Prenatal and postnatal genetic influence on lung function development

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Background: It is unknown to what extent adult lung function genes affect lung function development from birth to childhood.
Objective: Our aim was to study the association of candidate genetic variants with neonatal lung function and lung function development until age 7 years.
Methods: Lung function measurement by means of spirometry with the raised-volume thoracoabdominal compression technique and bronchial responsiveness to methacholine challenge were assessed in 411 high-risk newborns from the Copenhagen Prospective Study on Asthma in Childhood 2000 ($COPSAC_{2000}$) cohort. Measures were repeated at age 7 years. Genetic risk scores were calculated based on reported single nucleotide polymorphisms for adult lung function (FEV_1/forced expiratory vital capacity [FVC] ratio and FEV_1) as the number of risk alleles weighted on known effect size. These genetic risk scores were analyzed against lung function measures as *z* scores at birth (forced expiratory volume in 0.5 seconds [$FEV_{0.5}$], forced expiratory flow at 50% of functional vital capacity [FEF_{50}], and provocative dose of methacholine causing a 15% decrease in lung function [PD15]) and at age 7 years (FEV_1, FEF_{50}, and provocative dose of methacholine causing a 20% decrease in lung function [PD_{20}]) and with development from birth to age 7 years ($FEV_{0.5/1}$, FEF_{50}, and $PD_{15/20}$).
Results: The genetic risk scores were not associated with lung function measures at age 1 month, but the FEV_1/FVC genetic risk score was associated with reduced FEF_{50} values at age 7 years ($P = .01$) and similarly with reduced growth in FEF_{50} from birth to age 7 years ($P = .02$). This score was also associated with increased bronchial responsiveness (reduced PD_{20}) at age 7 years ($P = .02$) and change in responsiveness from birth to age 7 years ($P = .05$).
Conclusion: Lung function genetic variants identified in adults were not associated with neonatal lung function or bronchial responsiveness but with the development of these lung function measures during early childhood, suggesting a window of opportunity for interventions targeting these genetic mechanisms. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Child, asthma, genetics, respiratory function tests*

Reduced lung function in healthy children seems to track throughout life, with potential consequences for later respiratory health.[1-3] In the Copenhagen Prospective Study on Asthma in Childhood 2000 ($COPSAC_{2000}$) study neonatal lung function was tracked to school age independent of asthma,[4] and similar tracking from birth to adulthood was reported in the Tucson study.[5]

Disease processes can start prenatally, as evidenced by reduced lung function at birth in children with later asthma,[4] emphasizing the importance of studying risk factors and lung function in early life.[6] Genetic variation influences lung function, and several loci affecting adult lung function have been identified.[7-9] However, it is unknown at which point in life these genetic risk variants affect lung function development. Are the effects prenatal or postnatal? The answer to this question is important to determine the potential window of opportunity for preventing lung function deterioration and maintaining respiratory health.

The aim of this study was to evaluate the influence of genetic variants associated with adult lung function on neonatal lung function and bronchial responsiveness and the development of these parameters to age 7 years in $COPSAC_{2000}$.

METHODS

Study population: $COPSAC_{2000}$

The $COPSAC_{2000}$ study is a single-center, prospective clinical birth cohort study of 411 children born to asthmatic mothers with the primary objective of investigating gene-environment-phenotype interactions in the development of asthma and related diseases. Children attended the research unit at the age of 1 month and subsequently every 6 months for scheduled clinical investigations. Additional visits were arranged immediately on onset of any respiratory symptoms. Key exclusion criteria were gestational age of less than 36 weeks, severe congenital abnormality, neonatal mechanical ventilation, and symptoms of lower airway infection before inclusion. The study was approved by the Copenhagen Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754). Informed consent was obtained from the parents at enrollment.[10]

Genotyping was performed in both the child and parents by using high-throughput genome-wide single nucleotide polymorphism (SNP) genotyping with the Illumina Infinium II HumanHap550 BeadChip technology (Illumina, San Diego, Calif), as described previously.[11-13] Imputation of missing genotyped SNPs was performed with the Giant consortium[14] protocol by using a prephasing step before the imputation step with the MACH/Minimac software[15] and the 1000 Genomes reference panel (EUR, v3.20101123) and the HapMap (CEU, rel22) panel, selecting for each SNP the panel with the best imputation statistic (highest R^2 value). Please see the Methods section in this article's Online Repository at www.jacionline.org for preimputational quality control filters. All SNPs were used as dosage data, thereby taking into account the imputation uncertainty.

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Abbreviations used
$COPSAC_{2000}$: Copenhagen Prospective Study on Asthma in Childhood 2000
FEF_{50}: Forced expiratory flow at 50% of functional vital capacity
$FEV_{0.5}$: Forced expiratory volume in 0.5 seconds
FVC: Forced expiratory vital capacity
PD_{15}: Provocative dose of methacholine causing a 15% decrease in transcutaneous oxygen saturation
PD_{20}: Provocative dose of methacholine causing a 20% decrease in lung function
SNP: Single nucleotide polymorphism

Candidate gene SNPs were chosen based on publication of genome-wide association studies of lung function (FEV_1/forced expiratory vital capacity [FVC] ratio and FEV_1,[7-9] see Table E1 in this article's Online Repository at www.jacionline.org). Genetic risk scores were calculated for each group of SNPs (FEV_1/FVC risk score and FEV_1 risk score) and calculated for each subject as a weighted average: the published per-allele decreasing effect estimate on lung function was multiplied, with the dose of risk alleles for each SNP summed and divided by the total sum of published effect estimates, resulting in a normally distributed genetic risk score ranging from 0 to 2.[16] For similarity between SNP weights in the genetic risk scores, we chose SNPs and risk estimates from the largest genome-wide association study published.[8] There were 26 candidate FEV_1/FVC SNPs and 8 FEV_1 SNPs.

Lung function outcome measurements in $COPSAC_{2000}$ were obtained at 1 month of age by using the rapid raised-volume thoracoabdominal compression technique, assessing forced expiratory volume in 0.5 seconds ($FEV_{0.5}$) and forced expiratory flow at 50% of functional vital capacity (FEF_{50}). Spirometry was repeated at age 7 years, measuring FEV_1, FVC, and FEF_{50}. The lung function measurements have been described in detail elsewhere.[4,17,18]

Bronchial responsiveness outcomes were performed with repeated spirometric assessments and continuous transcutaneous oxygen ($PtcO_2$) measurement in the neonates during methacholine challenge with quadrupling dose steps. The provocative dose causing a 15% decrease in $PtcO_2$ (PD15) was estimated from the dose-response curves fitted with a nonlinear logistic function. Methacholine challenge was repeated at age 7 years, measuring the provocative dose of methacholine causing a 20% decrease in lung function (PD_{20}) as the test outcome.[4,17,18]

Current asthma at age 7 years was assessed, diagnosed, and treated at the COPSAC clinical research unit based on diary cards and according to predefined criteria, as previously described.[19]

Maternal factors potentially influencing neonatal lung function included maternal sensitization determined by using specific IgE measurement (ImmunoCAP; Phadia AB, Uppsala, Sweden)[20] on 0.35 kU/L or more to any common inhalant allergens,[21] worsening of asthma during pregnancy, and similarity of genetic risk scores similar to childhood risk scores.

Statistics

All lung function measures were calibrated for body size and sex, as detailed earlier,[4,17,18] and used as *z* scores. SNP allele dosages (taking into account imputation accuracy) were used, assuming additive genetic models.

Three time points/models were used and associated with both genetic risk scores and secondary to the SNPs separately: (1) lung function ($FEV_{0.5}$ and FEF_{50}) and bronchial responsiveness (PD_{15}) at age 1 month; (2) lung function (FEV_1, FEV_1/FVC and FEF_{50}) and bronchial responsiveness (PD_{20}) at age 7 years; and (3) development of lung function (FEF_{50} and $FEV_{0.5/1}$) and bronchial responsiveness ($PD_{15/20}$). Linear regressions were used for continuous outcomes, and the latter development model (model 3) was used as the SNP effect on the lung function measures at age 7 years adjusted for the neonatal measure.[4]

For the single-SNP analyses, observed against expected *P* values under the null hypothesis with a uniform distribution were plotted to visualize possible deviations and enrichment of lower *P* values compared with the expected values separately for each group of candidate SNPs (ie, FEV_1/FVC SNPs and FEV_1 SNPs). *P* values for enrichment were calculated as a 1-sample, 1-sided *t* test on the *t* scores from the single SNP analyses. Assuming similar direction of effects for the SNPs, as previously published, we used 1-sided tests. For selected loci with suggestive evidence of interaction with smoking exposure during pregnancy (*HHIP* rs11100860 and *TNS1* rs2571445),[22] an interaction term between the SNP (additive genetic model) and smoking exposure was included in the model, with lung function measures at 1 month and 7 years of age as response variables.

Statistics were performed with R Project software (version 2.15.1).[23]

RESULTS

Baseline

Four hundred eleven children were included in the $COPSAC_{2000}$ cohort, and 403 neonates completed lung function measurement at age 1 month. Genotyping was completed in 359 children after removal of siblings and quality control of data. In total, 352 (86% of the total cohort) children had both genotyping data and a completed neonatal lung function measurement. Spirometry at age 7 years was performed in 284 children with available genotyping. Methacholine challenge at age 7 years was completed in 229 of the genotyped children. Genotyping or imputation was completed for all candidate SNPs. On the basis of the imputation quality, 18 SNPs were imputed with the HapMap reference population and 16 SNPs were imputed with the 1000 Genomes reference population. All imputed SNPs had a sufficient quality with an R^2 value of greater than 0.3.[24] Baseline characteristics of the genotyped study population are shown in Table I.

FEV_1/FVC SNP set and outcomes at birth, at 7 years of age, and with growth

Risk score. The FEV_1/FVC genetic risk score was not associated with any of the neonatal lung function or neonatal bronchial responsiveness measures (Table II).

At age 7 years, there was a negative relationship between the FEV_1/FVC risk score and FEF_{50} ($\beta = -1.09$, $P = .01$), FEV_1/FVC ratio ($\beta = -0.85$, $P = .04$), and bronchial responsiveness PD_{20} ($\beta = -1.03$, $P = .02$).

In the analyses of lung function and bronchial responsiveness development (growth), the FEV_1/FVC risk score was associated with FEF_{50} growth ($\beta = -0.97$, $P = .02$) and relative increase in bronchial responsiveness PD ($\beta = -0.90$, $P = .05$), meaning that for children with similar neonatal lung function/responsiveness, an increase in genetic risk score of 1 was associated with an approximately 1-point *z* score reduction at age 7 years (Fig 1 and Table II).

Adjusting the results for asthma did not materially alter the results (see Table E2 in this article's Online Repository at www.jacionline.org).

Enrichment of low *P* values. We observed no enrichment of lower *P* values compared with expected values for FEV_1/FVC SNPs and any of the neonatal outcomes (see Fig E1 in this article's Online Repository at www.jacionline.org).

At age 7 years, there was a greater enrichment of lower *P* values than expected by chance in the Q-Q plot for the FEV_1/FVC SNPs and associations with FEF_{50} and FEV_1/FVC ratio, with a trend for bronchial responsiveness PD (Fig 2) after adjusting for current asthma (see Fig E2 in this article's Online Repository at