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ORIGINAL ARTICLE

Determinants of Lung Fissure Completeness

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Abstract

Rationale: New advanced bronchoscopic treatment options for patients with severe chronic obstructive pulmonary disease (COPD) have led to increased interest for COPD phenotyping, including fissure completeness.

Objectives: We investigated clinical, environmental, and genetic factors contributing to fissure completeness in patients with and without COPD.

Methods: We used data from 9,926 participants of the COPDGene study who underwent chest computed tomographic (CT) scans. Fissure completeness was calculated from CT scans after quantitative CT analysis at baseline and 5-year follow-up. Clinical and environmental factors, including sex, race, smoking, COPD, emphysema, maternal smoking during pregnancy and maternal COPD, were tested for impact on fissure completeness. Genome-wide association analyses were performed separately in non-Hispanic White subjects and African American subjects.

Measurements and Main Results: African American subjects had significantly higher fissure completeness than non-Hispanic White subjects for all three fissures ($P < 0.001$). There was no change in fissure completeness between baseline and 5-year follow-up. For all fissures, no clinically relevant differences in fissure completeness were found for other clinical or environmental factors, including COPD severity. Rs2173623, rs264866, rs2407284, rs7310342, rs4904145, rs6504172, and rs7209556 showed genome-wide significant associations with fissure completeness in non-Hispanic White subjects. In African American subjects, rs264866, rs4904145 and rs6504172 were identified as significant associations. Rs2173623, rs6504172, and rs7209556 lead to WNT5A and HOXB antisense RNA expression, which play an important role during embryogenesis.

Conclusions: Fissure completeness is genetically determined and not dependent on age, sex, smoking status, the presence and severity of COPD (including exacerbation frequency), maternal smoking during pregnancy, or maternal COPD.

Keywords: COPD; epidemiology; genetics

Over the past decade, several bronchoscopic treatment modalities have been developed as advanced treatment options for patients with severe chronic obstructive pulmonary disease (COPD), most notably the bronchoscopic lung volume reduction with endobronchial

valves (EBVs) (1). The development of these treatment modalities has increased attention for COPD phenotyping and anatomic variation.

In patients with severe emphysema, EBV treatment can result in significantly

improved exercise capacity, lung function, and quality of life (2). However, to benefit from this treatment, absence of interlobar collateral ventilation (movement of air from the adjacent lobes to the target lobe across the interlobar fissure) is essential (3).

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Author Contributions: M.C.v.d.M.: study design, data analysis, writing (original draft). J.E.H.: study design and writing (review and editing). C.J.V.: data analysis and writing (review and editing). M.v.d.B.: writing (review and editing). A.F.: writing (review and editing). H.A.M.K.: writing (review and editing). J.-P.C.: software and writing (review and editing). L.E.G.W.V.: writing (review and editing). D.-J.S.: study design and writing (review and editing).

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At a Glance Commentary

Scientific Knowledge on the

Subject: The development of advanced bronchoscopic treatment modalities for patients with severe chronic obstructive pulmonary disease (COPD), such as a bronchoscopic lung volume reduction with endobronchial valves, has led to increased interest for COPD phenotyping and fissure completeness over the past decade.

Although complete fissures are essential to benefit from the treatment with endobronchial valves, there are no therapeutic options to increase fissure integrities to date. Several smaller studies have investigated the prevalence of complete and incomplete fissures, in both study populations with COPD and study populations without pulmonary diseases. However, studies are lacking that investigate which clinical, environmental, and genetic factors contribute to fissure completeness, nor are there longitudinal studies that assess changes in fissure completeness over time.

What This Study Adds to the Field:

This is the largest study population in which fissure completeness was assessed. Moreover, we are the first to conclude that fissure completeness is genetically determined, and not influenced by clinical factors such as smoking, the presence and severity of COPD (including exacerbation frequency), maternal smoking during the pregnancy, or aging. In addition, we are the first to identify SNPs that are associated with fissure completeness.

Interlobar collateral ventilation has been shown to be highly dependent on the completeness of interlobar fissures (regions of the lung that separate each lobe) (4, 5), but interlobar fissures are often incomplete (defined as a fissure completeness score <95%) in both individuals with and without lung disease (6), and to date there are no established therapeutic options to increase fissure integrities (7). Thus, the interlobar fissure completeness is highly clinically

relevant to patients with severe COPD who may qualify for treatment with EBVs (3).

Thus far, there are limited data about the natural history of fissure completeness (8). It is currently unknown whether incomplete interlobar fissures are an aspect of the emphysematous destruction of the lung tissue associated with COPD, whether it is affected by, for example, age, sex, smoking, and race, or whether it merely reflects variation of the anatomy of the lung that occurs in the general population. If the completeness of interlobar fissures is due to natural variation in the population, it is likely under the regulation of genetics with or without interaction with external environmental influences.

In the current study, we assessed fissure completeness in participants of the COPDGene study. We aimed to investigate the clinical, environmental, and genetic factors contributing to fissure completeness in both patients with and without COPD, including a 5-year follow-up.

Methods

Study Population

The study was conducted with data of participants of the COPDGene study (NCT000608764, www.copdgene.org) (9). In COPDGene, initially 10,000 subjects were included at baseline with a minimum of 10 pack-years of smoking. At a later time, an additional cohort of 455 never-smokers was included. Former and current smokers with an FEV₁/FVC of 0.7 or more and FEV₁ of ≥80% predicted were defined as Global Initiative for Chronic Obstructive Lung Disease (GOLD) 0, and former and current smokers with an FEV₁/FVC of less than 0.7 were defined as GOLD 1 to GOLD 4 on the basis of the FEV₁. All participants enrolled at baseline were invited for a 5-year follow-up visit.

Exclusion criteria of the COPDGene study included known alpha-1 antitrypsin deficiency or the presence of significant lung diseases other than obstructive lung diseases, or a history of lung volume reduction surgery or lung transplantation (9). For our analysis, we included all 9,926 patients who underwent a computed tomographic (CT) scan. Of the participants, 4,175 were classified as participants with COPD, 5,244 as GOLD 0, and 447 as never-smokers.

Interlobar Fissure Analysis

Inspiratory chest CT scans were obtained at the baseline and 5-year follow-up study visits. For this study, interlobar fissure integrities were automatically calculated from the CT scans with the use of Thirona's lung quantitative software (LungQ; Thirona) (10, 11). With the use of this software, the lungs, lobes, and fissures are automatically segmented. Fissures are detected on the basis of pattern classification among the lobar borders. Fissure integrities were expressed as percentages of the lobar border and were scored separately for the left major fissure, right major fissure, and right minor fissure. The percentage emphysema was defined as the percentage of voxels with an attenuation < -950 Hounsfield units on inspiratory CT scans. Following the current guidelines, complete fissures were defined as fissures with completeness scores ≥95% (7).

Genotyping

All participants were whole genome genotyped using the Illumina Omni-Express Chip (Illumina Inc.). The genotyping techniques, quality control steps, and imputation were performed in the COPDGene study as previously described (12).

Expression Quantitative Trait Loci Analysis

The following online bioinformatic tools were used to explore the SNPs of interest: GTEx Portal (<https://gtexportal.org/home/>), UCSC browser (<https://genome.ucsc.edu/ENCODE/>), LDproxy Tool (<https://ldlink.nci.nih.gov/>; Utah Residents from North and West Europe), Genome Aggregation Database (<https://gnomad.broadinstitute.org/>), and NCBI database (<https://www.ncbi.nlm.nih.gov/gene/>).

Statistical Analysis

Clinical determinants of fissure completeness. Data analyses were performed using IBM SPSS statistics (version 23), and *P* values of less than 0.0167 were considered to be statistically significant after Bonferroni correction for multiple testing given the three different fissures.

All clinical variables were included that could potentially affect fissure completeness based on literature, the medical experience of the authors, and the variables that were available. Spearman correlation coefficients were calculated to

test for associations between completeness scores of the different interlobar fissures. The following variables were tested for potential univariate associations with fissure completeness: race, sex, age, number of pack-years, exacerbation frequency, number of severe exacerbations, number of treatments with steroids, FEV₁% predicted, FEV₁/FVC ratio, and percentage of emphysematous destruction. Variables with significant associations with at least one of the fissures ($P < 0.0167$) were included in the multivariate linear regression model to test these as independent predictors of fissure completeness. Student's t tests were used to test for significant differences in fissure integrities between the following variables: sex, race, smoking, COPD, emphysema destruction score $\geq 30\%$ (7, 13), fissure completeness at baseline and 5-year follow-up, maternal smoking during pregnancy, and maternal COPD. Student's t tests and χ^2 tests were performed to test for statistically significant differences between the groups with fissure integrities of $<95\%$ and $\geq 95\%$. Kruskal-Wallis testing was performed to test for differences in fissure integrities among the COPD GOLD stages.

Genetic determinants of fissure completeness. Genome-wide association analyses were performed using PLINK 1.9 (www.cog-genomics.org/plink2) (14). Because race is an important confounder in genome-wide association studies, the analyses were performed separately in non-Hispanic white subjects and African American subjects. P values of less than 1.67×10^{-8} were considered genome-wide significant after Bonferroni correction for multiple testing. Associations with P values of less than 3.3×10^{-6} (after Bonferroni correction) were considered exploratory.

Arcsine-transformed fissure completeness scores were analyzed by linear regression, using sex and principal components of ancestry as covariates in the model. In both ethnicities, the genomic inflation factor was less than 1.03 for all fissures. We defined categories by setting subjects with fissure integrities of $<95\%$ as cases and subjects with fissure integrities of $\geq 95\%$ as controls. Using the same model as the linear regression, categories were subjected to logistic regression.

Results

Study Population and Fissure Integrities

Baseline CT scans were performed in 9,926 participants, and 5-year follow-up CT scans were performed in 5,532 participants. Overall, median fissure integrities were 97.9% for the left major fissure, 92.7% for the right major fissure, and 72.2% for the right minor fissure at baseline. Complete fissures (fissure completeness scores $\geq 95\%$) were seen in 6,471 participants (65.2%) for the left major fissure, 3,945 (39.7%) for the right major fissure, and 1,489 (15.0%) for the right minor fissure.

A moderate correlation was found between the integrities of the left and right major fissures ($\rho = 0.39$, $P < 0.001$). The correlation coefficients between the right minor fissure completeness and the right or left major fissure completeness were both $\rho = 0.27$ ($P < 0.001$). Characteristics of patients with complete and incomplete fissures are shown in Table 1.

Clinical Determinants of Fissure Completeness

Race. For all fissures, African American patients had significant higher median fissure completeness scores than non-Hispanic White patients (left major fissure: 98.9% vs. 97.3% [$P < 0.001$]; right major fissure: 95.0% vs. 91.6% [$P < 0.001$]; right minor fissure: 81.9% vs. 68.4% [$P < 0.001$]). In the multiple linear regression model, race was an independent predictor with regard to the completeness of all three fissures (see Table E1 in the online supplement).

Sex. No significant differences were seen for the left major and right minor fissures (97.8% vs. 98.0% [$P = 0.06$] and 72.1% vs. 72.5% [$P = 0.58$]) between men and women. For the right major fissure, the median fissure completeness score was higher in women than in men (92.2% vs. 93.0% [$P < 0.001$]). However, men more often had a complete right major fissure than women did (Table 1). In the multiple linear regression model, sex was an independent predictor with regard to the completeness of both the left and the right major fissures (Table E1).

Smoking. A significant difference in fissure completeness score was found between never-smokers and (ex-) smokers for the right minor (79.3% vs. 71.9% [$P < 0.001$]), but no differences were found for the left and right major fissures. We

found no association between number of pack-years and completeness of the left major fissure and weak associations with completeness of the right major and minor fissures ($\rho = -0.036$ [$P < 0.001$] and $\rho = -0.044$ [$P < 0.001$], respectively). In the multiple linear regression model, the number of pack-years was only an independent predictor with regard to the completeness of the right minor fissure (Table E1).

COPD. Participants without COPD had higher median fissure completeness compared with participants with COPD for the left major fissure (98.1% vs. 97.7% [$P < 0.001$]), but there were no significant differences between both groups for the right major and minor fissures (92.9% vs. 92.3% [$P = 0.02$] and 72.2% vs. 72.3% [$P = 0.96$]).

No significant differences were found in fissure completeness between the different COPD GOLD stages (Table 2) nor were significant associations found between fissure completeness and FEV₁% predicted. Weak associations were found between fissure completeness of the left and right major fissures and the FEV₁/FVC ratio ($\rho = 0.053$ [$P < 0.001$] and $\rho = 0.032$ [$P < 0.001$], respectively), but, in the multiple linear regression model, the FEV₁/FVC ratio was not an independent predictor for one of the fissures.

For all fissures, no significant associations were found between fissure completeness and exacerbation frequency, number of severe exacerbations, or number of treatments with steroids.

Significant associations were found between the percentage of emphysematous destruction and fissure completeness for all fissures (left major: $\rho = -0.124$ [$P < 0.001$]; right major: $\rho = -0.100$, [$P < 0.001$]; right minor: $\rho = -0.049$ [$P < 0.001$]). No differences in fissure completeness were found between patients with emphysematous scores of $<30\%$ and the clinical threshold of $\geq 30\%$ (left major fissure: 97.9% vs. 97.7% [$P = 0.11$]; right major fissure: 92.7% vs. 92.6% [$P = 0.65$]; right minor fissure: 72.2% vs. 72.3% [$P = 0.56$]). In addition, emphysematous destruction was not an independent predictor for one of the fissures in a multiple linear regression model (Table E1).

Longitudinal change in fissure completeness during 5-year follow-up. At 5-year follow-up, the mean change in fissure completeness was -0.05% for the left major fissure (97.9% vs. 97.9% [$P < 0.001$]), $+0.15\%$ for the right major fissure (92.8% vs. 92.7%

Table 1. Characteristics of Patients with Complete and Incomplete Fissures

	Left Major Fissure			Right Major Fissure			Right Minor Fissure		
	Incomplete (<95%)	Complete (≥95%)	P Value	Incomplete (<95%)	Complete (≥95%)	P Value	Incomplete (<95%)	Complete (≥95%)	P Value
Age (n=9,926), y	59.8	59.5	0.12	59.8	59.2	<0.001	59.8	58.4	<0.001
Sex (n=9,926), M:F, %	45.1:54.9	47.8:52.2	0.01	45.5:54.5	49.0:51.0	<0.001	46.7:53.3	47.7:52.3	0.50
Race, (n=9,926), non-Hispanic White:African American, %	74.8:25.2	64.0:36.0	<0.001	73.2:26.8	59.4:40.6	<0.001	71.7:28.3	45.5:54.5	<0.001
Pack-years (n=9,924)	42.4	42.0	0.46	42.6	41.4	0.02	42.3	41.2	0.13
COPD:no COPD (n=9,866), %	43.3:56.7	42.0:58.0	0.22	43.3:56.7	41.1:58.9	0.04	42.8:57.2	40.4:59.6	0.08
FEV ₁ % predicted	77.8	77.8	0.87	78.0	77.5	0.38	77.8	77.8	0.97
Emphysema (n=9,926), ≤30%:>30%, %	95.6:4.4	95.3:4.7	0.52	95.5:4.5	95.4:4.6	0.82	95.3:4.7	96.1:3.9	0.19
Maternal smoking during pregnancy, (n=5,532), yes:no, %	29.4:70.6	30.8:69.2	0.24	30.2:69.8	30.5:69.5	0.77	30.5:69.5	29.3:70.7	0.45
Maternal COPD, (n=5,532), yes:no, %	8.8:91.2	9.4:90.6	0.35	9.0:91.0	9.5:90.5	0.41	9.0:91.0	10.2:89.8	0.20

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

Complete fissures are defined as fissure integrities ≥95%. Age is presented as median. Student's *t* tests and χ^2 tests were performed to test for statistically significant differences between the groups.

[$P < 0.001$]), and +0.45% for the right minor fissure (71.0% vs. 71.8% [$P < 0.001$]). In a multiple linear regression model including sex, race, and pack-years, age was an independent predictor of fissure completeness for the right minor fissure (Table E1).

Maternal factors. Maternal smoking during pregnancy did not result in differences in fissure completeness (left major fissure: 97.7% vs. 97.8% [$P = 0.36$]; right major fissure: 92.6% vs. 92.3% [$P = 0.13$]; right minor fissure: 70.7% vs. 71.4% [$P = 0.80$]), nor did the presence of maternal COPD (left major fissure: 97.7% vs. 97.8% [$P = 0.36$]; right major fissure: 92.9% vs. 92.5% [$P = 0.06$]; right minor fissure: 71.4% vs. 71.3% [$P = 0.43$]).

Genetic Determinants of Fissure Completeness

Non-Hispanic White patients. Loci with genome-wide significant associations ($P < 1.67 \times 10^{-8}$) were identified on chromosomes 2, 3, 5, 12, 14, and 17 (Figure 1). Loci on chromosomes 3, 5, 12,

and 17 showed associations ($P < 3.3 \times 10^{-6}$) with intactness of more than one fissure. Loci on chromosome 2 were only significant for the left major fissure completeness, and loci on chromosome 14 were only significant only for right minor fissure completeness. A logistic regression model using fissure completeness scores ≥95% resulted in loci with genome-wide significance on chromosomes 12 and 17 (Figure E1).

For the left and right major fissure, a strong association with fissure completeness was found on chromosome 3 on rs2173623 (left major fissure: $P = 2.39 \times 10^{-8}$, odds ratio = 0.81; right major fissure: $P = 7.75 \times 10^{-6}$, odds ratio = 0.84). Located on chromosome 5, rs264866 (in linkage disequilibrium [LD] with rs39800, rs2559974, and rs3797713) showed associations with the fissure integrities of all fissures (left major fissure: $P = 2.24 \times 10^{-6}$, odds ratio = 0.83; right major fissure: $P = 9.41 \times 10^{-6}$, odds ratio = 0.84; right minor fissure: $P = 4.81 \times 10^{-7}$, odds ratio = 0.74). On chromosome 12, rs7310342

(in LD with rs1080902) and rs2407284 (in LD with rs1514669) showed significant associations with left and right major fissure completeness. On chromosome 14, rs4904145 showed a significant association with right minor fissure completeness. On chromosome 17, the following two loci were found to be associated with left major fissure completeness: rs7209556 ($P = 1.42 \times 10^{-8}$, odds ratio = 0.81; in LD with rs7216202) and rs6504172 ($P = 2.07 \times 10^{-7}$, odds ratio = 0.81). The significance level and odds ratios for all associations are given in Table 3.

African American patients. Loci with genome-wide significant associations ($P < 1.67 \times 10^{-8}$) were identified on chromosomes 5 and 14 (Figure 1). A logistic regression model using fissure completeness scores ≥95% resulted in loci with genome-wide significant associations on chromosomes 5 and 14 (Figure E1). On chromosome 5, genome-wide significant associations were identified between rs264866 and left major and right minor fissure completeness (left major fissure: $P = 1.14 \times 10^{-8}$, odds ratio = 0.71; right minor fissure: $P = 1.02 \times 10^{-9}$, odds ratio = 0.74). On chromosome 14, a genome-wide significant association was found between rs4904145 and right minor fissure completeness ($P = 2.45 \times 10^{-9}$, odds ratio = 1.43). In addition, rs6504172 on chromosome 17 (rs6504172) was associated with left major fissure completeness ($P = 1.96 \times 10^{-6}$, odds ratio = 0.75). The significance level and odds ratios for all associations that were

Table 2. Fissure Integrity Scores among Patients with GOLD 1–4 COPD

	GOLD 1 (n = 749)	GOLD 2 (n = 1,809)	GOLD 3 (n = 1,076)	GOLD 4 (n = 541)	P Value
Left major, %	97.6	97.9	97.6	97.3	0.237
Right major, %	91.6	92.6	92.4	92.7	0.093
Right minor, %	71.5	71.8	73.6	72.3	0.697

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease.

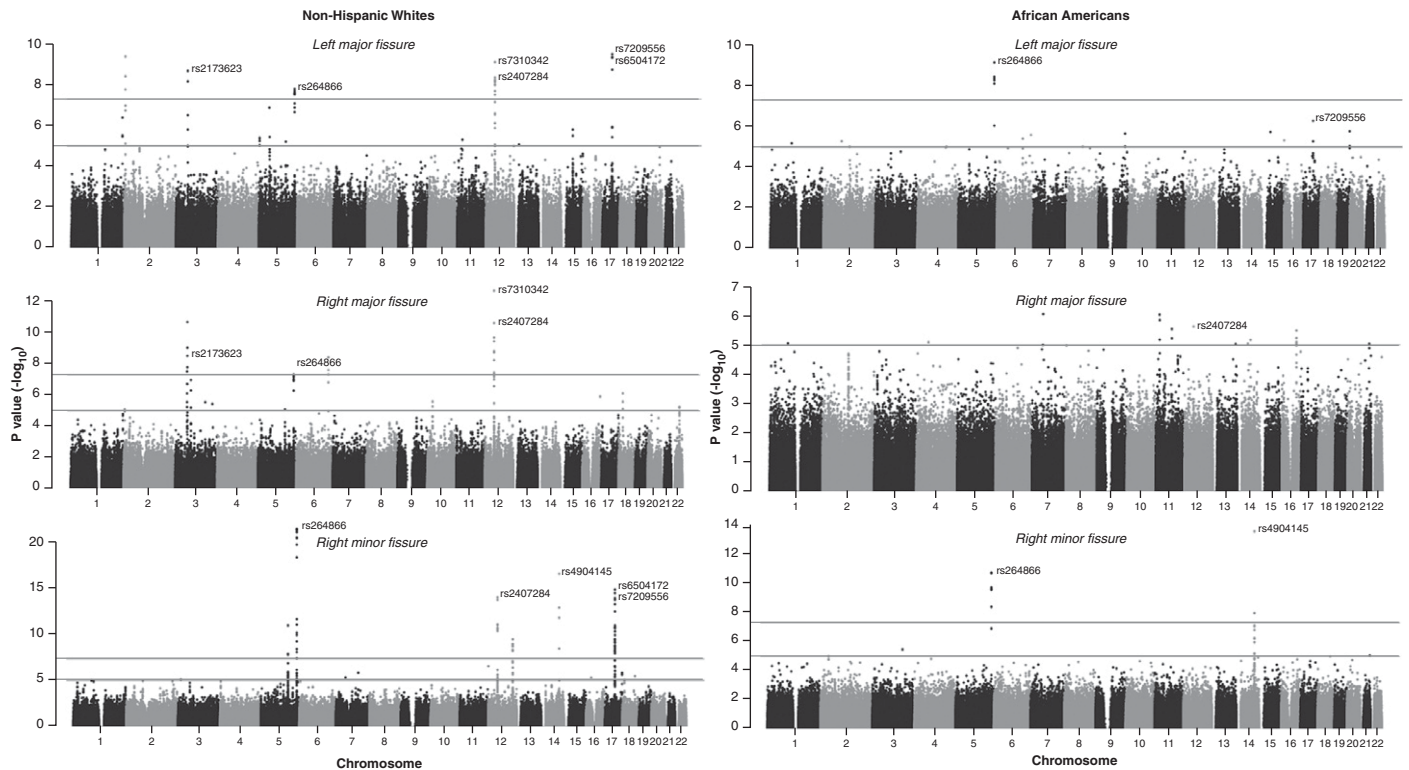


Figure 1. Manhattan plots of the left major, right major, and right minor fissures in non-Hispanic White and African American subjects. The results are shown for all non-Hispanic White and African American subjects. The upper horizontal line represents the general threshold of genome-wide significance ($P = 5 \times 10^{-8}$); the lower horizontal line represents the threshold of $P = 1 \times 10^{-5}$.

Table 3. Loci with Genome-wide Significance for Fissure Integritys

Location			Allele		Left Major >95%			Right Major >95%			Right Minor >95%		
Chr	Marker	Position	Ref	Alt	Alt Fq	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
Non-Hispanic White Patients													
12	rs7310342	43222842	G	A	0.38	1.26 (1.05–1.51)	2.07×10^{-9}	1.27 (1.05–1.53)	1.41×10^{-9}	1.06 (0.94–1.20)	N.S.		
17	rs7209556	48388801	C	T	0.46	0.81 (0.68–0.96)	1.42×10^{-8}	0.93 (0.84–1.03)	N.S.	0.80 (0.63–1.00)	N.S.		
3	rs2173623	55562888	T	C	0.43	0.81 (0.68–0.96)	2.39×10^{-8}	0.84 (0.72–0.99)	7.75×10^{-6}	0.87 (0.73–1.04)	N.S.		
12	rs2407284	43216289	G	A	0.27	0.80 (0.66–0.96)	6.52×10^{-8}	0.83 (0.69–0.98)	7.24×10^{-7}	0.80 (0.63–1.01)	N.S.		
17	rs6504172	48330072	T	C	0.28	0.81 (0.67–0.97)	2.07×10^{-7}	0.94 (0.85–1.04)	N.S.	0.76 (0.59–0.99)	N.S.		
5	rs264866	169635918	C	T	0.69	0.83 (0.70–0.98)	2.24×10^{-6}	0.84 (0.71–0.99)	9.41×10^{-6}	0.74 (0.57–0.97)	4.81×10^{-7}		
14	rs4904145	83871856	A	G	0.31	1.00 (0.98–1.03)	N.S.	0.95 (0.87–1.04)	N.S.	1.46 (1.06–2.00)	7.10×10^{-8}		
African American Patients													
12	rs7310342	43222842	G	A	0.26	1.19 (0.97–1.46)	N.S.	1.16 (0.97–1.40)	N.S.	1.05 (0.94–1.18)	N.S.		
17	rs7209556	48388801	C	T	0.64	1.27 (1.00–1.61)	N.S.	1.03 (0.95–1.12)	N.S.	1.08 (0.94–1.23)	N.S.		
3	rs2173623	55562888	T	C	0.52	1.18 (0.97–1.42)	N.S.	1.16 (0.98–1.38)	N.S.	1.03 (0.95–1.12)	N.S.		
12	rs2407284	43216289	G	A	0.41	0.80 (0.64–1.01)	N.S.	0.79 (0.63–0.98)	N.S.	0.89 (0.75–1.05)	N.S.		
17	rs6504172	48330072	T	C	0.36	0.75 (0.57–0.97)	1.96×10^{-6}	0.98 (0.92–1.05)	N.S.	0.92 (0.79–1.06)	N.S.		
5	rs264866	169635918	C	T	0.57	0.71 (0.53–0.94)	1.14×10^{-8}	0.83 (0.69–1.01)	N.S.	0.74 (0.56–0.96)	1.02×10^{-9}		
14	rs4904145	83871856	A	G	0.50	0.96 (0.87–1.06)	N.S.	0.97 (0.89–1.05)	N.S.	1.43 (1.07–1.92)	2.45×10^{-9}		

Definition of abbreviations: Alt = alternate allele; Alt Fq = alternate allele frequency; Chr = chromosome; CI = confidence interval;

N.S. = $P > 1 \times 10^{-5}$; OR = odds ratio; Ref = reference allele.

Data are presented as median. Kruskal-Wallis test was performed to test for statistically significant differences between the groups.

Subjects with fissure integritys <95% were set as cases, and subjects with fissure integritys $\geq 95\%$ were set as controls.

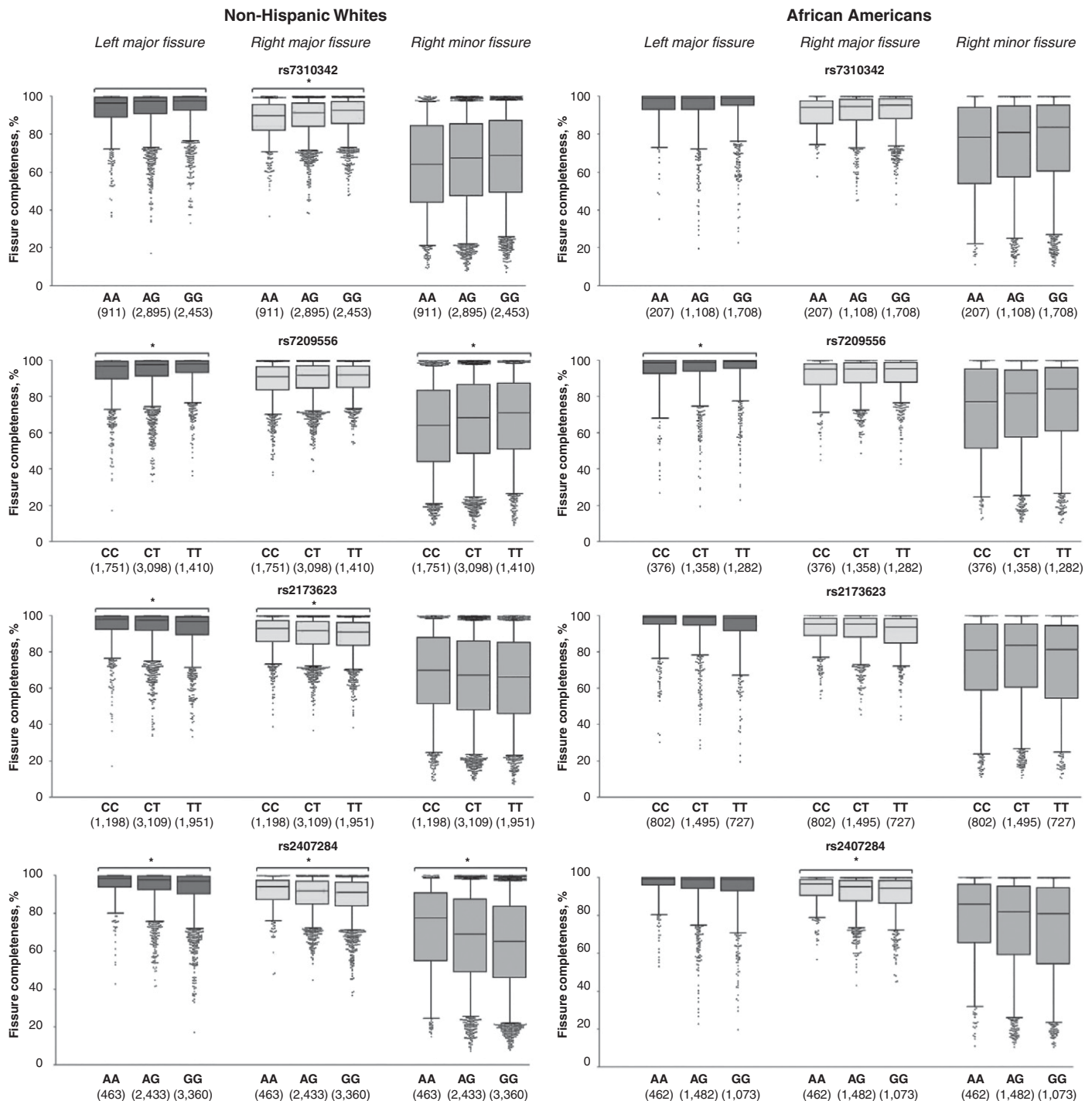


Figure 2. Fissure completeness of the left major, right major, and right minor fissures per genotype of the top SNPs in non-Hispanic White and African American subjects. Boxplots are shown of fissure completeness scores for all non-Hispanic White and African American subjects. SNPs with an association interaction with fissure completeness ($P \leq 1 \times 10^{-5}$) are indicated with an asterisk.

significant in the non-Hispanic white subjects are given in Table 3.

Expression quantitative trait loci analysis. The fissure completeness per genotype of the top SNPs is shown in Figure 2. The most consistent association

we found is located on chromosome 5; rs264866 showed genome-wide significant associations with all fissure integrities in non-Hispanic White subjects and with the left major and right minor fissure completeness in African American

subjects. Rs264866 is located in the intron region within the *SPDL1* gene, which encodes a protein that plays a role in the mitotic spindle formation and mitotic checkpoint signaling (15). Rs264866 is in complete linkage with rs3797713 and

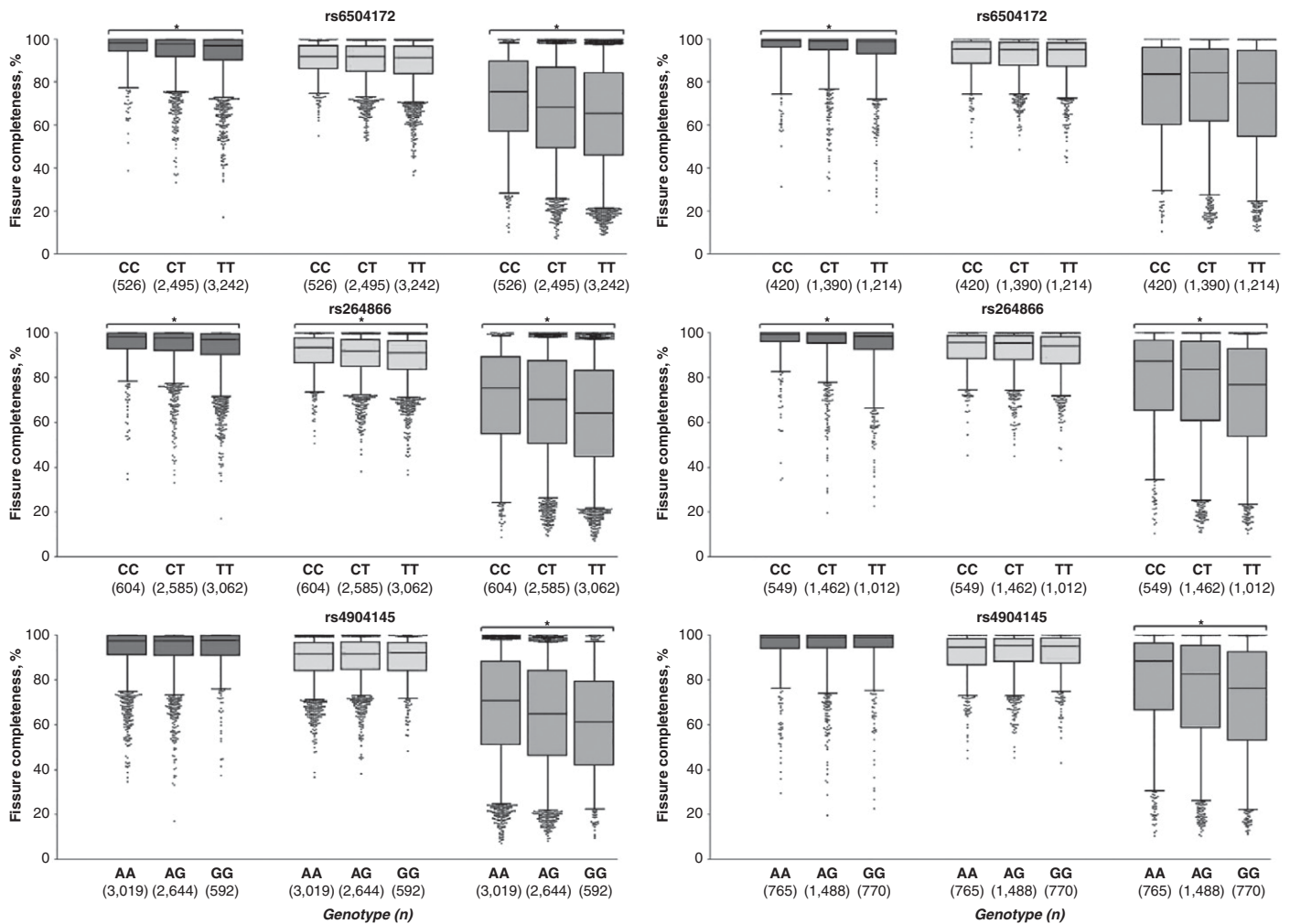


Figure 2. (Continued).

rs3777084, which are both missense coding proteins (Figure E2), although these mutations likely have no effect on gene function according to the Genome Aggregation Database (16, 17).

On chromosome 12, rs2407284 had genome-wide significant associations with all fissure integrities in non-Hispanic White subjects and a significant association with the right major fissure completeness in African American subjects. For this locus, no data on tissue expression are known. However, rs2407284 is located near the *ADAMTS20* gene, which encodes a protease that releases a peptide that may be involved in tissue remodeling (18). On the same chromosome, rs7310342 was genome-wide significantly associated with completeness of the left and right major fissure in non-Hispanic White subjects. This locus is in low LD with rs2407284

($r^2 = 0.15$) but is located near the *ADAMTS20* gene as well.

In both non-Hispanic White and African American subjects, rs4904145 showed genome-wide significant associations for the right minor fissure. For this locus, no data on tissue expression or transcription were known.

Rs2173623 on chromosome 3 had a genome-wide significant association with completeness of the left and right major fissures among non-Hispanic White subjects. Rs2173623 is located within the intron region of the *ERC2* gene but is associated with the expression of *WNT5A* in esophagus mucosa. *ERC2* encodes a protein that functions as a regulator of neurotransmitter release (19). *WNT5A* encodes signaling proteins that play a role in the regulation of development during embryogenesis.

The following two loci on chromosome 17 showed genome-wide significant

associations with completeness of the left and right major fissures: rs6504172 and rs7209556 ($r^2 = 0.46$). Although both loci are located within the intron region of the *SKAP1* gene, rs6504172 is associated with the expression of *HOXB* cluster antisense RNA 1, RP11-6N17.4, and secernin 2 in lung tissue and pyridoxamine 5'-phosphate oxidase in esophagus mucosa. Rs7209556 leads to the expression of *SKAP1* in lung tissue and to the expression of pyridoxamine 5'-phosphate oxidase and *HOXB* cluster antisense RNA 1 in esophagus mucosa. *HOXB* cluster antisense RNA is a regulator of *HOXB* cluster genes. *HOXB* cluster genes play an important role in internal organ development and tissue differentiation (20). *SKAP1* encodes a T-cell adaptor protein that plays a role in the activation of integrins (21). The impact of these SNPs on gene expression is shown in Figure 3.

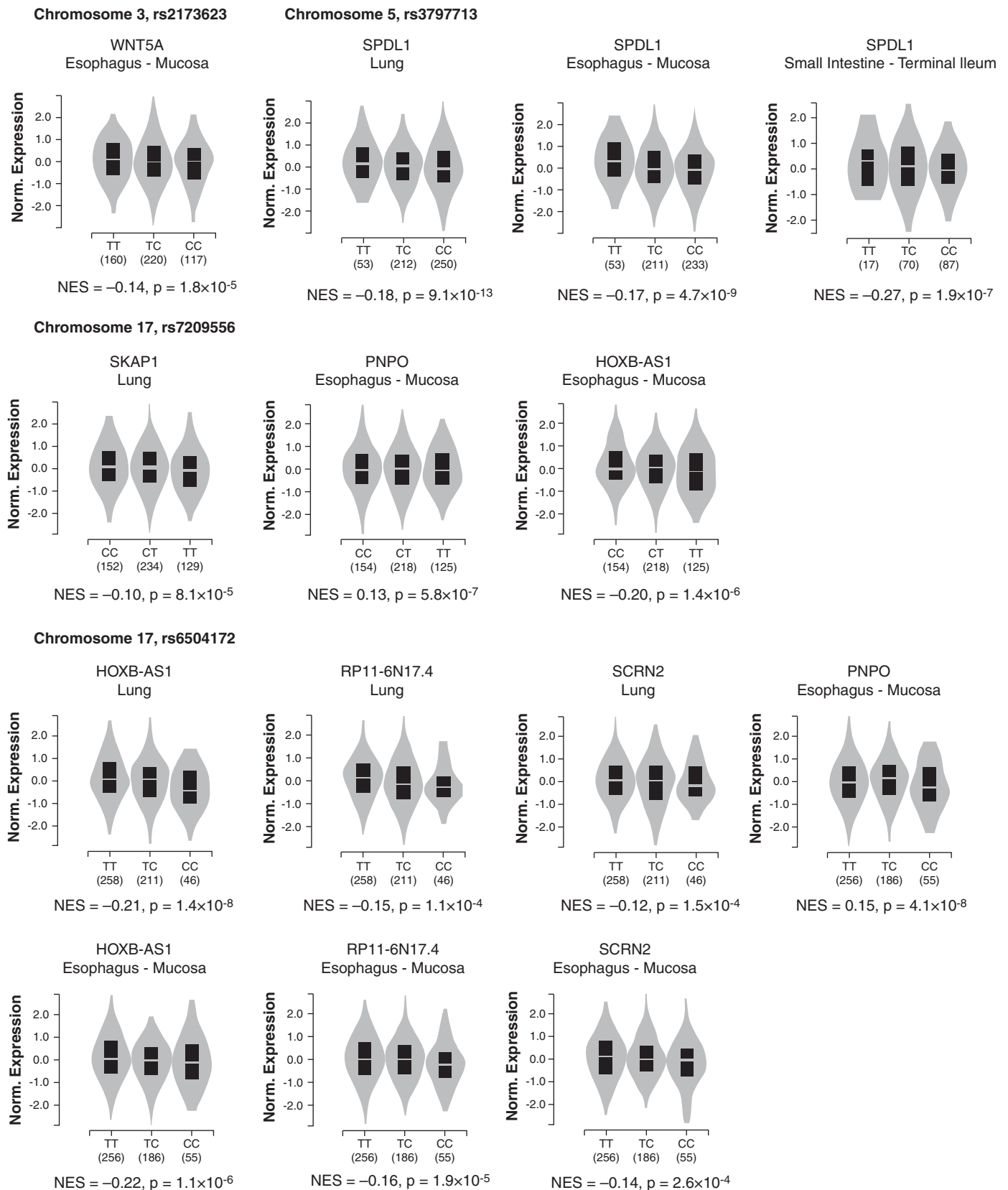


Figure 3. Expression quantitative trait loci violin plots for the top SNPs in lung, esophagus, and intestine tissue. The results are derived from GTExPortal. NES = normalized effect size.

Discussion

Thus far, data about fissure completeness were limited. Several smaller studies have evaluated the prevalence of complete fissures in both healthy individuals and patients with COPD, but data were lacking about the natural history of fissures and other factors that could potentially contribute to fissure completeness (6, 8, 21).

The main conclusions of our study are that fissure completeness is genetically determined and did not change as a result of aging, the number of pack-years of smoking, maternal smoking during the pregnancy, or COPD severity.

The mean change over 5 years in fissure completeness was minimal and in both directions (−0.05% for the left major fissure, +0.15% for the right major fissure, and +0.45% for the right minor fissure) and most likely all within the margin of error of CT scan–based fissure analysis (10).

Although we found small and clinically irrelevant differences in fissure completeness between participants with and without COPD, we did not find differences in fissure completeness between patients with higher and lower emphysematous destruction scores nor between the different COPD GOLD stages or with FEV₁ as a continuous variable. We therefore conclude that presence and severity of COPD severity have no clinically relevant impact on fissure completeness, which is in line with earlier findings (8, 22).

We found evident differences in fissure completeness between non-Hispanic White subjects and African American subjects (97.3% vs. 98.9% for the left major fissure, 91.6% vs. 95.0% for the right major fissure, and 68.4% vs. 81.9% for the right minor fissure), which is supportive to our finding that fissure completeness is genetically

determined. Following genome-wide association analyses, we found multiple genome-wide significant associations with intactness of at least one fissure. Most of these significant associations were preserved in a logistic regression model using a $\geq 95\%$ fissure completeness threshold to define complete fissures. Moreover, the calculated odd ratios revealed consistency in their direction for the different fissures and the different ethnicities.

We found multiple genome-wide significant associations that are known to influence to gene expression, including *WNT5A* and *HOXB* cluster antisense RNA. *WNT5A* encodes signaling proteins that play a role in the regulation of development during embryogenesis (19). *HOXB* cluster antisense RNA is a regulator of *HOXB* cluster genes, which are one of the main genes of organ position and structure during embryogenesis. In lung tissue, *HOXB* gene expression has been demonstrated to effect both lung positioning and tissue differentiation during embryogenesis (20). These findings suggest that lung fissure completeness is already determined during early embryonal development.

Although multiple genome-wide significant associations were found, their impact on phenotyping and gene expression is relatively small (Figures 2 and 3). Therefore, the genetic determination of fissure completeness is likely to be an interplay of multiple genes. However, because fissure completeness is most likely determined during embryogenesis (23, 24), it is possible that the most appropriate tissue to assess for gene expression is pleural tissue. Moreover, it is not known whether the genes that are responsible for fissure development are still expressed after embryogenesis.

Our results and interpretations have limitations. To our knowledge, there is no

other cohort study with available genome-wide association study analyses and fissure completeness data. Therefore, we have no replication cohort. However, we found consistent results across non-Hispanic White and African American subjects and for the different fissures, which shows the robustness of our data. Although we studied associations between age and fissure completeness and compared fissure completeness at baseline and 5-year follow-up, a follow-up with a longer time interval would strengthen our results. Furthermore, as we only analyzed American participants, it is unclear whether our results can be extrapolated to other ethnic groups.

In conclusion, on the basis of our results, we conclude that fissure completeness is genetically determined and not dependent on age, sex, smoking status, or the severity of COPD. These findings imply that fissure completeness is not a feature related to COPD and is not predictable from clinical characteristics. Future studies should focus on modalities to increase fissure completeness rather than preserve of fissure intactness. Currently, several studies are already ongoing that investigate the possibility of closing fissure gaps to prevent collateral ventilation by applying a polymer sealant (NCT04256408 and NCT04559464) (25). In addition, in swine, an experiment was successful to eliminate collateral ventilation by surgical stapling incomplete fissures (26).

Finally, in patients with complete fissures, collateral ventilation is unlikely to occur over time. As emphysematous destruction and lung function change with time, but fissures do not, it might be beneficial to postpone an EBV treatment in patients if the lung function does not completely meet the requirements yet. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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