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

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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 2014;133:68-76.)

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

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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

persists through the rest of childhood and adolescence.[11-14] Hence 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).

TABLE II. Characteristics of the selected SNPs

SNP	Nearest gene	Chromosome	Position	Major > minor allele	MAF PIAMA	Risk allele*	Function	Phenotype	References
rs1130866	*SFTPB*	2	85893741	T>C	0.47	C	Missense	COPD	Hersh et al[21]
rs2571445	*TNS1*	2	218683154	C>T	0.36	T	Missense	FEV_1	Repapi et al[22]
rs6734100	*SERPINE2*	2	224841995	C>G	0.13	C	Intronic	COPD	DeMeo et al[23] and Zhu et al[24]
rs729631	*SERPINE2*	2	224844919	C>G	0.16	C	Intronic	COPD	DeMeo et al[23] and Zhu et al[24]
rs7671167	*FAM13A*	4	89883979	T>C	0.46	T	Intronic	COPD	Cho et al[25]
rs10516526	*GSTCD*	4	106908353	A>G	0.06	A	Intronic	FEV_1	Repapi et al[22] and Hancock et al[26]
rs1032295	*HHIP*	4	145654034	T>G	0.40	T	Intergenic	FEV_1/FVC	Hancock et al[26]
rs1828591	*HHIP*	4	145700230	A>G	0.42	A	Intergenic	FEV_1/FVC, COPD	Wilk et al[27] and Pillai et al[28]
rs9686327	*ANKH*	5	15071537	G>A	0.17	?	Intergenic	COPD	Pillai et al[28]
rs735243	*ANKH*	5	15092327	G>A	0.14	?	Intergenic	COPD	Pillai et al[28]
rs3995090	*HTR4*	5	147826008	A>C	0.44	A	Intronic	FEV_1	Repapi et al[22]
rs2070600	*AGER*	6	32151443	C>T	0.03	C	Missense	FEV_1/FVC	Hancock et al[26] and Repapi et al[22]
rs2276109	*MMP12*	11	102745791	A>G	0.12	A	5′UTR	COPD, FEV_1	Hunninghake et al[29]
rs12899618	*THSD4*	15	71645120	G>A	0.17	A	Intronic	FEV_1/FVC	Repapi et al[22]
rs2568494	*IREB2*	15	78740964	G>A	0.31	A	Intronic	COPD	DeMeo et al[30]
rs2656069	*IREB2*	15	78745707	T>C	0.24	T	Intronic	COPD	DeMeo et al[30]
rs8034191	*AGPHD1*	15	78806023	T>C	0.31	C	Intronic	COPD	Pillai et al[28]
rs1051730	*CHRNA3*	15	78894339	C>T	0.31	T	Exon: synonymous	COPD	Pillai et al[28]
rs6957	*TGFB1*	19	41830606	T>C	0.15	C	3′UTR	COPD	Smolonska et al[20]
rs1800469	*TGFB1*	19	41860296	T>C	0.30	T	Promoter	COPD	Smolonska et al[20]

MAF, Minor allele frequency (proportion); *UTR*, untranslated region.
*Allele with highest risk of COPD or lowest lung function level; *?* = risk alleles are inconsistently associated with COPD.

TABLE III. Association of SNPs in known COPD genes with TEW, FEV_1, FVC, and FEV_1/FVC ratio in the PIAMA study

SNP	Nearest gene	Effect allele	Genetic model	Trait	Effect (95% CI)	*P* value
rs2571445	*TNS1*	T	Additive	TEW	1.24 (1.05 to 1.47)	.014
rs6734100	*SERPINE2*	C	Dominant	FEV_1	4.77 (0.25 to 9.29)	.039
				FVC	5.52 (0.92 to 10.1)	.019
rs729631	*SERPINE2*	C	Dominant	FEV_1	5.12 (0.93 to 9.30)	.017
				FVC	4.57 (0.34 to 8.81)	.034
rs7671167	*FAM13A*	T	Recessive	FEV_1	2.02 (0.33 to 3.70)	.019
				FVC	2.33 (0.64 to 4.03)	.007
rs1828591	*HHIP*	A	Additive	TEW	1.27 (1.07 to 1.50)	.005
rs2070600	*AGER*	C	Additive	FEV_1/FVC	−2.52 (−4.34 to −0.70)	.007
rs2276109	*MMP12*	A	Dominant	FVC	11.21 (1.60 to 20.8)	.022

Results are presented as ORs for TEW and as the mean difference in FEV_1 or FVC percent predicted between children with and without the effect genotype.

Outcome and exposure variables

TEW was defined as parentally reported wheeze in the last 12 months at age 1 or 2 years but not at age 4 and 6 years because KOALA only collected data at these time points. Children without wheeze at ages 1, 2, 4, and 6 years served as the reference group. FEV_1 and FVC were measured at age 8 years in PIAMA and ALSPAC and age 6 years in KOALA by using spirometry, with the methods detailed in the Methods section in this article's Online Repository. Percent predicted FEV_1, FVC, and the FEV_1/FVC ratio were calculated according to previously published prediction equations.[32]

Maternal smoking during pregnancy was defined as smoking by the mother in the last trimester of pregnancy. Environmental tobacco smoke (ETS) exposure in the first year was defined as smoking of any household member in the house in PIAMA, smoking in the vicinity of the child in KOALA, and parental smoking during the first year of the child's life in ALSPAC.

Statistical analysis

Genetic association testing for each SNP separately was performed by using a codominant (general) model. Only when the overall *P* value was less than .10 was the best fitting genetic model (dominant, recessive, or additive) further explored. Logistic and linear regression analysis was performed by analyzing TEW and lung function parameters as the outcome, respectively. Gene–smoke exposure interactions were tested for significance ($P < .05$) by including an interaction term in the regression models. The presence of relevant confounding of the associations was evaluated by including variables (day care attendance, the presence of older siblings, and sex) in the regression model. No variables caused a greater than 10% change in coefficients. Therefore we did not include any covariates in the presented models. These analyses were performed in both PIAMA and KOALA. Results from KOALA are presented in the Methods section in this article's Online Repository; because lung function was available only in a small proportion of that population (n = 366), we could not draw firm conclusions.

SNPs showing significant associations were tested for main associations or interactions for all outcomes using imputed dosages, which would be equivalent to additive genetic models in ALSPAC. Associations were considered replicated when a significant effect in the same direction was observed for any of the outcomes.

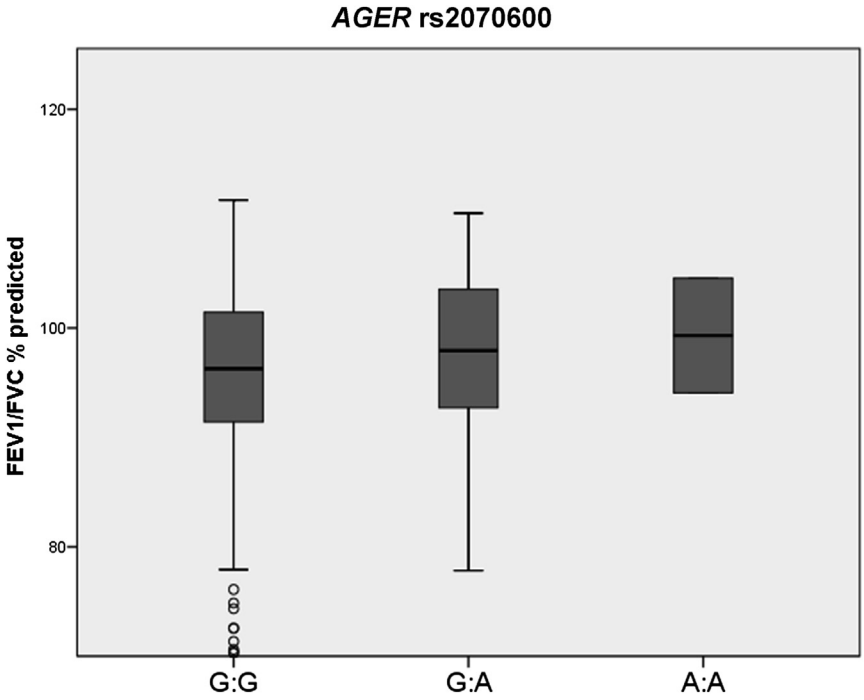

FIG 1. Box plot of FEV_1/FVC percent predicted by genotype of *AGER* rs2070600 in PIAMA.

TABLE IV. Significant interaction of SNPs in COPD genes with maternal smoking during pregnancy or environmental smoke exposure in the first year of life with respect to lung function parameters observed in PIAMA

					Effect (95% CI) of genotype on lung function (in presence or absence of exposure)					
					Maternal smoking during pregnancy			Environmental smoke in first year		
SNP	Nearest gene	Effect allele	Genetic model	Trait	Yes	No	*P*-int	Yes	No	*P*-int
rs2571445	*TNS1*	T	Recessive	FEV_1				**−4.89 (−9.18 to −0.60)**	1.75 (−0.94 to 4.44)	.010
rs729631	*SERPINE2*	C	Recessive	FVC	**6.22 (1.35 to 11.1)**	−0.76 (−2.52 to 1.00)	.008	2.98 (−0.31 to 6.26)	−1.16 (−3.11 to 0.79)	.034
rs1032295	*HHIP*	T	Dominant	FEV_1	**7.27 (1.46 to 13.1)**	−1.30 (−3.48 to 0.87)	.007			
				FEV_1/FVC	**5.58 (1.76 to 9.39)**	−0.63 (−2.06 to 0.79)	.003			
rs1828591	*HHIP*	A	Dominant	FEV_1	**5.48 (0.09 to 10.9)**	**−2.58 (−4.73 to −0.44)**	.006			
				FEV_1/FVC	**4.46 (0.93 to 7.99)**	**−1.68 (−3.08 to −0.27)**	.002			
rs6957	*TGFB1*	C	Dominant	FEV_1	**5.51 (0.43 to 10.6)**	−0.63 (−2.33 to 1.07)	.025			
				FVC	**6.14 (1.02 to 11.3)**	−0.69 (−2.41 to 1.03)	.013			

Results are presented as the mean difference in FEV_1 or FVC percent predicted between children with and without the effect genotype.
P-int, *P* value interaction term.
$P < .05$ (boldface).

RESULTS

The characteristics of the populations are shown in Table I. TEW was present in 23%, 22%, and 31% of the children from PIAMA, KOALA, and ALSPAC, respectively. The mean FEV_1 percent predicted was higher in PIAMA than in KOALA and ALSPAC.

Main associations of COPD SNPs with TEW

The *TNS1* SNP rs2571445 (odds ratio [OR] per allele, 1.24 [95% CI, 1.05 to 1.47]; $P = .01$) and the *HHIP* SNP rs1828591 (OR per allele, 1.27 [95% CI, 1.07 to 1.50]; $P = .005$) were significantly associated with TEW compared with the "never wheeze" category in PIAMA.

Main effects of COPD SNPs on childhood lung function

SNPs in 4 genes were significantly associated with FEV_1, FVC, or FEV_1/FVC ratio (Table III). Children with 1 or more COPD risk alleles of the *SERPINE2* SNPs or 2 risk alleles of the *FAM13A*

TABLE V. Effects of different combinations of genotype and exposure on outcomes for SNPs involved in the response to oxidative stress significantly interacting with exposure

SNP	Nearest gene	Trait	Major > minor	COPD RA	Reference genotype (≤1 RA)	Exposure	Reference genotype		COPD genotype	
							Not exposed	Exposed	Not exposed	Exposed
rs2571445	*TNS1*	FEV_1	C>T	T	CC/CT	ETS	Reference	0.55 (−1.34 to 2.43)	1.75 (−0.94 to 4.44)	**−4.34 (−8.42 to −0.27)**
rs729631	*SERPINE2*	FVC	C>G	C	GG/GC	IUS	Reference	**−5.68 (−10.0 to −1.33)**	−0.76 (−2.52 to 1.00)	0.54 (−2.49 to 3.57)
rs1032295	*HHIP*	FEV_1	T>G	T	GG	IUS	Reference	**−9.17 (−14.8 to −3.51)**	−1.30 (−3.48 to 0.87)	−1.89 (−5.03 to 1.24)
		FEV_1/FVC			GG			**−6.67 (−10.4 to −2.96)**	−0.63 (−2.06 to 0.79)	−1.09 (−3.15 to 0.97)
rs1828591	*HHIP*	FEV_1	A>G	A	GG	IUS	Reference	**−8.75 (−14.0 to −3.54)**	**−2.58 (−4.73 to −0.44)**	**−3.27 (−6.38 to −0.15)**
		FEV_1/FVC			GG			**−6.24 (−9.65 to −2.83)**	**−1.68 (−3.08 to −0.27)**	−1.78 (−3.82 to 0.26)
rs6957	*TGFB1*	FEV_1	T>C	C	TT	IUS	Reference	**−3.73 (−6.42 to −1.05)**	−0.63 (−2.33 to 1.07)	1.77 (−2.73 to 6.27)
		FVC						−2.03 (−4.75 to 0.68)	−0.69 (−2.41 to 1.03)	4.10 (−0.43 to 8.64)

Effect estimates (95% CIs) are presented for the specific combination compared with the reference group with 1 or fewer COPD risk alleles and without exposure calculated as ORs for TEW and the mean difference in percent predicted FEV_1, FVC, and FEV_1/FVC.
IUS, Cigarette smoke exposure *in utero*; *RA*, risk allele.
$P < .05$ (boldface).

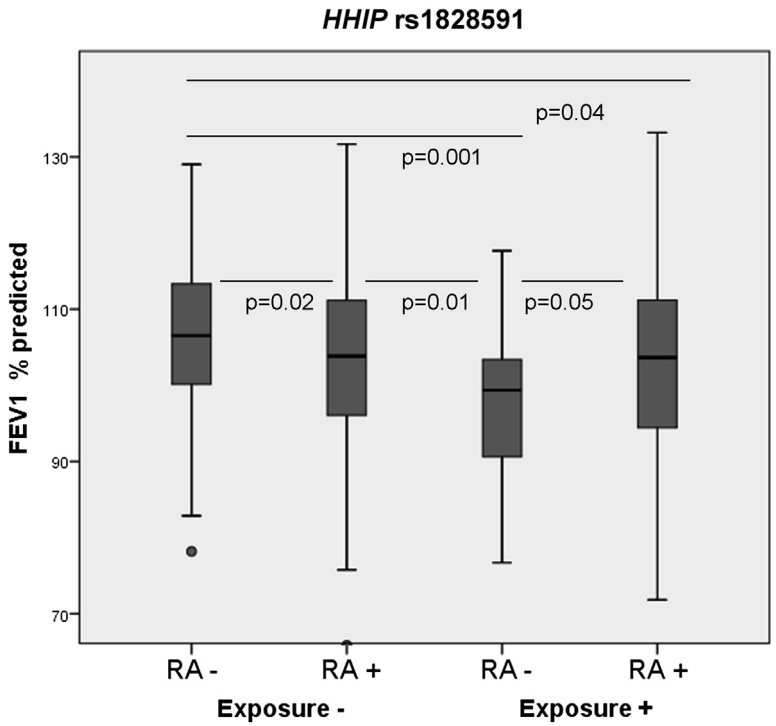

FIG 2. Box plots of FEV_1 percent predicted for children without *(RA−)* or with *(RA+)* risk allele A of *HHIP* SNP rs1828591 in PIAMA stratified by exposure to maternal smoking during pregnancy.

SNP rs7671167 had a significantly higher mean FEV_1 and FVC (Table III). Children with 1 or more COPD risk allele of *MMP12* SNP rs2276109 had a significantly higher mean FVC than children without COPD risk alleles (Table III). A similar nonsignificant effect of *MMP12* was observed for FEV_1.

The FVC was higher and the FEV_1/FVC ratio was lower in children with a higher number of COPD risk alleles of the *AGER* SNP rs2070600 (Fig 1, and Table III). We found no association of the other genes with lung function.

Main associations of exposure with outcomes

Maternal smoking during pregnancy was not significantly associated with TEW in PIAMA (OR, 1.1 [95% CI, 0.8 to 1.5]; $P = .71$). Mean FEV_1 was significantly lower (−2.3% [95% CI, −4.6% to −0.1%], $P = .04$) in exposed children than in nonexposed children. Maternal smoking during pregnancy was not significantly associated with FVC (data not presented). ETS exposure in the house was not significantly associated with TEW or lung function levels. Associations of exposure with outcomes for the replication cohorts are shown in the Results section in this article's Online Repository at www.jacionline.org.

Effect modification by maternal smoking during pregnancy

SNPs in 3 genes interacted significantly with exposure to maternal smoking during pregnancy (Table IV). *In utero* exposure was only associated with lung function in children without (*HHIP* and *TGFB1*) or with zero or 1 *(SERPINE2)* COPD risk alleles (Table V). As a result, COPD risk alleles of these genes were associated with better lung function in exposed children (Table IV). The presence of 2 COPD risk alleles of *SERPINE2* SNP rs729631 was significantly associated with a higher mean FVC only in children exposed to maternal smoking during pregnancy (Table IV).

The presence of 1 or more COPD risk alleles of the *HHIP* SNPs rs1032295 or rs1828591 was associated with a significantly higher FEV_1 and a higher FEV_1/FVC ratio only in children whose mother smoked during pregnancy (Table IV). In nonexposed children a significant opposite effect was observed for rs1828591 (ie, the presence of ≥1 COPD risk allele was associated with lower levels; Table IV). Both exposed and nonexposed children with 1 or more COPD risk alleles of rs1828591 had significantly lower FEV_1 levels than the reference group of nonexposed children without COPD risk alleles (Table V). Fig 2 shows this by showing the FEV_1 levels for the 4 combinations of genotypes and exposure. Exposure was significantly associated with lower FEV_1 levels in children without risk alleles and not in children with 1 or more risk alleles of rs1828591.

The presence of COPD risk alleles of the *TGFB1* SNP rs6957 was associated with higher FEV_1 levels in children exposed to smoke *in utero* as well but not in children without exposure. A similar effect was found on FVC in children exposed to smoke *in utero*.

We found no significant interaction of the SNPs with maternal smoking during pregnancy in the association with TEW.

Effect modification by tobacco smoke exposure after birth

For the *TNS1* SNP, significant interaction ($P = .03$) with exposure was observed, showing worse lung function levels in children exposed to smoke in the home who had the genotype conferring a higher risk of COPD (Table V). Children with 2 COPD risk alleles exposed to smoke had the lowest mean FEV_1 percent predicted, 5% lower than children exposed to smoke without the risk genotype (Tables IV and V).

SERPINE2 SNP rs729631 significantly ($P = .03$) interacted with smoke exposure in the association with FVC in PIAMA (Table IV). ETS exposure was associated with a 6% lower FVC percent predicted in children without the COPD-related genotype (Table V).

We found no significant interaction between the SNPs and exposure to tobacco smoke in the first year of life in the association with TEW.

Replication results

In the ALSPAC study significant associations with lung function levels were found for 5 of the 7 associated COPD genes (Table VI). The main association of the *AGER* SNP rs2070600 with FEV_1/FVC ratio was strictly replicated. The other significant associations in ALSPAC were observed for different outcomes, different genetic models, or different interacting exposures.

FEV_1 decreased significantly with the number of COPD risk alleles of *TNS1* in ALSPAC (Table VI). In PIAMA the risk of TEW increased with the number of COPD risk alleles, whereas the presence of 2 COPD risk alleles in *TNS1* associated with lower FEV_1 levels in ETS-exposed children only (Tables IV and V).

The significant effects of *SERPINE2* on FEV_1 were opposite in direction in ALSPAC (Table VI) compared with PIAMA (Table III). The effect of *HHIP* rs1828591 on FEV_1 and FVC was significantly modified by ETS exposure in the first year, with significantly lower FVC levels with increasing COPD risk alleles in ALSPAC children not exposed to ETS. In PIAMA effect modification in the same direction on FEV_1 and FEV_1/FVC ratio was observed for *in utero* smoke exposure.

For *MMP12*, a higher number of COPD risk alleles was associated with a higher FEV_1/FVC ratio in ALSPAC children *in utero* exposed to smoke. In PIAMA the presence of 1 or 2 *MMP12* COPD risk alleles associated with a higher FVC in the total population.

None of the associations were replicated in KOALA (see the Results section in this article's Online Repository).

DISCUSSION

To our knowledge, this is the first study to test the hypothesis that genetic variants known to be associated with COPD and lower lung function in adults are associated with lung function and/or TEW, a marker of low lung function in early childhood. We found significant associations for 7 of the 15 genes in the PIAMA cohort; this was very unlikely to have occurred by chance. For 5 of these 7 genes, significant associations were observed with lung function levels in the ALSPAC cohort as well. COPD risk alleles of SNPs in *TNS1*, *HHIP*, and *AGER* pointed toward reduced airway caliber, as reflected by either TEW, lower FEV_1, or lower FEV_1/FVC ratio in both PIAMA and ALSPAC. Conversely, COPD risk alleles of SNPs in *SERPINE2*, *FAM13A*, and *MMP12* were associated with higher FEV_1 and FVC in PIAMA, results that were not replicated and might be due to chance. Moreover, smoke exposure during pregnancy or early life significantly interacted with effects of SNPs in *TNS1*, *SERPINE2*, *HHIP*, *MMP12*, and *TGFB1* in PIAMA and with *HHIP* and *MMP12* in ALSPAC. Thus our findings indicate relevant involvement of at least 3 COPD genes in lung development and lung growth.

The results for *TNS1* and *AGER* are most consistent with findings in adults. Tensin 1 is involved in signal transduction and cell migration.[33] The *TNS1* locus at 2q35 was initially discovered in a genome-wide association study on FEV_1.[22] The main hit rs2571445, a nonsynonymous coding SNP, has recently been reported to be associated with the presence of GOLD stage 2 to 4 COPD as well.[33]

Our findings on the *AGER* SNP rs2070600 affecting the FEV_1/FVC ratio are in line with the results from Hancock et al.[26] The

TABLE VI. Replication results in the ALSPAC cohort

SNP	Nearest gene	Effect allele	MAF	Trait	Mean difference per allele	*P* value
rs2571445	*TNS1*	A↔T	A (0.40)	FEV_1	**−0.48 (−0.96 to −0.01)**	.047
				FVC	−0.47 (−0.96 to 0.01)	.054
rs975278 (proxy)	*SERPINE2*	C	T (0.19)	FEV_1	**−0.72 (−1.33 to −0.11)**	.021
rs1032295	*HHIP*	T	G (0.40)	FEV_1		
				FVC		
rs1828591	*HHIP*	A	G (0.40)	FEV_1		
				FVC		
rs2070600	*AGER*	C	T (0.07)	FEV_1/FVC	**−0.71 (−1.27 to −0.16)**	.012
rs17368659 (proxy)	*MMP12*	G	T (0.13)	FEV_1/FVC		

Results are presented as the mean difference in FEV_1 or FVC percent predicted per effect allele (additive genetic model). Only results with *P* values of less than .10 are shown.
A↔T, Genotyped on the opposite strand; *MAF*, minor allele frequency; *P-int*, *P* value interaction term.

presence of 2 risk alleles of this nonsynonymous SNP in the gene coding for the receptor for advanced glycation end products *(RAGE)* has been reported to be strongly associated with higher levels of soluble forms of this protein in a Dutch population.[34] *RAGE* expression is most abundant in the lung, and the intensity of expression in respiratory epithelial cells varies during lung morphogenesis.[35] It has been shown to play a role in critical processes directly involved in perinatal transitioning of the embryonic lung into a mature functional organ.[35]

Hedgehog-interacting protein *(HHIP)* has been implicated in organ development and repair in multiple tissues[36] and is therefore a strong candidate gene for COPD. Our findings of *HHIP* SNP rs1828591 risk alleles being associated with an increased risk of TEW and lower lung function support our hypothesis.

In addition, we have made an intriguing observation that for some genes exposure effects on lung function were only observed in children without or with only 1 risk allele or alleles of genes known to be associated with COPD. As a result, COPD risk alleles of these genes were associated with better lung function in exposed children. We have observed these effects in PIAMA for *SERPINE2*, *HHIP*, and *TGFB1*, genes that are all strong candidates for our hypothesis because they play an important role in lung morphogenesis.[1,23,37,38] A significant interaction in the same direction was only replicated for *HHIP* in ALSPAC. Therefore we cannot exclude that these findings are false positive. Further research has to be performed because the exposure effects were not minor. Adverse effects of 4% to 9% in FEV_1 percent predicted with *in utero* exposure were observed in PIAMA children without COPD-related genotypes. If these observations are replicated in future studies and found for other genes replicated as well, we put forward that this might be due to the fact that genetic effects during lung development *in utero* might be different from effects at adult age, where the lung has been fully developed and effects might be targeting lung tissue destruction.

Our hypothesis was that COPD genes influence early lung development, such as branching morphogenesis *in utero*, hence showing an indirect relationship with TEW, a marker of lower lung function. Alternatively, COPD genes might influence susceptibility to early-life respiratory tract infections leading to TEW without a relationship with later COPD. However, we did not observe a direct relationship of *TNS1* and *HHIP* with the occurrence of lower respiratory tract infections in PIAMA (data not shown), making this alternative explanation of the association of these genes with TEW unlikely.

Low lung function in childhood is not specific for COPD and can also be caused by other diseases, such as bronchopulmonary dysplasia, cystic fibrosis, and asthma.[39] Furthermore, some of the genes we have studied are associated not only with COPD but also with asthma.[40-42] We cannot fully exclude that observed associations with lung function in our birth cohort might be ascribed to asthma. However, this is unlikely because none of the SNPs were associated with doctor-diagnosed asthma at age 8 years (data not shown). Our findings support the general concept of the "Dutch hypothesis" by showing common genetic origins of obstructive airway disease in childhood and COPD at older age related to environmental pressure.[4]

Our aim was to detect all possible relevant associations of variants in the 15 COPD genes with any of the outcome parameters. We acknowledge that our strategy of avoiding type II errors implies accepting a higher risk of type I errors. We have calculated the probability of observing a *P* value as small as our minimum *P* value under the null hypothesis of no main associations to be 20%.[43] Thus we cannot fully exclude that some of the statistically significant test results observed in the PIAMA study are false positive because of multiple testing. Because the effect sizes we actually found were at a level that would be very relevant, we evaluated our positive findings in the larger ALSPAC cohort. We are convinced that we have found evidence that at least some of the genes known to be involved in COPD play a role in lung function development and growth in childhood. Otherwise, it would be very unlikely to find so many statistically significant associations. For example, the probability to find 10 or more significant main associations with any of the outcomes for the 20 SNPs by chance in PIAMA would be only 0.7%. Further research on this topic has to be performed to pinpoint the variants in the genes studied and the underlying mechanisms.

Because our aim was to link childhood lung function with adult COPD, we have chosen to limit our study to SNPs previously associated with COPD that mostly emerged from studies covering the complete gene by haplotype tagging SNPs. Because of this strategy, we cannot exclude that other functional genetic variants in the genes that were not related to COPD associate with childhood lung function or TEW.

TABLE VI. *(Continued)*

Mean difference (95% CI) in lung function per COPD risk allele (in presence or absence of exposure)					
Maternal smoking pregnancy			ETS exposure first year		
Yes	No	*P*-int	Yes	No	*P*-int
−0.93 (−2.14 to 0.28)	0.29 (−0.26 to 0.85)	.065	−0.91 (−1.94 to 0.12)	0.33 (−0.38 to 1.04)	.047
			−0.44 (−1.47 to 0.59)	0.66 (−0.06 to 1.38)	.082
0.68 (−0.49 to 1.85)	−0.40 (−0.94 to 0.15)	.095	0.92 (−0.09 to 1.92)	−0.37 (−1.07 to 0.32)	.036
			0.50 (−0.50 to 1.51)	**−0.73 (−1.44 to −0.03)**	.046
1.12 (0.08 to 2.16)	−0.52 (−1.01 to 0.03)	.005			

We had enough statistical power to find relevant differences in lung function levels and occurrence of symptoms between genotypes. For example, we had more than 90% statistical power to find additive effects on FEV_1 of 2% per allele in PIAMA children at an α value of .05. However, a reason why the findings from the PIAMA study could not be replicated in KOALA could be that the number of lung function measurements in KOALA was too small to detect relatively small effects (<5%) under a recessive mode of inheritance, as observed in PIAMA. Moreover, lung function was measured at a younger age in KOALA, and we observed many differences in characteristics between both studies that might have played a role. The exceptionally low prevalence of maternal smoking during pregnancy in the KOALA cohort might have hampered the statistical power to find interactions in this study.

Because KOALA did not collect data on the third year of life, TEW could not be defined by using the standard definition of wheeze in the first 3 years and not at age 6 years.[11] However, the results were not altered when using this definition in PIAMA or when analyzing TEW as the outcome, which is one of the previously identified and validated phenotypes from longitudinal latent class analysis in PIAMA (data not shown).[44] In the analyses on TEW we have excluded children with symptoms of wheeze at school age (5.6% with late onset and 11.4% with persistent wheeze in PIAMA), an outcome known to be closely related to asthma and allergic sensitization.[11] This might have affected the results. However, analyses, including these children in the reference group produced similar results with significant ORs for *TNS 1* (OR, 1.23 [95% CI, 1.04 to 1.25]) and *HHIP* (OR, 1.33 [95% CI, 1.13 to 1.57]), showing the robustness of our findings.

A challenge to directly assess relationships between COPD genes, early lung function deficits, and COPD is to overcome the long time period between early childhood events and COPD onset. This might require long-term follow-up or, alternatively, well-documented historical data on relevant exposures and wheeze history in retrospective cohorts or case-control studies of COPD onset with genotyping of the relevant genes.

In summary, our results support the general hypothesis that COPD has its origins in early childhood by demonstrating associations between several important COPD-associated genes and transient early symptoms of wheeze and lower lung function in the PIAMA and ALSPAC studies. These effects were not minor. Differences of 4% to 7% in FEV_1 percent predicted between children with and without the COPD genotypes were observed in PIAMA children exposed to maternal smoking during pregnancy. Our results suggest that COPD genes are involved in the infant's lung response to maternal smoking during pregnancy.

Key messages

- **We show suggestive evidence for the general hypothesis that COPD has origins in early childhood.**
- **Several important COPD-associated genes are associated with transient early symptoms of wheeze and lung function in children from 2 large birth cohorts.**
- **The results suggest that COPD genes are involved in the infant's lung response to smoke exposure.**

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METHODS

Study population: PIAMA

Recruitment of participants took place in 1996-1997 through 52 antenatal clinics in 3 different regions: the north (Groningen and surroundings), central (Utrecht and Wageningen), and southwest (Rotterdam) of The Netherlands. A total of 4146 pregnant women were recruited from the general population by using a validated short screening questionnaire, and their children were followed from birth in 1996-1997. Children from mothers reporting symptoms of allergic disease were defined as high risk. All these 1327 children and a random sample of 663 children from nonallergic mothers were selected for lung function measurements. Spirometry was successfully performed in 1058 children at age 8 years. Genotypes were obtained from 1996 children from PIAMA after exclusion of 53 children from non-Dutch mothers. Complete data on genotypes and spirometry were available from 914 children.

The medical ethics committees approved the study, and all participants provided written informed (parental) consent.

Study population: KOALA

For the KOALA birth cohort, 2343 children from healthy pregnant women were recruited in weeks 10 to 14 of pregnancy from an ongoing prospective cohort study on pregnancy-related pelvic girdle pain. In addition, pregnant women were recruited through posters in organic food shops, anthroposophical physicians' offices, and midwives at 10 to 14 weeks of pregnancy; these subjects comprised the alternative recruitment group (n = 491). Recruitment of participants took place in 2001-2003 in the south of The Netherlands. Children of women who had provided blood samples in pregnancy and had subsequent home visits at age 2 years (n = 829) were candidates for home visits at age 6 to 7 years. Spirometry was measured in 443 of these children. Genotypes were obtained from 1572 children from KOALA after exclusion of 71 children from non-Dutch mothers. Complete data on genotypes and spirometry were available from 366 children.

Study population: ALSPAC

ALSPAC is a longitudinal, population-based birth cohort study that recruited pregnant women residing in Avon, United Kingdom, with expected dates of delivery from April 1, 1991, to December 31, 1992. The study methodology has been described in detail previously.[E1] Ethical approval was obtained from the ALSPAC Law and Ethics Committee and the local research ethics committees. Further details of the ALSPAC study are available at: http://www.bristol.ac.uk/alspac.

TEW was defined at 6, 18, 42 and 69 months after birth based on responses to the following question: "In the past 12 (6 for first questionnaire) months has (your child) had wheezing/wheezing with whistling on the chest?"

Genotyping in PIAMA and KOALA

Genotyping in PIAMA and KOALA was performed by means of competitive allele-specific PCR with KASPar genotyping chemistry (K-Biosciences, Herts, United Kingdom). The genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium ($P < .002$).

Genotyping in ALSPAC

Genotyping was carried out at 2 different centers (the Wellcome Trust Sanger Centre, Cambridge, United Kingdom, and Laboratory Corporation of America, Burlington, NC) by using the Illumina HumanHap 550 array (Illumina, San Diego, Calif). Subjects were excluded on the basis of the following: sex mismatches, minimal or excessive heterozygosity, disproportionate levels of individual missingness (>3%), cryptic relatedness measured as a proportion of identity by descent (IBD > 0.1), and insufficient sample replication (IBD < 0.8). The remaining subjects were assessed for evidence of population stratification by using multidimensional scaling analysis and compared with HapMap II (release 22) European descent (CEU), Han Chinese, Japanese, and Yoruba reference populations; all subjects with non-European ancestry were removed. SNPs with a minor allele frequency of less than 1%, a call rate of less than 95%, or evidence for violations of Hardy-Weinberg equilibrium ($P < 5 \times 10^{-7}$) were removed. Autosomal genotypic data were subsequently imputed with Markov Chain Haplotyping software (MACH v.1.0.16, Li et al[E2]) and phased haplotype data from CEU subjects (HapMap release 22, phase II NCBI B36, dbSNP 126) based on a cleaned dataset of 8,365 subjects and 464,311 autosomal SNPs. After imputation, all SNPs with indications of poor imputation quality ($r^2 < 0.30$) were removed.

Lung function measurements

A Jaeger pneumotachograph (Viasys Healthcare) was used for lung function testing in PIAMA. In ALSPAC and KOALA spirometry was performed with the Vitalograph Spirotrac IV system (Vitalograph, Maids Moreton, United Kingdom) and the handheld Medikro Spirostar USB spirometer (Medikro, Kuopio, Finland), respectively, by using methods described previously.[E3,E4] The machines were calibrated every day on which medical examinations took place. FVC and FEV_1 were measured in the sitting position while wearing a nose clip by trained personnel according to the American Thoracic Society/European Respiratory Society guidelines.[E5] For each child, at least 3 acceptable maneuvers had to be obtained. The best results of 3 acceptable and repeatable (FVC ± 150 mL) flow-volume curves were accepted after *post hoc* quality control by a respiratory physician.

RESULTS

Main associations of outcomes with exposure in KOALA and ALSPAC

Maternal smoking during pregnancy increased the risk of TEW in KOALA (OR, 3.0 [95% CI, 1.5 to 5.8]; $P = .001$) and ALSPAC (OR, 1.3 [95% CI, 1.1 to 1.6]; $P = .001$). Maternal smoking during pregnancy was not significantly associated with FEV_1 (−3.2% [95% CI, −9.0% to 2.6%]; $P = .28$) or FVC (−3.6% [95% CI, −9.5% to 2.2%]; $P = .22$) in KOALA or ALSPAC (FEV_1: −0.62% [95% CI, −1.47% to 0.23%]; $P = .15$; FVC: 0.48% [95% CI, −0.38% to 1.34%]; $P = .28$). ETS exposure in the vicinity of the child (KOALA) after birth was not significantly associated with TEW or lung function levels in KOALA. In ALSPAC parental smoking during the first year of life was significantly associated with TEW (OR, 1.2 [95% CI, 1.0 to 1.4]; $P = .03$) but not with FEV_1 (OR, 0.53% [95% CI, −1.35% to 0.29%]; $P = .20$) or FVC (OR, 0.35% [95% CI, −0.48% to 1.18%]; $P = .41$).

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TABLE E1. Linkage disequilibrium (r^2 values) between SNPs within loci

Gene	*SERPINE2*	*HHIP*	*ANKH*	*IREB2*, *AGPHD1*, *CHRNA3*			*TGFB1*
SNP	rs729631	rs1828591	rs735243	rs2568494	Rs2656069	rs1051730	rs1800469
rs6734100	0.73						
rs1032295		0.55					
rs9686327			0.73				
rs2568494				—	0.14	0.68	
rs2656069				0.14	—	0.12	
rs8034191				0.75	0.13	0.89	
rs6957							0.01

TABLE E2. Association of SNPs in known COPD genes with TEW, FEV_1, FVC, and FEV_1/FVC ratio in the KOALA study

SNP	Nearest gene	Effect allele	Genetic model	Trait	Effect (95% CI)	*P* value
rs2571445	*TNS1*	T	Additive	TEW	0.91 (0.73 to 1.14)	.42
rs6734100	*SERPINE2*	C	Dominant	FEV_1	−1.34 (−10.7 to 8.01)	.78
				FVC	−0.56 (−9.96 to 8.85)	.91
rs729631	*SERPINE2*	C	Dominant	FEV_1	−1.30 (−7.41 to 4.81)	.68
				FVC	1.18 (−4.92 to 7.29)	.70
rs7671167	*FAM13A*	T	Recessive	FEV_1	2.39 (−0.72 to 5.50)	.13
				FVC	1.76 (−1.37 to 4.89)	.27
rs1828591	*HHIP*	A	Additive	TEW	0.98 (0.79 to 1.21)	.82
rs2070600	*AGER*	C	Additive	FEV_1/FVC	0.32 (−2.20 to 2.84)	.80
rs2276109	*MMP12*	A	Dominant	FVC	5.97 (−10.3 to 22.2)	.47

Results are presented as ORs for TEW and as the mean difference in FEV_1 or FVC percent predicted between children with and without the effect genotype.

TABLE E3. Replication results of SNPs in COPD genes interacting with maternal smoking during pregnancy or environmental smoke exposure in the first year of life in KOALA

					Effect (95% CI) of genotype on lung function (in presence or absence exposure)					
					Maternal smoking during pregnancy			Environmental smoke in first year		
SNP	Nearest gene	Effect allele	Genetic model	Trait	Yes	No	*P*-int	Yes	No	*P*-int
rs2571445	*TNS1*	T	Recessive	FEV_1				1.86 (−9.33 to 13.0)	−0.25 (−4.43 to 3.93)	.73
rs729631	*SERPINE2*	C	Recessive	FVC	1.76 (−15.4 to 18.9)	**−3.78 (−6.11 to −0.45)**	.57	−1.25 (−9.23 to 6.73)	−3.31 (−6.37 to −0.25)	.64
rs1032295	*HHIP*	T	Dominant	FEV_1	1.90 (−21.7 to 25.5)	−0.01 (−3.72 to 3.70)	.88			
				FEV_1/FVC	−1.39 (−15.1 to 12.3)	−1.69 (−3.83 to 0.45)	.97			
rs1828591	*HHIP*	A	Dominant	FEV_1	1.90 (−21.9 to 25.7)	0.29 (−3.53 to 4.09)	.90			
				FEV_1/FVC	−1.39 (−14.9 to 12.1)	−0.72 (−2.87 to −1.43)	.92			
rs6957	*TGFB1*	C	Dominant	FEV_1	4.20 (−8.89 to 17.3)	−1.52 (−4.39 to 1.34)	.40			
				FVC	−2.97 (−16.2 to 10.2)	−1.33 (−4.22 to 1.56)	.81			

Results are presented as the mean difference in FEV_1 or FVC percent predicted between children with and without the effect genotype.
P-int, *P* value interaction term.
$P < .05$ (boldface).