Analysis of Exonic Elastin Variants in Severe, Early-Onset Chronic Obstructive Pulmonary Disease

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The destruction of elastic fibers has been implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). Emphysema has been described in autosomal dominant cutis laxa, which can be caused by mutations in the elastin gene. Previously, a rare functional mutation in the terminal exon of elastin was found in a case of severe, early-onset COPD. To test the hypothesis that other similar elastin mutations may predispose to COPD, we screened 90 probands from the Boston Early-Onset COPD Study and 90 smoking control subjects from the Normative Aging Study for mutations in elastin exons using high-resolution DNA melt analysis followed by resequencing. Rare nonsynonymous single-nucleotide polymorphisms (SNPs) seen only in cases were examined for segregation with airflow obstruction within pedigrees. Common nonsynonymous SNPs were tested for association with COPD in a family-based analysis of 949 subjects from the Boston Early-Onset COPD Study, and in a case-control analysis in 389 COPD cases from the National Emphysema Treatment Trial and 472 control subjects from the Normative Aging Study. Of 28 elastin variants found, 3 were nonsynonymous SNPs found only in cases. The previously described Gly773Asp mutation was found in another proband. The other two SNPs did not clearly segregate with COPD within families. Two common nonsynonymous SNPs did not demonstrate significant associations in either a family-based or case-control analysis. Exonic SNPs in the elastin gene do not appear to be common risk factors for severe COPD.

Keywords: elastin; chronic obstructive pulmonary disease; emphysema; genetic polymorphism

The development of chronic obstructive pulmonary disease (COPD) is highly variable among those with similar pack-years of smoking (1). Numerous studies suggest that genetic factors may influence COPD susceptibility; however, few findings—with the exception of α_1 -antitrypsin deficiency—have been

CLINICAL RELEVANCE

Exonic elastin variants are unlikely to be common risk factors for severe chronic obstructive pulmonary disease (COPD). Clinicians and researchers should be aware that exonic elastin mutations leading to increased severe COPD susceptibility are rare.

reliably and consistently replicated (2). While much attention in complex disease genetics has recently been focused on identification of common variants in genome-wide association studies, several studies have identified susceptibility variants for complex diseases by studying genes that are mutated in Mendelian diseases that include the phenotype of interest within the syndrome constellation (3, 4). In contrast to the common variants typically found in genome-wide association studies (5), most of these variants have been rare, and only found through DNA resequencing.

Pulmonary emphysema is a known manifestation of congenital cutis laxa, a rare genetic disorder characterized by loose, redundant, and sagging skin. Autosomal dominant, autosomal recessive, and X-linked modes of inheritance have been described. Mutations in the elastin gene (ELN) can cause the autosomal dominant form (MIM: #123700), and emphysema has been described in several autosomal dominant cutis laxa cases (6–9). Elastin represents an excellent candidate gene for COPD for other reasons as well, as destruction of the elastic fiber has been clearly implicated in emphysema pathogenesis (10), and mice lacking elastin (*Eln*^{−/−}) have emphysema-like lesions with dilated distal airspaces (11).

Previously, we had identified a nonsynonymous variant in the terminal exon of elastin (Gly773Asp) in severe, early-onset COPD (12). This mutation was found to segregate with lung function within the pedigree, in that all smokers had evidence of airflow limitation. Transfection studies demonstrated that the mutant elastin protein had compromised ability to undergo normal elastic fiber assembly, reduced interaction with matrix receptors on cells, and altered proteolytic susceptibility. We hypothesized that other protein-altering mutations in elastin could predispose to severe COPD.

Some of the results of these studies have been previously reported in the form of an abstract (13).

MATERIALS AND METHODS

Details of subject recruitment and phenotyping in the Boston Early-Onset COPD Study (EOCOPD), the National Emphysema Treatment Trial (NETT), and the Normative Aging Study (NAS) have been reported previously (14–16). Probands in the Boston Early-Onset COPD Study had physician-diagnosed COPD, FEV₁ less than 40% predicted (16), age less than 53 years, and no severe α_1 -antitrypsin deficiency. NETT participants had physician-diagnosed COPD, FEV₁

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less than or equal to 45% predicted (14), evidence of hyperinflation on pulmonary function testing, and bilateral emphysema on chest computed tomography (CT) scan. None of the NETT subjects included in our study had severe α_1 -antitrypsin deficiency. NAS participants who served as control subjects for this study were healthy men recruited through the Veterans Administration (VA) health facilities of Greater Boston (15) with at least 10 pack-years of cigarette smoking, without airflow obstruction ($FEV_1 > 80\%$ predicted [17] and $FEV_1/FVC > 90\%$ predicted [18]). All NETT and NAS subjects, as well as the EOCOPD participants who underwent variant screening, were white. Participants in the Boston Early-Onset COPD Study and the NETT Genetics Ancillary study gave written informed consent. Anonymized data were used for the NAS participants, as approved by the Partners Healthcare Human Research Committee and the IRB of the VA Hospitals. The appropriate institutional review boards approved all studies.

Elastin amplicons covering exons from the reference sequence (NM_000501.2) were generated from whole genome amplified DNA (REPLI-g; Qiagen, Germantown, MD) and analyzed by high-resolution DNA melt using the 96-well LightScanner (Idaho Technology, Salt Lake City, UT) (19, 20). We have recently demonstrated that high-resolution melting curve analysis has high sensitivity in whole genome amplified DNA (21). At least one representative sample from each melt curve was selected for bidirectional resequencing. Samples were purified using the MinElute PCR Purification Kit (Qiagen). Bidirectional resequencing was performed on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA), and resulting data were analyzed with Phred/Phrap/Consed and Polyphred software (University of Washington, Seattle, WA) (22–24).

Nonsynonymous single-nucleotide polymorphisms (SNPs) were analyzed separately on the basis of their minor allele frequency. For rare nonsynonymous SNPs ($MAF < 5\%$), the number of nonsynonymous SNPs unique to cases was compared with the number unique to control subjects (4) using Fisher's exact test. In addition, the presence of the variant in the corresponding pedigree, as determined by bidirectional sequencing, was examined for segregation with airflow obstruction. For common nonsynonymous SNPs, genotyping in the entire NETT, NAS, and EOCOPD cohorts was performed using the SEQUENOM MassARRAY MALDI-TOF mass spectrometer (Sequenom, San Diego, CA). A case-control analysis was performed in 389 NETT cases and 472 NAS control subjects under an additive genetic model using the Cochran-Armitage Trend Test. In addition, in the NETT cohort, quantitative and qualitative measures of emphysema severity and distribution (*see the online supplement*) were tested under an additive genetic model in multivariate population-based analysis adjusting for age, sex, post-bronchodilator $FEV_1\%$ predicted, and pack-years of cigarette smoking (25). Population stratification in the case-control cohorts has been previously assessed, using a panel of unlinked SNPs; no compelling evidence of overt stratification was found (26). The family-based association analysis was performed in 949 subjects in 127 pedigrees in the EOCOPD cohort, with quantitative (FEV_1 and FEV_1/FVC) and qualitative (COPD defined by GOLD stage ≥ 2) outcomes, under additive genetic models and adjusting for age, sex, height, ever smoking status, and pack-years of smoking.

Family-based analysis was performed using the Pedigree-Based Association Test (PBAT version 3.6) (27); case-control analyses were performed using SAS 9.1 (SAS Institute, Cary, NC). Power calculations for discovery of novel exonic variants in cases were performed using the exact binomial for 180 independent chromosomes. Power calculations for our case-control analysis were performed using Quanto (28), under a log-additive model. Evolutionary conservation at a nucleotide position was assessed using the 17-way phastCons score (29) available through the UCSC genome browser (<http://genome.ucsc.edu>; March 2006 assembly) (30) and Galaxy (<http://g2.bx.psu.edu>) (31). Prediction of functional effect of nonsynonymous SNPs was performed using Polyphen (32).

Additional methods details are provided in the online supplement.

RESULTS

Baseline characteristics of the COPD cases and control subjects used for variation discovery are shown in Table 1. Of our 31 amplicons in 180 total subjects, 95% of DNA melts were successfully completed. A total of 254 sequencing reactions

TABLE 1. BASELINE CHARACTERISTICS OF SUBJECTS INCLUDED IN VARIATION DISCOVERY

Variable	Cases (N = 90)	Control Subjects (N = 90)
Age, yr	47.9 (4.5)	70.8 (6.7)
Male sex, %	26.7%	100%
Pack-years of smoking	40.4 (22.8)	40.0 (25.5)
FEV_1 , % predicted*	21.5 (8.3)	98.0 (12.3)

Listed as mean (SD), except for sex.

* Reference values for EOCOPD from Crapo and coworkers (18), for NAS from O'Connor and coworkers (17).

were performed on these subjects, resulting in identification of a total of 28 SNPs (Table 2). Of these SNPs, eight were present in dbSNP (build 129). A total of seven nonsynonymous SNPs were found. Three nonsynonymous SNPs (Gly109Asp, Gly216Val, and Gly773Asp) were unique to cases; two (Gly348Glu and Ala495Thr) were found only in control subjects. The difference in distribution of the nonsynonymous SNPs was not statistically significant.

Of the three nonsynonymous SNPs present only in cases, the Gly773Asp variant was found in two cases—one was the previously reported proband (12), and the other was newly identified. The remaining two nonsynonymous SNPs present only in cases were examined in early-onset COPD pedigrees. Neither of these demonstrated clear segregation with airflow obstruction within the family. For Gly109Asp, the only other available family member with the mutation was a paternal uncle, who had a normal FEV_1 , though he had only 1 pack-year of smoking. Two siblings who had COPD did not carry the mutation. For Gly216Val, four siblings and a daughter of the proband carried the mutation; all of these subjects had normal lung function, including two with significant (> 40 pack-year) smoking histories.

The two common nonsynonymous SNPs (rs2071307, Gly422Ser and rs17855988, Gly610Arg) were further tested for association with COPD in NETT cases and NAS control subjects. There was no evidence of association with case-control status for either of these variants. In addition, there was no evidence of association of either of these SNPs with any of the four CT emphysema phenotypes, though there was a trend toward association of rs2071307 with total emphysema measured at -950 Hounsfield units ($P = 0.06$). In the family-based analysis in EOCOPD, there was no association of these two SNPs with COPD (GOLD 2 and above) or with FEV_1 .

As additive models lack power when the true genetic model is recessive (33), a secondary analysis, using a recessive genetic model, was performed; none of the associations were significant ($P > 0.05$). *In silico* analysis by PolyPhen was performed; none of the nonsynonymous variants present only in cases was predicted to be possibly or probably damaging. Conservation across species by phastCons score was high (> 0.99) for Gly216Val and Gly773Asp, and moderate for Gly348Glu, while the other nonsynonymous SNPs had scores of approximately 0 (Table 2).

DISCUSSION

The role of elastin in the pathogenesis of emphysema has long been recognized (10). The recognition that rare elastin mutations can lead to emphysema in autosomal dominant cutis laxa led to the discovery of a functional variant (Gly773Asp) in severe, early-onset COPD (12), suggesting that other such mutations in elastin might be responsible for increased COPD susceptibility. In our detailed analysis of elastin exonic variants, we identified another proband with the Gly773Asp mutation, which, in combination with the allele frequency in NETT (12),

TABLE 2. ELASTIN VARIANTS IDENTIFIED BY DNA MELT

	Name	Position	Mutation	Location	rs Number	Cases	Control Subjects	PhastCons Score†
1	c.132+16	73087696	C→A	Intron 2		No	Yes	0.000
2	c.162+34	73088884	A→C	Intron 3		No	Yes	0.000
3	c.195+10	73090015	A→C	Intron 4		Yes	No	0.001
4	c.195+71	73090076	G→A	Intron 4	rs2301995	Yes	Yes	0.000
5	Gly109Asp	73095250	G→A	Exon 7		Yes	No	0.002
6	c.427+8	73095442	C→T	Intron 8		Yes	Yes	0.107
7	c.470-25	73097463	C→T	Intron 9	rs28424575	No	Yes	0.000
8	c.571+25	73098508	C→A	Intron 11		No	Yes	0.001
9	c.572-55	73098907	C→T	Intron 11		No	Yes	0.000
10	c.572-40	73098922	G→T	Intron 11		Yes	No	0.001
11	c.594	73098984	G→A	Exon 12		Yes	No	0.998
12	Gly216Val	73099944	G→T	Exon 13		Yes	No	1.000
13	c.686-76	73100332	G→A	Intron 13		No	Yes	0.001
14	Gly348Glu	73105522	G→A	Exon 18		No	Yes	0.738
15	Gly422Ser	73108650	G→A	Exon 20	rs2071307	Yes	Yes	0.001
16	c.1315+17	73108718	T→C	Intron 20	rs2856728	Yes	Yes	0.000
17	Ala495Thr	73109944	G→A	Exon 23		No	Yes	0.367
18	c.1501+24	73109986	C→T	Intron 23	rs28763986	Yes	Yes	0.000
19	c.1653	73112303	T→A	Exon 24		No	Yes	0.000
20	c.1664-22	73112384	C→T	Intron 24		No	Yes	0.000
21	Gly610Arg	73112761	G→C	Exon 26	rs17855988	Yes	Yes	0.056
22	c.1934-105	73113262	C→T	Intron 26		Yes	No	0.000
23	c.1972+22	73113427	T→C	Intron 27		No	Yes	0.000
24	c.2219-16	73118194	A→G	Intron 31		Yes	Yes	0.000
25	c.2272+5	73118268	G→C	Intron 32		No	Yes	0.318
26	c.2273-34	73118964	C→T	Intron 32	rs3757587	Yes	Yes	0.000
27	c.2318-29	73120894	C→G	Intron 33		No	Yes	0.001
28	Gly773Asp	73120923	G→A	Exon 34	rs41511151	Yes	No	0.994

Variants labeled according to reference in Tassabehji and coworkers (35), corresponding to P15502 (UniProtKB/Swiss-Prot). Position based on the March 2006 reference sequence (NCBI Build 36.1).

† Pointwise PhastCons score (29).

is consistent with a 1 to 2% carrier frequency in severe COPD. However, we were unable to find strong evidence of association of other protein-altering elastin mutations with COPD in our severe COPD cohorts, suggesting that such mutations are unlikely to be common risk factors for severe COPD.

Further evidence supporting the role of elastin in the pathogenesis of COPD may be found by examining other organs. In the skin, facial wrinkles appear to be correlated with COPD (34), and premature aging of the skin is also a characteristic seen in those with elastin mutations in Williams-Beuren Syndrome (MIM # 194050) and cutis laxa. In the vasculature, elastin mutations can cause supravalvular aortic stenosis (35); elastin degradation in the arteries is associated with increased arterial stiffness, and elastolytic activity is increased in arteriosclerosis (10, 36). Recently, a report demonstrated that emphysema severity is associated with arterial stiffness in patients with COPD, independent of airflow limitation and systemic inflammation (37).

Our analysis does not preclude other genetic abnormalities in elastin. A recent report described a tandem repeat, not detected by exonic sequencing, in autosomal dominant cutis laxa with emphysema (9). In addition, the amount of elastin incorporated in the lung, and not just the functional qualities, may be important for normal lung development and susceptibility to emphysema (38). Our study did not attempt to address the possibility of copy number variation or variants in regulatory regions that could be responsible for increased genetic susceptibility to COPD. Our decision to study nonsynonymous SNPs was based on work indicating that these SNPs are more likely to be deleterious (39), as well as the ability to interpret—albeit in a limited way—the implications of single-base pair changes. Functional work is needed, however, to investigate whether these nonsynonymous variants—including the variants present only in the control subjects—affect elastin function.

Our analysis also does not preclude variants in genes relating to elastic fiber assembly or related to elastin processing, at least one of which (*LTBP4*) has been associated with COPD-related phenotypes (40). Alternatively, an autoimmune mechanism of emphysema has been hypothesized, based on antielastin antibodies and T-helper type 1 responses correlating with emphysema severity (41), suggesting that differences in immune function and regulation could make individuals more susceptible to emphysema.

Lack of sufficient power to (1) find novel variants or (2) detect deleterious effects may also explain our negative results. Rare variants may be specific to an ethnic group, and our variant detection was in only white subjects. However, within this ethnic group, by sampling 180 alleles, we had a greater than 80% detection rate for a SNP with a minor allele frequency of 1% (42), and thus are unlikely to have missed any reasonably common coding variants. In addition, our cohort of severe, early-onset COPD (EOCOPD) probands represents an extreme form of the COPD spectrum, and thus may be more likely to be enriched for functional rare genetic variation (4). Our ability to detect a deleterious effect depends on effect size and allele frequency; for our rare variants, relying on segregation of the phenotype within the family requires a very strong effect size (43). On the other hand, for a more common variant (minor allele frequency of 10%), we had greater than or equal to 80% power to detect a moderately strong effect size of 1.6.

A number of recent studies have identified genetic susceptibility variants for complex disease through examination of a gene implicated in a Mendelian disease with shared characteristics (3, 4, 44). These variants are often rare and found only through resequencing, but they demonstrate that a comprehensive survey of a monogenic candidate gene can identify important susceptibility variants. Our analysis of elastin suggests that while a functional protein coding variant (Gly773Asp) is

present in a small subset of severe, early-onset COPD, such variants are not common risk factors for COPD.

Conflict of Interest Statement: B.A.R. received \$7,500 in 2008 from Novartis serving as a course instructor for faculty course on Human Genetics. E.K.S. received an honorarium from Wyeth for a talk on COPD genetics in 2004, and an honorarium from Bayer for a symposium at the ERS Meeting in 2005. He also received an honorarium for a talk on COPD genetics in 2006, and grant support and consulting fees from GlaxoSmithKline for two studies of COPD genetics. He received an additional honorarium for a talk at the Lund Symposium in 2007 and consulting fees in 2008 from AstraZeneca. None of the other authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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