

ORIGINAL ARTICLE

Epidemiology, Genetics & Prevention

WILEY

Influence of genetic variants on childhood lung function – The Generation R Study

Sara S. Shagiwal^{1,2,3} | Herman T. den Dekker^{1,2,3} | Johan C. de Jongste² |
Guy G. Brusselle^{3,4} | Vincent W. V. Jaddoe^{1,3,5} | Janine F. Felix^{1,3} | Liesbeth Duijts^{2,4,5}

¹The Generation R Study Group, Division of Neonatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

²Department of Pediatrics, Division of Respiratory Medicine and Allergology, Division of Neonatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

³Department of Epidemiology, Division of Neonatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

⁴Department of Respiratory Medicine and Allergology, Division of Neonatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

⁵Department of Pediatrics, Division of Neonatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Correspondence

Liesbeth Duijts, Erasmus MC - Sophia Children's Hospital, University Medical Center Rotterdam, Rotterdam, The Netherlands.

Email: l.duijts@erasmusmc.nl

Funding information

The Generation R Study is made possible by financial support from the Erasmus Medical Centre, Rotterdam, the Erasmus University Rotterdam, and the Netherlands Organization for Health Research and Development. Dr Vincent Jaddoe received grants from the Netherlands Organization for Health Research and Development (ZonMw-VIDI 016.136.361) and the European Research Council (ERC-2014-CoG-64916). Dr Janine Felix has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 633595 (DynaHEALTH). Dr Liesbeth Duijts received funding from the co-funded program ERA-Net on Biomarkers for Nutrition and Health (ERA HDHL) (ALPHABET project, Horizon 2020 (grant agreement no. 696295; 2017), ZonMW, the Netherlands (no 529051014; 2017)). The researchers are independent of the funders. The Generation R Study received funding from the European Union's Horizon 2020 research and innovation program (733206, LIFECYCLE). The study sponsors had no role in the study design, data analysis, interpretation of data, or writing of this report.

Abstract

Genetic variants associated with adult lung function could already exert the effects on childhood lung function. We aimed to examine the associations of adult lung function-related genetic variants with childhood lung function and asthma, and whether these associations were modified by atopic predisposition, tobacco smoke exposure, or early growth characteristics. In a population-based prospective cohort study among 3347 children, we selected 7 and 20 single nucleotide polymorphisms (SNPs) associated with adult forced expiratory volume in 1 second (FEV₁) and FEV₁/forced vital capacity (FEV₁/FVC), respectively. Weighted genetic risk scores (GRSs) for FEV₁ and FEV₁/FVC were constructed. At age 10, FEV₁, FVC, FEV₁/FVC, forced expiratory flow between 25% and 75% (FEF₂₅₋₇₅), and forced expiratory flow at 75% (FEF₇₅) of FVC were measured, and information on asthma was obtained by parental-reported questionnaires. The FEV₁-GRS was associated with lower childhood FEV₁, FEV₁/FVC, and FEF₇₅ (Z-score (95% CI): -0.03 (-0.05, -0.01), -0.03 (-0.05, -0.01), and -0.04 (-0.05, -0.01), respectively, per additional risk allele). The FEV₁/FVC-GRS was associated with lower childhood FEV₁/FVC and FEF₇₅ (Z-score (95% CI): -0.04 (-0.05, -0.03) and -0.03 (-0.05, -0.02), respectively, per additional risk allele). Effect estimates of FEV₁-GRS with FEF₂₅₋₇₅, FEV₁, FEF₇₅, and FVC, and of FEV₁/FVC-GRS with FEV₁/FVC and FEF₂₅₋₇₅ were stronger among children exposed to non-atopic mothers, smoking during pregnancy or in childhood, or those born with a lower birthweight, respectively (P-values for interaction < .05). Genetic risk scores were not associated with asthma. Adult lung function-related genetic variants were associated with childhood lung function. Maternal atopy, smoking during pregnancy or in childhood, and birthweight modified the observed effects.

KEYWORDS

asthma, child, genetics, lung function, spirometry

1 | INTRODUCTION

Chronic obstructive respiratory diseases, including asthma and chronic obstructive pulmonary disease (COPD), have their origins partly in early life.¹ COPDs are characterized by lower lung function measures of airway patency, such as forced expiratory volume in 1 second (FEV₁) and a lower FEV₁/forced vital capacity (FEV₁/FVC). Genetic factors are suggested to contribute to lung function in later life. Large genomewide association studies (GWAS) have identified the variants associated with lung function in European adults.²⁻⁸ The genes closest to these variants are expressed in lung tissue and are involved in the processes including airway inflammation, growth-related processes, and repair responses to injury.^{5,7} Previous studies have aimed to replicate adult lung function-related genetic variants, either individually or combined into genetic risk scores (GRSs), in European children aged 7-9 years.^{5,7,9,10} However, results across these studies are inconsistent.^{5,7,9,10} This might be due to the type of study population (high risk vs general population),^{9,10} low sample size (range: 389-411 subjects), number of single nucleotide polymorphisms SNPs assessed (range: 21-34), and age at which lung function was measured (infancy vs early childhood).^{9,10} Also, the observed associations of adult lung function-related genetic variants with childhood lung function and asthma might be modified by early life factors such as atopic predisposition and maternal smoking during pregnancy or in childhood,^{11,12} or explained by infant characteristics such as gestational age and birthweight through the underlying epigenetic mechanisms.¹ Whether and to what extent the previously reported associations of adult lung function-related variants with childhood function are modified by early life factors or mediated by infant characteristics is not known. Therefore, we examined among 3347 children the associations of adult lung function-related genetic variants, individually and combined into genetic risk scores, with lung function measures and current asthma at the age of 10 years in a population-based prospective cohort study, and explored whether the observed associations were modified by early life factors or mediated by infant characteristics.

2 | METHODS

This study among 3347 was embedded in the Generation R Study, a prospective cohort study from early fetal life onward, in Rotterdam, the Netherlands¹³ (Figure S1).

2.1 | Genetic variants and risk scores

Genomewide association studies published until December 2015 identified 44 SNPs associated with adult FEV₁ and FEV₁/FVC.²⁻⁸ In this study, these SNPs were extracted from a previously run GWA (genomewide association) scan performed on DNA isolated from cord blood samples or samples taken at the 6-year visit using the Illumina 610 Quad and 660 W platforms.¹⁴ Information on 33

previously identified SNPs was available from the GWAS dataset (Table S1). Seven SNP pairs were in strong linkage disequilibrium (LD) ($r^2 \geq .8$).¹⁵ Of each pair, the SNP with the strongest association with FEV₁ or FEV₁/FVC was included. One SNP (rs2045517) was monomorphic and not included. Proxies (rs8050059 and rs10470171) with perfect LD ($r^2 = 1.00$) were selected for two SNPs, which were not available (rs2865531 and rs9978142). In total, 27 SNPs were used. Unweighted GRSs were calculated by summation of risk alleles using the dosage data from the GWAS dataset.⁵ Weighted GRSs were calculated by weighing the dosage data of each SNP by the reported effect estimate in adults.⁵ Risk alleles were defined as the alleles associated with lower lung function. For similarity between SNP weights in the GRSs, SNPs and effect estimates from the largest adult GWA were used.⁵

2.2 | Childhood lung function and asthma

At a mean age of 10 years (standard deviation (SD): 0.3), FEV₁, FVC, FEV₁/FVC, forced expiratory flow between 25% and 75% (FEF₂₅₋₇₅), and forced expiratory flow at 75% (FEF₇₅) of FVC were measured and converted into sex-, height-, age-, and ethnicity-adjusted Z-scores.^{16,17} Current asthma was defined as ever physician-diagnosed asthma at the age of 10 years with parental-reported wheezing and/or the use of inhaled medication in the past 12 months obtained through questions adapted from the International Study on Asthma and Allergy in Childhood (ISAAC).¹⁸

2.3 | Covariates

Maternal history of asthma, eczema, or allergy was obtained by a questionnaire at enrollment. Maternal smoking during pregnancy was assessed by questionnaires in early, mid- and late pregnancy, and smoking in childhood was assessed by questionnaire at age 6. Child's sex, gestational age, and birthweight were retrieved from hospital and midwife registries. Height at the age of 10 years was measured using a stadiometer (Holtain Limited, Crosswell, Crymch, UK).

2.4 | Statistical analysis

Associations of the 27 adult SNPs, individually and combined into GRSs with childhood lung function and current asthma, were examined using regression analyses. The difference in the explained variance in the null (without the GRS) and alternative model (with the GRS) was considered as the variance in the outcome explained by the GRS. All analyses were performed in the full group. As a sensitivity analysis, analyses were repeated in the largest ethnic subgroup, Europeans. All models were adjusted for child's age, age² (to model the nonlinear associations by age), sex, and height (associated with adult lung function SNPs) as carried out in original adult GWA studies²⁻⁸; and for the first four principal components to account for population structure for either the full group or European only.¹⁹ Effect modification by maternal asthma, allergy

or eczema status, smoking during pregnancy and in childhood, and child's gestational age and birthweight was evaluated by adding the product term of the weighted GRSs and potential effect modifier as an independent variable into the model. Analyses were stratified for the potential effect modifier for the models in which a significant interaction (P -value $< .05$) was observed. Further, we assessed whether gestational age and birthweight explained associations between GRSs and lung function measures by additional adjustment for these variables in the models. To adjust for multiple testing, Bonferroni correction for the number of individual SNPs per outcome and number of tested outcomes in the analyses of weighted GRSs was applied. All measures of association are presented as change in Z-scores or odds ratios with corresponding 95% confidence intervals (95% CI). Statistical analyses were performed using SPSS, version 21.0, for Windows software (IBM, Chicago, Ill, USA).

3 | RESULTS

Table 1 shows the characteristics for all children in the full and European groups. In the full group, 50.9% were girls. Mean FEV₁ was 2.02 (SD 0.30) L, mean FVC 2.34 (SD 0.36) L, mean FEV₁/FVC 86.7% (SD 5.67) %, mean FEF₂₅₋₇₅ 2.70 (SD 0.64) L/s, and mean FEF₇₅ 1.15 (SD 0.34) L/s. Associations of SNPs with childhood lung function and current asthma are presented in Tables S2-S3.

TABLE 1 Characteristics of children and their mothers

	Full group n = 3347	Europeans n = 1924
Maternal characteristics		
Age (y)	31.0 (4.8)	32.0 (4.0)
History of asthma, eczema, or allergy, yes (%)	37.2 (1111)	37.8 (663)
Smoking during pregnancy, yes (%)	24.4 (743)	22.5 (403)
Smoking in childhood, yes (%)	6.6 (173)	5.5 (92)
Child characteristics		
Female sex (%)	50.9 (1705)	50.8 (977)
Gestational age at birth (wk)	40.1 (36.3-42.4)	40.2 (36.6-42.4)
Birthweight (kg)	3459 (514)	3536 (511)
Age (y)	9.8 (0.3)	9.8 (0.3)
Height (cm)	141.6 (6.5)	142.1 (6.4)
Lung function measures		
FEV ₁ (L)	2.02 (0.30)	2.05 (0.30)
FVC (L)	2.34 (0.36)	2.39 (0.35)
FEV ₁ /FVC (%)	86.7 (5.69)	86.1 (5.68)
FEF ₂₅₋₇₅ (L/s)	2.70 (0.64)	2.67 (0.63)
FEF ₇₅ (L/s)	1.15 (0.34)	1.15 (0.34)
Current asthma, yes (%)	7.6 (214)	6.1 (108)

FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of FVC; FEF₇₅, forced expiratory flow at 75% of FVC.

Values are means (SD), medians (2.5-97.5 percentile), or percentages (absolute numbers).

3.1 | Genetic risk scores and childhood lung function and current asthma

The weighted adult FEV₁-GRS was associated with lower childhood FEV₁, FEV₁/FVC, and FEF₇₅ (Z-score (95% CI): -0.03 (-0.05, -0.01), -0.03 (-0.05, -0.01), -0.04 (-0.06, -0.01), respectively, per additional adult FEV₁ lowering allele) (Table 2). The weighted adult FEV₁-GRS explained 0.2% and 2.5% of the variation in childhood FEV₁ and FEF₇₅, respectively. The weighted adult FEV₁/FVC-GRS was associated with lower childhood FEV₁/FVC and FEF₇₅ (Z-score (95% CI): -0.04 (-0.05, -0.0) and -0.03 (-0.05, -0.02) respectively, per additional FEV₁/FVC lowering allele). The weighted adult FEV₁/FVC-GRS explained 1.2% and 3.6% of the variation in childhood FEV₁/FVC and FEF₇₅, respectively. The results did not differ in European children (Table 2). The difference in mean FEV₁ between two extreme groups (≤ 5 and ≥ 12 risk alleles) was 0.3 SDS (Figure 1, A1). The difference in mean FEV₁/FVC between two extreme groups (≤ 14 and ≥ 27 risk alleles) was 0.8 SDS (Figure 1, C2). The weighted adult FEV₁- and FEV₁/FVC-GRSs were not associated with current asthma. Similar results were observed for the unweighted GRSs (Table S4).

3.2 | Modifying and mediating factors

The weighted adult FEV₁-GRS was associated with higher FEF₂₅₋₇₅ among children whose mothers were atopic than those with non-atopic mothers (0.04 (-0.00, -0.08) vs -0.03 (-0.07, -0.00) per

TABLE 2 Association of weighted genetic risk scores with childhood lung function and current asthma

	FEV ₁ Z-score (95% CI)	FVC Z-score (95% CI)	FEV ₁ /FVC Z-score (95% CI)	FEF ₂₅₋₇₅ Z-score (95% CI)	FEF ₇₅ Z-score (95% CI)	Current asthma Odds ratio (95% CI)
FEV ₁ genetic risk score						
Full cohort (n = 3347)	-0.03 (-0.04, -0.01)*	-0.01 (-0.03, 0.01)	-0.03 (-0.05, -0.01)*	-0.01 (-0.03, 0.02)	-0.04 (-0.06, -0.01)*	1.02 (0.92, 1.13)
Europeans (n = 1924)	-0.04 (-0.07, -0.01)*	-0.02 (-0.05, 0.01)	-0.03 (-0.06, 0.00)	-0.01 (-0.04, 0.02)	-0.03 (-0.06, -0.01)	1.04 (0.89, 1.21)
FEV ₁ /FVC genetic risk score						
Full cohort (n = 3347)	-0.01 (-0.03, -0.00)	0.01 (-0.00, 0.02)	-0.04 (-0.05, -0.03)*	-0.02 (-0.03, -0.00)	-0.03 (-0.05, -0.02)*	1.04 (0.97, 1.11)
Europeans (n = 1924)	-0.01 (-0.03, 0.00)	0.01 (-0.00, 0.03)	-0.04 (-0.06, -0.03)*	-0.02 (-0.04, 0.00)	-0.04 (-0.05, -0.02)*	1.02 (0.93, 1.11)

Values represent change in Z-score or odds ratio with their corresponding 95% confidence interval (CI) of forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), forced expiratory flow between 25% and 75% of FVC (FEF₂₅₋₇₅), forced expiratory flow at 75% (FEF₇₅) of FVC, or current asthma, per additional lowering allele of the genetic risk score. Models were adjusted for child's age, age², sex, height and the first four ancestry principal components.

*P-value < .01. Because of Bonferroni correction, a P-value < .01 (0.05/6) was considered significant.

additional FEV₁ lowering allele, P-value for interaction .01) (Table S6). The weighted adult FEV₁-GRS was associated with lower FEV₁ and FEF₇₅ among children exposed to maternal smoking in childhood than those who were not (-0.10 (-0.19, -0.02) vs -0.02 (-0.05, 0.00), -0.12 (-0.21, -0.04) vs -0.03 (-0.05, -0.00), respectively, per additional FEV₁ lowering allele, P-value for interaction, .04 and .02, respectively). The weighted adult FEV₁-GRS was associated with lower FEV₁ and FVC among children born with a lower birthweight than those who were not (-0.05 (-0.18, 0.09) vs -0.03 (-0.05, -0.01), -0.02 (-0.15, 0.11) vs -0.01 (-0.03, 0.01), respectively, per additional FEV₁ lowering allele, P-value for interaction, .01 and < .01, respectively). The weighted adult FEV₁/FVC-GRS was associated with lower FEV₁/FVC among children exposed to smoking during pregnancy and higher FEF₂₅₋₇₅ among children exposed to smoking in childhood, than those who were not (-0.07 (-0.10, -0.04) vs -0.03 (-0.05, -0.02), 0.05 (-0.02, 0.13) vs -0.02 (-0.04, -0.00), per additional FEV₁/FVC lowering allele, P-value for interaction .03 and .03, respectively).

Prior to mediation analyses, an association between weighted adult FEV₁/FVC-GRS and gestational age was observed (data not shown). Effect estimates of FEV₁/FVC-GRS with childhood lung function did not change when models were additionally adjusted for gestational age.

4 | DISCUSSION

In this study, weighted GRSs based on SNPs related to adult lung function were associated with lower childhood FEV₁, FEV₁/FVC, FEF₇₅, FEV₁/FVC, or FEF₇₅. Similar results were observed among European children only. Effect estimates of adult FEV₁-GRS with FEF₂₅₋₇₅, FEV₁, FEF₇₅, and FVC and of FEV₁/FVC-GRS with FEV₁/FVC and FEF₂₅₋₇₅, respectively, were stronger among children

exposed to non-atopic mothers, maternal smoking during pregnancy or in childhood, and those born with a lower birthweight, respectively.

4.1 | Comparison with previous studies

Previous GWAS identified SNPs in or near genes associated with lung function measures in healthy European adults of.²⁻⁸ These SNPs, either individually or combined into GRSs, were partly replicated with lung function among children (n = 389-411) aged 7-9 years in previous studies.^{9,10} In this study, the magnitude and direction of effect estimates were comparable to the adult GWAS in or near 23 genes.⁵ In addition to previous studies, we examined associations between the GRSs and peripheral airway function and current asthma and explored the modifying and mediating effects of early growth factors and infant characteristics, respectively. Among 3347 children, maternal atopy, maternal smoking in childhood, and birthweight modified the associations of weighted adult FEV₁-GRS with FEF₂₅₋₇₅, FEV₁, FEF₇₅, and FVC, respectively. Maternal smoking during pregnancy and in childhood modified the associations of weighted FEV₁/FVC-GRS with childhood FEV₁/FVC and FEF₂₅₋₇₅, respectively. However, these findings should be interpreted cautiously due to the relatively small number of children included in these analyses, either in general or when samples were stratified.

4.2 | Interpretation of results

Weighted GRSs based on SNPs related to adult FEV₁ and FEV₁/FVC were associated with childhood lung function measures. These findings indicate there is a polygenic effect on lung function in children and also suggest there is overlap in genetic background of lung function in children and adults. However, the biologic functions of genes associated with adult lung function are unclear. Also, to what

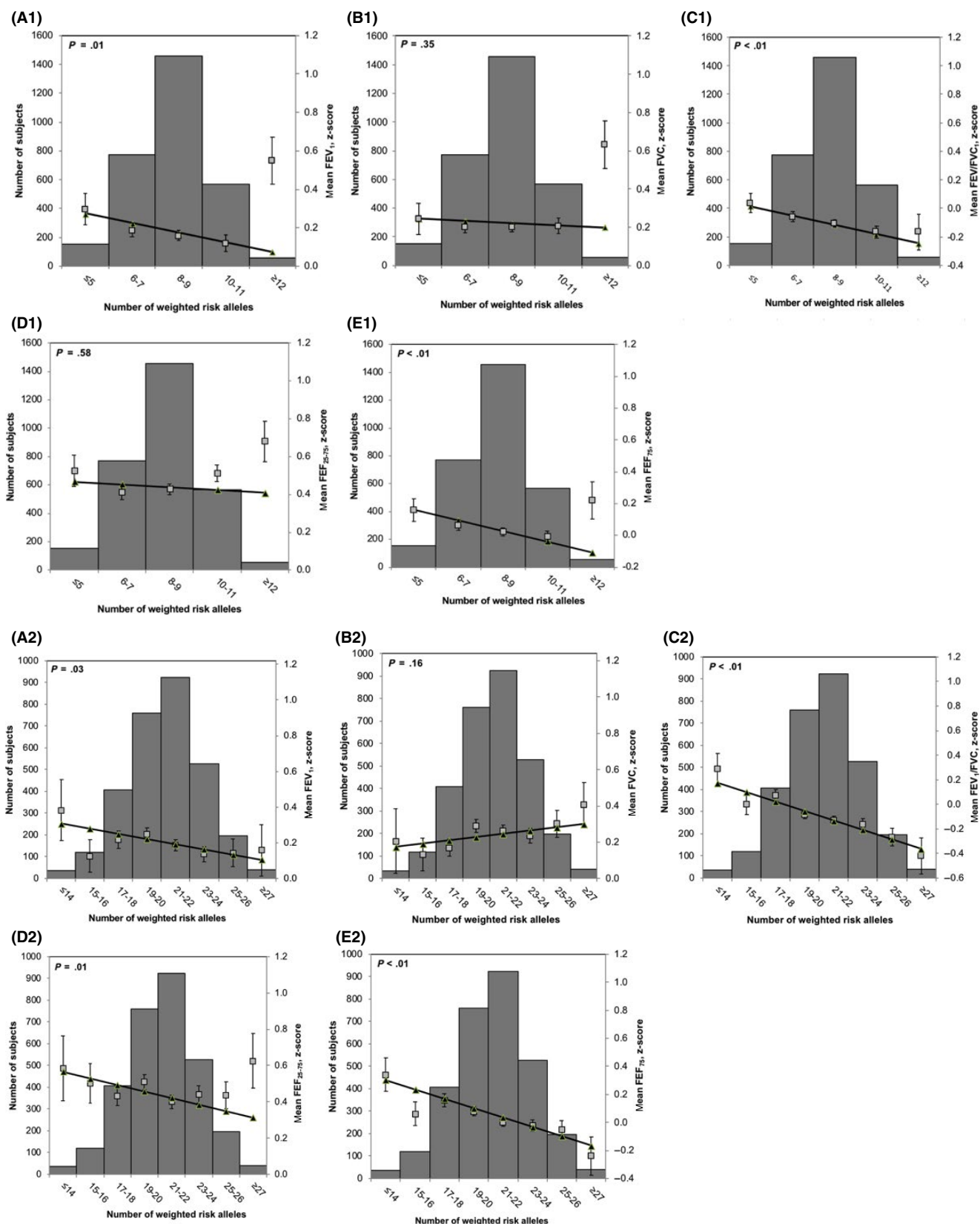


FIGURE 1 Associations of weighted FEV₁-GRS (A1-E1) and FEV₁/FVC-GRS (A2-E2) categorized by number of risk alleles with (A) forced expiratory volume in one second (FEV₁), (B) forced vital capacity (FVC), (C) FEV₁/FVC, (D) forced expiratory flow between 25-75% (FEF₂₅₋₇₅) and (E) forced expiratory flow at 75% (FEF₇₅) in childhood. The x-axis represents the distribution of the number of weighted risk alleles. The left y-axis shows the number of subjects as indicated by bars, the right y-axis the mean (SD) of each lung function measure as indicated by boxes. The line shows the regression of the mean of each lung function measure shown as triangles [Colour figure can be viewed at wileyonlinelibrary.com]

extent gene-gene and gene-environment interactions play a role is unclear. Studies are needed to determine whether the nearest genes are those with causal effects. Also, previous GWAS have focused on cross-sectional measured lung function phenotypes,²⁻⁸ and therefore, large pediatric populations with longitudinal data across life are required. Lung function measures have been shown to be correlated with each other and heritable.²⁰⁻²³ Yet, the percentage of variance explained by the GRSs and the individual SNPs was low in the current study, which could be due to the small-to-moderate effect estimates of the adult SNPs. In addition, rare SNPs, epigenetic factors, and gene/gene-environment interactions may account for this difference. Therefore, meta-analyses of genetic and epigenetic data are required. In this study, the variance explained by the weighted GRSs was higher for FEF₇₅ than that for FEV₁ and FEV₁/FVC. The GWAS that identified adult SNPs were restricted to FEV₁ and FEV₁/FVC. Therefore, our results suggest that adult SNPs might be associated even stronger with peripheral flow measures. Of the seven FEV₁ SNPs, one was individually associated with childhood FEV₁. This SNP, rs10516526, is located in intron 5 of glutathione S-transferase C-terminal domain containing protein (*GSTCD*). Studies have reported that the encoded protein could influence lung function via mechanisms involving the detoxification by glutathione S-transferases that might damage the lungs.³ It has also been shown to be expressed across fetal lung developmental stages.²⁴ Of the 20 FEV₁/FVC SNPs, one showed an individual association with childhood FEF₇₅ but not with FEV₁/FVC. This SNP, rs262129, is located in the gene *LOC153910*, of which the potential function remains unclear to date.^{3-5, 9,10} In previous studies, SNPs in or near *GSTCD* and *LOC153910* have been suggested to be associated with airflow obstruction and risk of COPD.²⁵⁻²⁸ Both reduced growth and accelerated lung function decline in childhood lead to lower lung function in adults. A majority of the adult lung function-related SNPs were not individually associated with childhood lung function measures. As such, the SNPs might affect lung growth and development rather than the accelerated decline of lung function. However, further studies at older ages when lung growth is fully completed are needed. Further, associations of individual SNPs or when combined into GRSs with childhood lung function measures in different ethnic subgroups were limited by our sample size; therefore, further large multi-ethnic studies are warranted. No associations between the GRSs and asthma were observed, which could be due to low power in the asthma subgroup. Alternatively, other SNPs than those associated with adult lung function may be involved in the development of asthma. No associations between SNPs related to adult lung function and asthma have been reported before.^{5,29}

4.3 | Strengths and limitations

This study was embedded in a large prospective cohort study with detailed childhood lung function measures. Some of the following limitations should be acknowledged: first, of all children with genetic information, information on lung function measures was available in 72%, which was mainly due to the follow-up response

of 79% of children at age 9 years. This could have led to selective loss to follow-up and some potential selection bias; second, we selected SNPs that reached genomewide significance levels and might have missed SNPs with true effects that failed to reach the stringent GWA threshold levels in previous studies; and last, the individual SNPs for lung function may have varying effect sizes across the life course. Therefore, weighing based on effect estimates from adult studies may not reflect the true effect sizes in children. However, in the current study, similar findings were observed for the unweighted and weighted GRSs, suggesting that adult weights used in the GRSs did not strongly influence the observed associations. In conclusion, our findings support the hypothesis that SNPs associated with adult lung function affect lung function already in childhood. Maternal atopy, maternal smoking during pregnancy and in childhood, and birthweight partly modify the associations of weighted FEV₁ and FEV₁/FVC-GRSs with lung function measures in children. Our findings could potentially improve risk prediction of lower lung function and asthma in children and support the development of prevention strategies in early life to reduce respiratory health problems in later life. Also, future studies are needed to explore the role of genetic variants in lung development and chronic obstructive respiratory diseases throughout life.

ACKNOWLEDGMENTS

The Generation R Study is conducted by the Erasmus Medical Centre in close collaboration with the School of Law and the Faculty of Social Sciences at the Erasmus University Rotterdam, the Municipal Health Service, Rotterdam area, and the Stichting Trombosedienst en Artsenlaboratorium Rijnmond (Star-MDC), Rotterdam. We gratefully acknowledge the contribution of children and parents' general practitioners, hospitals, midwives, and pharmacies in Rotterdam. The generation and management of GWAS genotype data for the Generation R Study were carried out at the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, the Netherlands. We would like to thank Karol Estrada, Dr. Tobias A. Knoch, Anis Abuseiris, Luc V. de Zeeuw, and Rob de Graaf, for their help in creating GRIMP, BigGRID, MediGRID, and Services@MediGRID/D-Grid (funded by the German Bundesministerium fuer Forschung und Technology; grants 01 AK 803 A-H, 01 IG 07015 G) for access to their grid computing resources. We thank Mila Jhamai, Manoushka Ganesh, Pascal Arp, Marijn Verkerk, Lizbeth Herrera, and Marjolein Peters for their help in creating, managing, and QC of the GWAS database. Also, we thank Karol Estrada for his support in creation and analysis of imputed data.

AUTHOR CONTRIBUTION

SS, HD, JJ, and LD contributed to the conception and design, acquisition of data, analyses and interpretation of the data; drafted the article; revised it critically for important intellectual content; and gave final approval of the version to be published. JJ, VJ, and

GB contributed to the conception and design and acquisition of data; revised the drafted manuscript critically for important intellectual content; and gave final approval of the version to be published.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Sara Shagiwal  <http://orcid.org/0000-0002-1259-5510>

REFERENCES

- Duijts L, Reiss IK, Brusselle G, de Jongste JC. Early origins of chronic obstructive lung diseases across the life course. *Eur J Epidemiol*. 2014;29:871-885.
- Wilk JB, Chen TH, Gottlieb DJ, et al. A genome-wide association study of pulmonary function measures in the Framingham Heart Study. *PLoS Genet*. 2009;5:e1000429.
- Hancock DB, Eijgelsheim M, Wilk JB, et al. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat Genet*. 2010;42:45-52.
- Repapi E, Sayers I, Wain LV, et al. Genome-wide association study identifies five loci associated with lung function. *Nat Genet*. 2010;42:36-44.
- Artigas M, Loth DW, Wain LV, et al. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet*. 2011;43:1082-1090.
- Loth DW, Soler Artigas M, Gharib SA, et al. Genome-wide association analysis identifies six new loci associated with forced vital capacity. *Nat Genet*. 2014;46:669-677.
- Soler Artigas M, Wain LV, Miller S, et al. Sixteen new lung function signals identified through 1000 genomes project reference panel imputation. *Nat Commun*. 2015;6:8658.
- Wain LV, Shrine N, Miller S, et al. Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. *Lancet Respir Med*. 2015;3:769-781.
- Kreiner-Moller E, Bisgaard H, Bonnelykke K. Prenatal and postnatal genetic influence on lung function development. *J Allergy Clin Immunol*. 2014;134:1036-1042.
- Panasevich S, Melen E, Hallberg J, et al. Investigation of novel genes for lung function in children and their interaction with tobacco smoke exposure: a preliminary report. *Acta Paediatr*. 2013;102:498-503.
- Sonnenschein-van der Voort AM, Howe LD, Granell R, et al. Influence of childhood growth on asthma and lung function in adolescence. *J Allergy Clin Immunol*. 2015;135:1435-1443. e7.
- den Dekker HT, Sonnenschein-van der Voort AM, de Jongste JC, et al. Tobacco smoke exposure, airway resistance, and asthma in school-age children: the Generation R Study. *Chest*. 2015;148:607-617.
- Kruithof CJ, Kooijman MN, van Duijn CM, et al. The Generation R Study: biobank update 2015. *Eur J Epidemiol*. 2014;29:911-927.
- Jaddoe VW, van Duijn CM, Franco OH, et al. The Generation R Study: design and cohort update 2012. *Eur J Epidemiol*. 2012;27:739-756.
- Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics*. 2008;24:2938-2939.
- Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319-338.
- Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J*. 2012;40:1324-1343.
- Asher MI, Keil U, Anderson HR, et al. International study of asthma and allergies in childhood (ISAAC): rationale and methods. *Eur Respir J*. 1995;8:483-491.
- Medina-Gomez C, Felix JF, Estrada K, et al. Challenges in conducting genome-wide association studies in highly admixed multi-ethnic populations: the Generation R Study. *Eur J Epidemiol*. 2015;30:317-330.
- DeMeo DL, Silverman EK. Genetics of chronic obstructive pulmonary disease. *Semin Respir Crit Care Med*. 2003;24:151-160.
- Pauwels RA, Buist AS, Ma P, Jenkins CR, Hurd SS, Global Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: National Heart, Lung, And Blood Institute And World Health Organization Global Initiative For Chronic Obstructive Lung Disease (GOLD): executive summary. *Respir Care*. 2001;46:798-825.
- Hubert HB, Fabsitz RR, Feinleib M, Gwinn C. Genetic and environmental influences on pulmonary function in adult twins. *Am Rev Respir Dis*. 1982;125:409-415.
- McClearn GE, Svartengren M, Pedersen NL, Heller DA, Plomin R. Genetic and environmental influences on pulmonary function in aging Swedish twins. *J Gerontol*. 1994;49:264-268.
- Obeidat M, Miller S, Probert K, et al. GSTCD and INTS12 regulation and expression in the human lung. *PLoS ONE*. 2013;8:e74630.
- Soler Artigas M, Wain LV, Repapi E, et al. Effect of five genetic variants associated with lung function on the risk of chronic obstructive lung disease, and their joint effects on lung function. *Am J Respir Crit Care Med*. 2011;184:786-795.
- Castaldi PJ, Cho MH, Litonjua AA, et al. The association of genome-wide significant spirometric loci with chronic obstructive pulmonary disease susceptibility. *Am J Respir Cell Mol Biol*. 2011;45:1147-1153.
- Pillai SG, Ge D, Zhu G, et al. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet*. 2009;5:e1000421.
- Cho MH, Boutaoui N, Klanderman BJ, et al. Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet*. 2010;42:200-202.
- Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med*. 2010;363:1211-1221.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Shagiwal SS, den Dekker HT, de Jongste JC, et al. Influence of genetic variants on childhood lung function – The Generation R Study. *Pediatr Allergy Immunol*. 2018;29:589–595. <https://doi.org/10.1111/pai.12931>

Influence of genetic variants on childhood lung function.

The Generation R Study.

Running title: Genetics of childhood lung function

Supplementary Material

Sara Shagiwal, MSc^{1,2,3}, Herman T. den Dekker, MD^{1,2,3}, Johan C. de Jongste, MD PhD²,
Guy G. Brusselle^{3,4}, Vincent W.V. Jaddoe, MD PhD^{1,3,5}, Janine F. Felix, MD, PhD^{1,3}, Liesbeth
Duijts, MD PhD^{2,4,6}

¹The Generation R Study Group, ²Department of Pediatrics, Division of Respiratory Medicine
and Allergology, ³Department of Epidemiology, ⁴Department of Respiratory Medicine and
Allergology, ⁵Department of Pediatrics, ⁶Department of Pediatrics, Division of Neonatology;
Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands.

Table S1. List of identified genetic variants associated with adult lung function.

Table S2. Associations of individual SNPs related to adult FEV₁ with childhood lung function and
current asthma.

Table S3. Associations of individual SNPs related to adult FEV₁/FVC with childhood lung function
and current asthma.

Table S4. Associations of unweighted genetic risk scores with childhood lung function and
current asthma.

Table S5. Associations of weighted genetic FEV₁ and FEV₁/FVC genetic risk scores with
childhood lung function, and interaction of potential effect modifiers

Table S6. Stratified analyses of the associations of weighted genetic FEV₁ and FEV₁/FVC genetic
risk scores with childhood lung function by significant effect modifiers.

Figure S1. Flow chart of participants included for analyses.

Table S1. List of identified genetic variants associated with adult lung function.

Phenotype	Chromosome	Position	SNP ID	Locus	EA*	Published effect*
FEV ₁	2	218391399	rs2571445	<i>TNS1</i>	A	0.047
FEV ₁	3	170782913	rs1344555	<i>MECOM</i>	T	0.025
FEV ₁	4	106908353	rs10516526	<i>GSTCD</i>	A	0.108
FEV ₁	5	147827981	rs1985524	<i>HTR4</i>	G	0.048
FEV ₁	6	28430275	rs6903823	<i>ZKSCAN3/ ZNF323</i>	G	0.029
FEV ₁	10	12317998	rs7068966	<i>CDC123</i>	C	0.022
FEV ₁	10	77985230	rs11001819	<i>C10orf11</i>	G	0.022
FEV ₁ /FVC	1	17179262	rs2284746	<i>MFAP2</i>	G	0.038
FEV ₁ /FVC	1	216926691	rs993925	<i>TGFB2</i>	C	0.023
FEV ₁ /FVC	2	239542085	rs12477314	<i>HDAC4</i>	C	0.031
FEV ₁ /FVC	3	25495586	rs1529672	<i>RARB</i>	C	0.038
FEV ₁ /FVC	4	145698589	rs11100860	<i>HHIP</i>	A	0.064
FEV ₁ /FVC	5	147822546	rs11168048	<i>HTR4</i>	T	0.047
FEV ₁ /FVC	5	156869344	rs11134779	<i>ADAM19</i>	G	0.042
FEV ₁ /FVC	5	95062456	rs153916	<i>SPATA9</i>	T	0.025
FEV ₁ /FVC	6	31676448	rs2857595	<i>NCR3</i>	A	0.028
FEV ₁ /FVC	6	109374743	rs2798641	<i>ARMC2</i>	T	0.03
FEV ₁ /FVC	6	32259421	rs2070600	<i>AGER</i>	C	0.126
FEV ₁ /FVC	6	142894837	rs262129	<i>LOC153910</i>	A	0.056
FEV ₁ /FVC	9	97244613	rs16909859	<i>PTCH1</i>	A	0.08
FEV ₁ /FVC	10	12317998	rs7068966	<i>CDC123</i>	C	0.023
FEV ₁ /FVC	12	55813550	rs11172113	<i>LRP1</i>	T	0.026
FEV ₁ /FVC	12	94795559	rs1036429	<i>CCDC38</i>	C	0.028
FEV ₁ /FVC	15	69467134	rs8033889	<i>THSD4</i>	T	0.072
FEV ₁ /FVC	16	56632783	rs12447804	<i>MMP15</i>	T	0.021
FEV ₁ /FVC	16	73947817	rs2865531	<i>CFDP1</i>	A	0.024
FEV ₁ /FVC	21	34574109	rs9978142	<i>KCNE2</i>	C	0.031

*Published effect, standard errors and p-values for the associations between SNPs and corresponding lung function measures by Artigas et al (5). *Published effect was used as a weight in the calculation of the genetic risk scores. EA, effect allele; SE, standard error; P; p-value

Table S2. Associations of individual SNPs related to adult FEV₁ with childhood lung function and current asthma.³⁹

SNP ID	Chr.	Position	EA/ OA	EAF	FEV ₁ Z-score (95% CI)	FVC Z-score (95% CI)	FEV ₁ /FVC Z-score (95% CI)	FEF ₂₅₋₇₅ Z-score (95% CI)	FEF ₇₅ Z-score (95% CI)	Current asthma Odds ratio (95% CI)
Full cohort n=3,347										
rs2571445	2	218391399	A/G	0.35	0.02 (-0.03,0.07)	0.01 (-0.04,0.06)	0.02 (-0.03,0.07)	-0.01 (-0.07,0.04)	0.02 (-0.03,0.06)	1.00 (0.78,1.28)
rs1344555	3	170782913	C/T	0.22	-0.01 (-0.08,0.05)	-0.00 (-0.06,0.06)	-0.03 (-0.09,-0.03)	-0.02 (-0.09,0.05)	-0.03 (-0.08,0.03)	1.03 (0.76,1.39)
rs10516526	4	106908353	A/G	0.94	-0.17 (-0.27,-0.06)**	-0.03 (-0.13,0.07)	-0.22 (-0.32,-0.12)**	-0.01 (-0.12,0.11)	-0.24 (-0.34,-0.14)**	1.24 (0.72,2.16)
rs1985524	5	147827981	C/G	0.48	0.01 (-0.04,0.06)	-0.00 (-0.05,0.04)	0.02 (-0.04,0.06)	-0.02 (-0.08,0.03)	0.02 (-0.03,0.06)	0.99 (0.78,1.27)
rs6903823	6	28430275	A/G	0.24	0.07 (0.01,0.14)	0.07 (0.01,0.13)	-0.00 (-0.06,0.05)	0.01 (-0.06,0.07)	0.05 (-0.00,0.11)	0.91 (0.68,1.21)
rs7068966	10	12317998	C/T	0.52	0.02 (-0.03,0.07)	0.05 (0.00,0.10)	-0.05 (-0.10,0.00)*	-0.00 (-0.06,0.05)	-0.02 (-0.07,0.02)	0.93 (0.72,1.19)
rs11001819	10	77985230	A/G	0.58	-0.03 (-0.08,0.02)	-0.03 (-0.08,0.02)	-0.01 (-0.06,0.04)	-0.02 (-0.07,0.03)	-0.02 (-0.06,0.03)	1.10 (0.87,1.41)
Europeans n=1,924										
rs2571445	2	218391399	A/G	0.37	0.03 (-0.04,0.09)	0.02 (-0.03,0.08)	0.00 (-0.06,0.07)	-0.01 (-0.09,0.06)	0.01 (-0.05,0.07)	1.00 (0.70,1.41)
rs1344555	3	170782913	C/T	0.20	0.04 (-0.04,0.12)	0.04 (-0.03,0.11)	0.00 (-0.08,0.08)	0.00 (-0.09,0.09)	0.00 (-0.07,0.08)	1.31 (0.81,2.10)
rs10516526	4	106908353	A/G	0.94	-0.21 (-0.34,-0.08)**	-0.08 (-0.20,0.04)	-0.21 (-0.34,-0.08)**	-0.04(-0.19,0.11)	-0.24 (-0.37,-0.12)**	1.12 (0.54,2.33)
rs1985524	5	147827981	C/G	0.46	0.02 (-0.04,0.08)	-0.00 (-0.06,0.05)	0.04 (-0.02,0.11)	-0.01 (-0.08,0.07)	0.03 (-0.03,0.09)	1.14 (0.80,1.61)
rs6903823	6	28430275	A/G	0.21	0.05 (-0.03,0.12)	0.02 (-0.05,0.09)	0.04 (-0.04,0.12)	0.04 (-0.05,0.12)	0.06 (-0.02,0.13)	0.91 (0.61,1.36)
rs7068966	10	12317998	C/T	0.48	0.02 (-0.04,0.08)	0.03 (-0.02,0.09)	-0.03 (-0.09,0.04)	-0.02 (-0.09,0.05)	0.01 (-0.05,0.07)	0.79 (0.56,1.12)
rs11001819	10	77985230	A/G	0.53	-0.03 (-0.09,0.03)	-0.02 (-0.08,0.03)	-0.01 (-0.08,0.05)	-0.02 (-0.09,0.05)	-0.01 (-0.07,0.04)	1.18 (0.85,1.65)

Values represent differences in Z-score or odds ratio with their corresponding 95% confidence interval (CI) for the effect allele (EA) compared to the other allele (OA). Models were adjusted for child's age, age², sex, height and the first four ancestry principal components. Chr. chromosome; EA, effect allele; OA, other allele; EAF, effect allele frequency; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25–75% of FVC; FEF₇₅, forced expiratory flow at 75% of FVC. ** p-value < 0.01. Because of Bonferroni-correction, a p-value < 0.01 (0.05/6) was considered significant.

Table S3. Associations of individual SNPs related to adult FEV ₁ /FVC with childhood lung function and current asthma ^{46a}										
SNP ID	Chr.	Position	EA/OA	EAF	FEV ₁ Z-score (95% CI)	FVC Z-score (95% CI)	FEV ₁ /FVC Z-score (95% CI)	FEF ₂₅₋₇₅ Z-score (95% CI)	FEF ₇₅ Z-score (95% CI)	Current asthma Odds Ratio (95% CI)
Full cohort n=3,347										
rs993925	1	216926691	C/T	0.67	0.05 (0.00,0.11)	0.05 (0.00,0.11)	0.01 (-0.05,0.05)	0.01 (-0.05,0.07)	0.02 (-0.03,0.07)	1.08 (0.83,1.40)
rs2284746	1	17179262	C/G	0.53	-0.03 (-0.08,0.02)	-0.01 (-0.07,0.04)	-0.03 (-0.08,0.02)	-0.08 (-0.14,-0.02)	-0.02 (-0.06,0.03)	1.05 (0.81,1.36)
rs12477314	2	239542085	C/T	0.82	-0.05 (-0.11,0.02)	-0.02 (-0.08,0.04)	-0.05 (-0.12,0.01)	-0.01 (-0.08,0.06)	-0.07 (-0.12,-0.01)	1.19 (0.86,1.65)
rs1529672	3	25495586	A/C	0.82	-0.05 (-0.12,0.02)	-0.00 (-0.07,0.06)	-0.07 (-0.13,0.00)	0.00 (-0.07,0.08)	-0.08 (-0.14,-0.02)	1.11 (0.80,1.56)
rs11100860	4	145698589	A/G	0.46	0.04 (-0.02,0.10)	0.06 (-0.00,0.11)	-0.03 (-0.09,0.03)	0.01 (-0.06,0.08)	-0.02 (-0.07,0.04)	1.04 (0.77,1.41)
rs11168048	5	147822546	C/T	0.59	-0.03 (-0.08,0.02)	-0.02 (-0.07,0.03)	-0.00 (-0.05,0.05)	-0.01 (-0.07,0.05)	-0.01 (-0.06,0.04)	0.99 (0.77,1.27)
rs11134779	5	156869344	A/G	0.39	0.04 (-0.01,0.09)	0.02 (-0.03,0.07)	0.04 (-0.01,0.09)	0.03 (-0.03,0.09)	0.05 (0.01,0.10)	1.28 (0.98,1.65)
rs153916	5	95062456	C/T	0.55	0.02 (-0.03,0.07)	0.04 (-0.00,0.09)	-0.04 (-0.09,0.01)	-0.02 (-0.08,0.03)	-0.02 (-0.07,0.02)	1.10 (0.87,1.41)
rs2857595	6	31676448	A/G	0.22	0.03 (-0.03,0.10)	-0.01 (-0.07,0.05)	0.08 (0.01,0.14)	-0.01 (-0.07,0.06)	0.06 (0.01,0.12)	0.91 (0.68,1.21)
rs2798641	6	109374743	C/T	0.17	0.06 (-0.01,0.13)	0.01 (-0.05,0.08)	0.08 (0.00,0.14)	0.08 (0.01,0.15)	0.08 (0.02,0.14)	1.02 (0.74,1.41)
rs2070600	6	32259421	C/T	0.97	0.04 (-0.11,0.19)	0.15 (0.01,0.29)	-0.21 (-0.36,-0.07)	-0.05 (-0.21,0.12)	-0.13 (-0.26,0.00)	1.82 (0.73,4.53)
rs262129	6	142894837	A/G	0.62	0.05 (-0.01,0.10)	-0.01 (-0.06,0.04)	0.08 (0.03,0.14)	0.02 (-0.04,0.09)	0.10 (0.05,0.15)**	1.02 (0.78,1.34)
rs16909859	9	97244613	A/G	0.91	0.05 (-0.05,0.16)	0.03 (-0.07,0.14)	0.01 (-0.10,0.11)	-0.03 (-0.14,0.09)	0.04 (-0.05,0.14)	1.35 (0.77,2.36)
rs7068966	10	12317998	C/T	0.52	0.02 (-0.03,0.07)	0.05 (-0.00,0.10)	-0.05 (-0.10,0.00)	-0.01 (-0.07,0.04)	-0.02 (-0.07,0.02)	0.93 (0.72,1.19)
rs11172113	12	55813550	C/T	0.60	0.03 (-0.02,0.09)	0.04 (-0.01,0.09)	-0.02 (-0.07,0.02)	-0.00 (-0.06,0.06)	-0.00 (-0.05,0.05)	1.18 (0.92,1.51)
rs1036429	12	94795559	C/T	0.79	0.01 (-0.06,0.07)	0.00 (-0.06,0.06)	0.02 (-0.04,0.08)	0.01 (-0.06,0.07)	0.01 (-0.05,0.06)	1.17 (0.85,1.61)
rs8033889	15	69467134	G/T	0.23	0.02 (-0.04,0.08)	-0.01 (-0.06,0.05)	0.06 (-0.00,0.11)	0.03 (-0.03,0.10)	0.05 (-0.00,0.10)	0.81 (0.62,1.06)
rs12447804	16	56632783	C/T	0.17	0.10 (0.03,0.16)	0.06 (-0.00,0.13)	0.06 (-0.01,0.12)	0.02 (-0.05,0.09)	0.09 (0.03,0.15)	0.79 (0.61,1.02)
rs8050059 [§]	16	73947817	A/T	0.55	0.06 (0.01,0.11)	0.02 (-0.03,0.07)	0.07 (0.02,0.12)	0.04 (-0.01,0.10)	0.06 (0.02,0.11)	1.15 (0.90,1.47)
rs10470171 [#]	21	34574109	C/T	0.14	0.02 (-0.06,0.09)	0.00 (-0.07,0.07)	0.02 (-0.05,0.09)	0.01 (-0.07,0.09)	-0.01 (-0.08,0.05)	1.01 (0.71,1.45)
Europeans n=1,924										
rs993925	1	216926691	C/T	0.66	0.06 (-0.00,0.13)	0.05 (-0.01,0.11)	0.02 (-0.04,0.08)	0.02 (-0.06,0.10)	0.03 (-0.03,0.09)	1.27 (0.87,1.84)
rs2284746	1	17179262	C/G	0.47	-0.02 (-0.08,0.04)	-0.00 (-0.06,0.06)	-0.03 (-0.09,0.04)	-0.10 (-0.17,-0.02)	-0.01 (-0.07,0.05)	1.04 (0.73, 1.47)
rs12477314	2	239542085	C/T	0.79	-0.01 (-0.08,0.07)	0.00 (-0.07,0.07)	-0.03 (-0.11,0.04)	-0.03 (-0.11,0.06)	-0.03 (-0.10,0.04)	0.94 (0.63,1.41)
rs1529672	3	25495586	A/C	0.84	-0.05 (-0.14,0.04)	-0.03 (-0.11,0.05)	-0.04 (-0.12,0.04)	-0.00 (-0.10,0.10)	-0.06 (-0.14,0.01)	0.98 (0.61,1.55)
rs11100860	4	145698589	A/G	0.43	0.07 (-0.01,0.14)	0.09 (0.02,0.16)	-0.04 (-0.11,0.04)	0.03 (-0.06,0.12)	-0.01 (-0.08,0.06)	1.15 (0.76, 1.73)
rs11168048	5	147822546	C/T	0.57	-0.02 (-0.09,0.04)	-0.00 (-0.06,0.06)	-0.03 (-0.09,0.04)	0.00 (-0.07,0.08)	-0.02 (-0.08,0.03)	0.93 (0.66,1.31)
rs11134779	5	156869344	A/G	0.33	0.05 (-0.02,0.11)	0.01 (-0.06,0.07)	0.08 (0.01,0.14)	0.03 (-0.05,0.10)	0.08 (0.01,0.14)	1.10 (0.76,1.59)
rs153916	5	95062456	C/T	0.54	0.01 (-0.05,0.08)	0.05 (-0.01,0.11)	-0.05 (-0.12,0.01)	-0.01 (-0.09,0.06)	-0.04 (-0.10,0.02)	1.05 (0.75,1.46)
rs2857595	6	31676448	A/G	0.21	0.02 (-0.06,0.10)	-0.02 (-0.09,0.05)	0.06 (-0.02,0.14)	0.02 (-0.07,0.11)	0.04 (-0.03,0.12)	1.01 (0.66,1.54)
rs2798641	6	109374743	C/T	0.18	0.10 (0.01,0.18)	0.05 (-0.03,0.12)	0.08 (-0.00,0.16)	0.07 (-0.02,0.17)	0.10 (0.03,0.18)	1.19 (0.75,1.90)
rs2070600	6	32259421	C/T	0.97	0.08 (-0.10,0.27)	0.12 (-0.04,0.29)	-0.09 (-0.27,0.09)	0.07 (-0.14,0.27)	-0.06 (-0.23,0.10)	2.34 (0.57,9.69)
rs262129	6	142894837	A/G	0.71	0.06 (-0.01,0.13)	0.00 (-0.06,0.07)	0.08 (0.00,0.15)	0.05 (-0.03,0.13)	0.09 (0.02,0.15)	0.93 (0.63,1.37)
rs16909859	9	97244613	A/G	0.91	0.02 (-0.11,0.15)	-0.02 (-0.14,0.10)	0.10 (-0.03,0.23)	-0.05 (-0.20,0.10)	0.08 (-0.04,0.21)	1.21 (0.58,2.56)
rs7068966	10	12317998	C/T	0.48	0.02 (-0.04,0.08)	0.03 (-0.02,0.09)	-0.02 (-0.08,0.04)	-0.02 (-0.09,0.05)	0.01 (-0.05,0.07)	0.79 (0.56,1.12)
rs11172113	12	55813550	C/T	0.58	0.04 (-0.03,0.10)	0.05 (-0.01,0.11)	-0.03 (-0.10,0.03)	0.00 (-0.07,0.07)	-0.01 (-0.07,0.05)	1.33 (0.93,1.89)
rs1036429	12	94795559	C/T	0.80	-0.02 (-0.10,0.06)	0.00 (-0.07,0.07)	-0.02 (-0.09,0.06)	-0.00 (-0.10,0.08)	-0.04 (-0.11,0.03)	0.89 (0.58,1.35)
rs8033889	15	69467134	G/T	0.24	0.05 (-0.03,0.12)	0.01 (-0.06,0.07)	0.07 (-0.00,0.14)	0.03 (-0.05,0.11)	0.07 (-0.00,0.14)	0.88 (0.60,1.29)
rs12447804	16	56632783	C/T	0.20	0.09 (0.01,0.17)	0.07 (-0.00,0.14)	0.03 (-0.05,0.10)	0.03 (-0.06,0.11)	0.06 (-0.01,0.13)	0.97 (0.64,1.48)

rs8050059 ^{\$}	16	73947817	A/T	0.59	0.07 (0.00,0.13)	0.02 (-0.04,0.07)	0.08 (0.02,0.15)	0.04 (-0.03,0.11)	0.08 (0.02,0.14)	0.95 (0.67,1.35)
rs10470171 [#]	21	34574109	C/T	0.15	-0.01 (-0.09,0.08)	-0.01 (-0.09,0.07)	-0.01 (-0.10,0.07)	-0.02 (-0.12,0.08)	-0.01 (-0.10,0.07)	1.37 (0.82,2.29)

Values represent differences in Z-score or odds ratio with their corresponding 95% confidence interval (CI) for the effect allele (EA) compared to the other allele (OA).

Models were adjusted for child's age, age², sex, height and the first four ancestry principal components. Chr. chromosome; EA, effect allele; OA, other allele; EAF, effect allele frequency; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25–75% of FVC; FEF₇₅, forced expiratory flow at 75% of FVC. ^{\$}proxy for rs2865531, [#]proxy for rs9978142. ** p-value <0.0025. Because of Bonferroni-correction, a p-value of <0.0025 (0.05/20) was considered significant.

Table S4. Association of unweighted genetic risk scores with childhood lung function and current asthma.

	FEV₁, Z-score (95% CI)	FVC Z-score (95% CI)	FEV₁/FVC, Z-score (95% CI)	FEF₂₅₋₇₅ Z-score (95% CI)	FEF₇₅, Z-score (95% CI)	Current asthma Odds ratio (95% CI)
FEV₁ Genetic risk scores						
Full cohort (n=3,347)	-0.02 (-0.04,0.00)	-0.01 (-0.03,0.01)	-0.02 (-0.04,0.00)	-0.01 (-0.03,0.01)	-0.02 (-0.04,-0.00)	1.02 (0.92,1.13)
Europeans (n=1,924)	-0.02 (-0.05,0.00)	-0.01 (-0.04,0.01)	-0.01 (-0.04,0.01)	-0.01 (-0.04,0.02)	-0.01 (-0.04, 0.01)	1.00 (0.87,1.16)
FEV₁/FVC Genetic risk scores						
Full cohort (n=3,347)	-0.02 (-0.03,-0.00)	0.01 (-0.00,0.02)	-0.04 (-0.05,-0.03)**	-0.02 (-0.03,-0.00)	-0.03 (-0.05,-0.02)**	1.04 (0.97,1.11)
Europeans (n=1,924)	-0.01 (-0.03,-0.00)	0.01 (-0.00,0.03)	-0.04 (-0.06,-0.03)**	-0.02 (-0.04,0.00)	-0.04 (-0.05,-0.02)**	1.01 (0.92,1.10)

Values represent change in Z-score or odds ratio with their corresponding 95% confidence interval (CI) of forced expiratory volume in one second (FEV₁), FEV₁/ to

forced vital capacity (FEV₁/FVC), per additional lowering allele of the genetic risk score using linear and logistic regression models. Regression models were adjusted for child's age, age², sex, height and the first four ancestry principal components. Chr. chromosome; EA, effect allele; OA, other allele; EAF, effect allele frequency; FEF₂₅₋₇₅, forced expiratory flow between 25–75% of FVC; FEF₇₅, forced expiratory flow at 75% of FVC. ** p-value <0.01. Because of Bonferroni-correction, a p-value of < 0.01 (0.05/6) was considered significant.

Table S5. Associations of weighted genetic FEV₁ and FEV₁/FVC genetic risk scores with childhood lung function, and interaction of potential effect modifiers

	Lung function Z-score (95% CI)	P_{interaction}
FEV₁ genetic risk score		
Maternal atopy	FEV ₁	0.90
	FVC	0.57
	FEV ₁ /FVC	0.73
	FEF₂₅₋₇₅	0.01*
	FEF ₇₅	0.73
Maternal smoking during pregnancy	FEV ₁	0.58
	FVC	0.34
	FEV ₁ /FVC	0.55
	FEF ₂₅₋₇₅	0.97
	FEF ₇₅	0.65
Maternal smoking in childhood	FEV₁	0.04*
	FVC	0.36
	FEV ₁ /FVC	0.08
	FEF ₂₅₋₇₅	0.36
	FEF₇₅	0.02
Gestational age at birth	FEV ₁	0.45
	FVC	0.32
	FEV ₁ /FVC	0.68
	FEF ₂₅₋₇₅	0.15
	FEF ₇₅	0.61
Birth weight (g)	FEV₁	0.01*
	FVC	<0.01**
	FEV ₁ /FVC	0.28
	FEF ₂₅₋₇₅	0.95
	FEF ₇₅	0.64
FEV₁/FVC genetic risk score		
Maternal atopy	FEV ₁	0.47
	FVC	0.53
	FEV ₁ /FVC	0.51
	FEF ₂₅₋₇₅	0.52
	FEF ₇₅	0.69
Maternal smoking during pregnancy	FEV ₁	0.22
	FVC	0.83
	FEV₁/FVC	0.03*
	FEF ₂₅₋₇₅	0.75
	FEF ₇₅	0.06
Maternal smoking in childhood	FEV ₁	0.45
	FVC	0.88
	FEV ₁ /FVC	0.47
	FEF₂₅₋₇₅	0.03*
	FEF ₇₅	0.73
Gestational age at birth	FEV ₁	0.60
	FVC	0.99

	FEV ₁ /FVC	0.45
	FEF ₂₅₋₇₅	0.23
	FEF ₇₅	0.23
Birth weight (g)	FEV ₁	0.32
	FVC	0.41
	FEV ₁ /FVC	0.70
	FEF ₂₅₋₇₅	0.60
	FEF ₇₅	0.81

Pinteraction: P-value for the statistical interaction of the genetic risk score for lung function measures with effect modifiers. FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25–75% of FVC; FEF₇₅, forced expiratory flow at 75% of FVC. *P-value < 0.05, ** p-value < 0.01.

Table S6. Stratified analyses of the associations of weighted genetic FEV₁ and FEV₁/FVC genetic risk scores with childhood lung function by significant effect modifiers

	Lung function Z-score (95% CI)	P_{interaction}
FEV₁ genetic risk score		
Maternal atopy	FEF₂₅₋₇₅	0.01
No	-0.03 (-0.07,-0.00)*	
Yes	0.04 (-0.00, 0.08)	
Maternal smoking in childhood	FEV₁	0.04
No	-0.02 (-0.05,0.00)	
Yes	-0.10 (-0.19,-0.02)*	
	FEF₇₅	0.02
No	-0.03 (-0.05, -0.00)*	
Yes	-0.12 (-0.21, -0.04)**	
Birth weight (g)	FEV₁	0.01
<2500	-0.05 (-0.18,0.09)	
>2500	-0.03 (-0.05,-0.01)*	
	FVC	<0.01
<2500	-0.02 (-0.15,0.11)	
>2500	-0.01 (-0.03,0.01)	
FEV₁/FVC genetic risk score		
Maternal smoking during pregnancy	FEV₁/FVC	0.03
No	-0.03 (-0.05,-0.02)**	
Yes	-0.07 (-0.10,-0.04)**	
Maternal smoking in childhood	FEF₂₅₋₇₅	0.03
No	-0.02 (-0.04,-0.00)*	
Yes	0.05 (-0.02,0.13)	

Values represent change in Z-score with its corresponding 95% confidence interval (CI) of lung function measure per additional lowering allele of the genetic risk score. P_{interaction}: P-value for the statistical interaction of the genetic risk score for lung function measures with effect modifiers. Models were adjusted for child's age, age², sex, height and the first four ancestry principal components. FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25–75% of FVC; FEF₇₅, forced expiratory flow at 75% of FVC *p-value < 0.05, ** p-value < 0.01

Figure S1. Flow chart of participants included for analyses.

