gerard H. Koppelman, MD, PhD,[i] and Dirkje S. Postma, MD, PhD[j] *Groningen, Bilthoven, Utrecht, Rotterdam, and Maastricht, The Netherlands, and Bristol, United Kingdom*

Background: It has been hypothesized that a disturbed early lung development underlies the susceptibility to chronic obstructive pulmonary disease (COPD). Little is known about whether subjects genetically predisposed to COPD show their first symptoms or reduced lung function in childhood.

Objective: We investigated whether replicated genes for COPD associate with transient early wheeze (TEW) and lung function levels in 6- to 8-year-old children and whether cigarette smoke exposure *in utero* and after birth (environmental tobacco smoke [ETS]) modifies these effects.

Methods: The association of COPD-related genotypes of 20 single nucleotide polymorphisms in 15 genes with TEW, FEV_1, forced vital capacity (FVC), and FEV_1/FVC ratio was studied in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort (n = 1996) and replicated in the Child, parents and health: lifestyle and genetic constitution (KOALA) and Avon Longitudinal Study of Parents and Children (ALSPAC) cohorts.

Results: *AGER* showed replicated association with FEV_1/FVC ratio. *TNS1* associated with more TEW in PIAMA and lower FEV_1 in ALSPAC. *TNS1* interacted with ETS in PIAMA, showing lower FEV_1 in exposed children. *HHIP* rs1828591 interacted with cigarette smoke exposure *in utero* in PIAMA and with ETS in ALSPAC, with lower lung function in nonexposed children. *SERPINE2*, *FAM13A*, and *MMP12* associated with higher FEV_1 and FVC, and *SERPINE2*, *HHIP*, and *TGFB1* interacted with cigarette smoke exposure *in utero* in PIAMA only, showing adverse effects of exposure on FEV_1 being limited to children with genotypes conferring the lowest risk of COPD.

Conclusion: Our findings indicate relevant involvement of at least 3 COPD genes in lung development and lung growth by demonstrating associations pointing toward reduced airway caliber in early childhood. Furthermore, our results suggest that COPD genes are involved in the infant's lung response to smoke exposure *in utero* and in early life. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *Chronic obstructive pulmonary disease, transient early wheeze, lung function growth,* in utero *exposure*

More than 30 years ago, Burrows suggested that disturbed early development of the lungs might underlie the susceptibility to chronic obstructive pulmonary disease (COPD), a hypothesis that has recently been put forward again by several researchers.[1-3] There is suggestive epidemiologic evidence that early-life events program a child to be at increased risk for future COPD development.[4] Oxidant pollutants have been shown to influence both lung development *in utero* and growth and maturation of the lungs after birth.[5] Additionally, *in utero* exposure to maternal tobacco smoke results in deficits in lung function measured soon after birth, which can persist from childhood to adult life.[6] Postnatal exposure to tobacco smoke might have an additional adverse effect on lung growth, although conflicting findings have been reported.[7,8]

COPD can have not only its origins but also its first signs and symptoms in early childhood, which might offer opportunities for early identification of subjects susceptible to COPD. Several studies have shown that an early-life history of respiratory disease increases the mortality caused by COPD.[2,9] Especially the occurrence of transient early wheeze (TEW) in childhood can constitute a first sign of disturbed early lung development and lung growth because TEW has been shown to associate with reduced lung function already soon after birth, which is probably caused by genetic constitution, *in utero* exposures (eg, cigarette smoke), or both.[10] Furthermore, these airway developmental abnormalities related to TEW have been shown to associate with lower lung function when symptoms have disappeared, which then persists through the rest of childhood and adolescence.[11-14] Hence

From the Departments of [a]Epidemiology, [i]Paediatric Pulmonology, and [j]Pulmonology, and [b]the GRIAC Institute, University of Groningen, University Medical Center Groningen; [c]the School of Social and Community Medicine, University of Bristol; [d]the Centre for Prevention and Health Services Research, National Institute for Public Health and the Environment, Bilthoven; [e]the Institute for Risk Assessment Science, Utrecht University; [f]the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht; [g]the Department of Pediatrics, Division of Respiratory Medicine, Erasmus University Medical Center/Sophia Children's Hospital, Rotterdam; and [h]the Department of Epidemiology, Maastricht University School for Public Health and Primary Care CAPHRI, Maastricht.

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Corresponding author: Marjan Kerkhof, MD, PhD, Department of Epidemiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, Groningen 9713 GZ, The Netherlands. E-mail: m.kerkhof@umcg.nl.

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Abbreviations used
ALSPAC: Avon Longitudinal Study of Parents and Children
COPD: Chronic obstructive pulmonary disease
ETS: Environmental tobacco smoke
FVC: Forced vital capacity
KOALA: Child, parents and health: lifestyle and genetic constitution
OR: Odds ratio
PIAMA: Prevention and Incidence of Asthma and Mite Allergy
RAGE: Receptor for advanced glycation end products
SNP: Single nucleotide polymorphism
TEW: Transient early wheeze

TEW might relate to later development of COPD. Our hypothesis is that COPD genes influence structural and functional airway development *in utero* and hence the occurrence of TEW and that the presence of TEW might be a forerunner of a lifelong lower-than-average lung function in a subset of children, eventually predisposing them to COPD.

A major limiting factor in research on the hypothesis that COPD has its origins in early childhood is the huge logistic difficulty of studying the effect of early-life events with respect to a disorder that only becomes apparent 50 to 60 years later. Therefore research must rely on indirect evidence, which can be obtained by investigating potential common underlying genes. We have previously published data connecting genes that are important for lung growth, such as *ADAM33*, with susceptibility to COPD.[15,16] We here show our investigation into whether replicated genes associated with COPD are additionally associated with TEW, the phenotype that is most relevant to our hypothesis, because it is characterized by a reduced lung function in later childhood, as well as with the level of lung function in children in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort and replicated significant associations in the KOALA and Avon Longitudinal Study of Parents and Children (ALSPAC) cohorts.

METHODS

Study populations

The PIAMA, KOALA, and ALSPAC birth cohorts have been described in detail elsewhere.[17-19] A description of the selection of the study populations is provided in the Methods section in this article's Online Repository at www.jacionline.org. Children with a mother of non-Dutch origin were excluded from the analyses.

Complete data on spirometry and genotypes were available for 914 PIAMA children, 366 KOALA children, and 4851 ALSPAC children (Table I).

Single nucleotide polymorphism selection and genotyping

We searched the literature up to March 2010 and selected 21 single nucleotide polymorphisms (SNPs) fulfilling 1 or more of the following criteria in white populations:

1. Replicated SNPs from genome-wide association studies on COPD, FEV_1, or FEV_1/forced vital capacity (FVC) ratio;
2. SNPs significantly associated with COPD in a published meta-analysis[20]; and
3. SNPs published to show significant association with COPD in 3 or more independent populations.

We selected SNPs in the following 15 genes: *SFTPB*,[21] *TNS1*,[22] *SERPINE2*,[23,24] *FAM13A*,[25] *GSTCD*,[22,26] *HHIP*,[26-28] *ANKH*,[28] *HTR4*,[22] *AGER*,[22,26] *MMP12*,[29] *THSD4*,[22] *IREB2*,[30] *AGPHD1*,[28] *CHRNA3*,[28] and *TGFB1*.[20] SNP (rs1800470) in the *TGFB1* gene failed genotyping. The 20 analyzed SNPs are shown in Table II.[20-30] Genotyping methods and patterns of linkage disequilibrium (Table E1) within loci are described in the Methods section in this article's Online Repository. We have selected proxy-SNPs for unavailable SNPs in ALSPAC by using HapMap release 22[31]: rs975278 in *SERPINE2* for rs729631 ($r^2 = 1$) and rs6734100 ($r^2 = 0.82$), rs17368659 in *MMP12* for rs2276109 ($r^2 = 1$), and rs8109167 in *TGFB1* for rs6957 ($r^2 = 1$).

TABLE I. Characteristics of the birth cohorts

	PIAMA	KOALA	ALSPAC
No. genotyped	1996	1572	4851
Male sex	51.6 (1030)	50.4 (792)	50.6 (2455)
Mother allergic	37.5 (749)	32.9 (517)	47.7 (2220)
Father allergic	30.8 (615)	36.5 (562)	40.2 (1410)
Mother smoking during pregnancy	12.0 (240)	4.4 (69)	19.6 (915)
ETS exposure in first year	25.0 (477)	11.4 (168)	34.7 (1210)
Older siblings at birth	51.7 (927)	58.4 (901)	52.9 (2440)
Day care attendance	24.6 (489)	35.4 (538)	11.1 (489)
Premature birth	4.6 (91)	3.7 (58)	5.0 (234)
Duration of breast-feeding			
Never	15.7 (311)	14.7 (231)	17.7 (793)
<3 mo	35.6 (706)	20.7 (325)	30.6 (1375)
≥3 mo	48.7 (967)	64.6 (1016)	51.8 (2326)
Mother's educational level			
Low	20.0 (398)	8.4 (126)	20.1 (949)
Intermediate	42.3 (842)	37.3 (561)	34.3 (1615)
High	37.7 (751)	54.4 (818)	45.6 (2151)
Wheezing phenotypes			
Never wheeze	60.2 (1132)	63.2 (773)	68.7 (2023)
TEW	22.8 (429)	22.2 (272)	31.3 (921)
FEV_1 (% predicted), mean ± SD (n)	104.0 ± 10.8 (914)	96.4 ± 11.5 (366)	98.3 ± 11.7 (4851)
FVC (% predicted), mean ± SD (n)	102.7 ± 10.9 (914)	101.4 ± 11.7 (366)	97.7 ± 11.9 (4851)
FEV_1/FVC (% predicted), mean ± SD (n)	97.6 ± 7.1 (914)	92.0 ± 6.7 (366)	99.9 ± 7.3 (4851)

Values are presented as percentages (numbers) or means ± SDs (numbers).