Progress in Chronic Obstructive Pulmonary Disease Genetics

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Familial aggregation of chronic obstructive pulmonary disease (COPD) has been demonstrated, suggesting that genetic factors likely influence the variable development of chronic airflow obstruction in response to smoking. A variety of approaches have been used to identify novel COPD susceptibility genes, including association studies, linkage analysis, and rare variant analysis. Future directions for COPD research include genomewide association studies and animal model genetic studies.

Keywords: association analysis; chronic obstructive pulmonary disease; genetics; linkage analysis

Although cigarette smoking is clearly the major environmental risk factor for the development of chronic obstructive pulmonary disease (COPD), several lines of evidence strongly suggest that genetic factors influence COPD susceptibility as well. First, there is marked variability in the development of chronic airflow obstruction among cigarette smokers (1, 2). Second, studies performed in families and in twins who were not selected for respiratory problems have demonstrated familial aggregation of spirometric measures, suggesting that genetic factors influence variation in pulmonary function (3, 4). Third, studies in families of COPD cases have found higher rates of airflow obstruction in first-degree relatives of patients with COPD compared with control subjects, implicating genetic determinants in COPD (5, 6). Fourth, severe α_1-antitrypsin deficiency is a proven genetic determinant of COPD in a small fraction of COPD cases. Although it does not account for most COPD cases, α_1-antitrypsin deficiency does demonstrate that genetic factors can influence the development of COPD.

This review discusses the approaches that can be used to identify the genetic determinants of a complex disease like COPD, and the progress that has been made in the application of each of these approaches to COPD genetic research.

APPROACHES TO THE IDENTIFICATION OF COPD SUSCEPTIBILITY GENES

The completion of the Human Genome Project, the catalog of common human genetic variation from the HapMap project, and technological advances in single nucleotide polymorphism (SNP) genotyping, expression array analysis, and DNA sequencing have provided an impressive array of tools to use in the search for complex disease susceptibility genes. However, the identification of susceptibility genes for complex diseases like COPD, which are likely influenced by multiple genetic factors, multiple environmental factors, as well as gene-by-gene and gene-by-environment interactions, remains challenging. As listed in Table 1, there are currently seven major approaches that could be used to identify COPD susceptibility genes. The most commonly applied approach is to select a candidate gene from known or suspected COPD pathophysiology, and to test genetic variants within that gene for association to COPD—typically in cases versus controls. Genomewide linkage analysis could also be performed to identify the general locations of COPD susceptibility genes, followed by association analysis with assessment of the following: (*1*) positional candidate genes from COPD pathophysiology, (*2*) positional candidate genes selected from gene expression studies, or (*3*) dense SNP panels across regions of linkage. Rare variant analysis involves systematically searching for all variants in a gene of interest, often followed by functional studies. Although not yet published in COPD, animal model genetic analysis and genomewide association analysis have great potential to assist in COPD susceptibility gene identification.

GENETIC ASSOCIATION ANALYSIS OF PATHOPHYSIOLOGIC CANDIDATE GENES

A reasonably large number of COPD genetic association studies have compared the distribution of variants within candidate genes selected based on COPD pathophysiology in COPD cases versus control subjects (summarized in Reference 7). For example, the risk for COPD among PI MZ heterozygotes at the α_1-antitrypsin (SERPINA1) locus remains controversial. As confirmed in a recent meta-analysis (8), comparisons of COPD cases versus control subjects have typically found an increased risk for COPD among PI MZ subjects, whereas population-based studies of pulmonary function values have usually reported similar FEV_1 values in PI MZ and PI M subjects. The basis for these inconsistencies remains unresolved, but it is possible that a subset of PI MZ subjects are at increased risk for COPD due to additional genetic modifiers. Variants in other candidate genes have also been studied in COPD case-control genetic association studies, and variants in several candidate genes have been significantly associated with COPD in multiple studies, including glutathione S-transferase P1 (GSTP1) (9, 10), glutathione S-transferase M1 (GSTM1) (11, 12), α_1-antichymotrypsin (SERPINA3) (13, 14), surfactant protein B (SFTPB) (7, 15), tumor necrosis factor α (TNFα) (16, 17), microsomal epoxide hydrolase (EPHX1) (18, 19), and vitamin D binding protein (GC) (20, 21). However, the evidence supporting these associations has not been consistent; potential contributors to inconsistent replication include genetic heterogeneity between different study populations, phenotypic differences between study populations (both the criteria used to define cases and control subjects and the relative proportions of different COPD subtypes), population stratification (typically related to differences in genetic ancestry between cases and control subjects), multiple statistical testing (testing many genetic variants and/or many phenotypes and not properly adjusting the

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TABLE 1. APPROACHES TO IDENTIFY NOVEL CHRONIC OBSTRUCTIVE PULMONARY DISEASE SUSCEPTIBILITY GENES

Approach	Status	Reference
Association analysis of pathophysiologic candidate genes	Widely performed, but results have been inconsistent	Hersh and colleagues, 2005 (7)
Linkage analysis followed by association analysis of pathophysiologic candidate genes	Not widely performed; TGFB1 has been reported	Celedon and colleagues, 2004 (27)
Linkage analysis followed by association analysis of gene expression candidate genes	Not widely performed; SERPINE2 has been reported	DeMeo and colleagues, 2006 (30)
Linkage analysis followed by dense fine mapping	Not yet reported	
Rare variant analysis	Not widely performed; elastin variant has been reported	Kelleher and colleagues, 2005 (34)
Animal model quantitative trait locus analysis followed by association analysis in humans	Not yet reported	Shapiro and colleagues, 2004 (40)
Genomewide association analysis	Not yet reported	

level of statistical significance), small sample sizes, and random error (22).

In an effort to determine if some of the previously reported significant genetic associations to COPD susceptibility could be confirmed, Hersh and colleagues assessed 29 variants in 12 candidate genes for COPD in both a family-based study of extended pedigrees ascertained through severe, early-onset COPD probands (949 members of 127 extended pedigrees in the Boston Early-Onset COPD Study) and a case-control study (304 COPD cases from the National Emphysema Treatment Trial [NETT] and 441 smoking control subjects with normal spirometry from the Normative Aging Study [NAS]) (7). A nonsynonymous SNP in SFTPB (Thr131Ile) was associated with airflow obstruction in Boston Early-Onset COPD Study families, but this SNP was only associated with COPD in the case-control study when a gene-by-smoking interaction term was included. Different alleles at a short tandem repeat (STR) marker in heme oxygenase 1 (HMOX1) were associated with airflow obstruction in Boston Early-Onset COPD Study families and with COPD in NETT cases versus NAS control subjects. None of the 27 other genetic variants showed significant evidence for replication across the two studies. Thus, none of the previously associated genetic variants was replicated in a compelling fashion. A variety of potential factors could contribute to inconsistent replication in this study, including phenotypic differences in the Boston Early-Onset COPD Study (qualitative and quantitative airflow obstruction measurements) and NETT–NAS (presence vs. absence of COPD); the unique nature of the early-onset COPD cases, who could have a different genetic basis for COPD; and assessment of only a limited number of SNPs in each candidate gene.

LINKAGE ANALYSIS FOLLOWED BY ASSOCIATION ANALYSIS OF POSITIONAL PATHOPHYSIOLOGIC CANDIDATE GENES

Thus far, the only published genetic linkage analyses in COPD have been performed in the Boston Early-Onset COPD Study. Genomewide linkage analysis was performed in 585 members of 72 extended pedigrees with 377 STR markers (23–26). Both qualitative and quantitative COPD-related phenotypes were analyzed. Post-bronchodilator quantitative spirometric phenotypes, which were available for 560 subjects, provided increased LOD scores in several genomic regions compared with pre-bronchodilator spirometric phenotypes (25). Post-bronchodilator spirometric phenotypes may provide stronger evidence for linkage because day-to-day variability in the level of bronchoconstriction is reduced by uniformly analyzing spirometric values after β_2-agonist treatment.

Genome-scan linkage analysis of quantitative spirometric phenotypes was performed with multipoint variance component linkage analysis [using Sequential Oligogenic Linkage Analysis Routines (SOLAR)] for the quantitative post-bronchodilator phenotypes FEV_1 and FEV_1/FVC, with covariates including pack-years of smoking (24). Post-bronchodilator FEV_1 was linked to multiple regions, most significantly to markers on chromosome 8p (LOD = 3.30) and 1p (LOD = 2.24). Post-bronchodilator FEV_1/FVC was also linked to multiple regions, most significantly to markers on chromosome 2q (LOD = 4.42) and 1p (LOD = 2.52).

After the initial genome-scan linkage analysis, flanking STR markers were genotyped to increase the information available for linkage. In addition, linkage analysis in smokers only, which included a smaller number of subjects but selected for genetic determinants influenced by gene-by-smoking interactions, was performed (26, 27). The combination of flanking STR markers and smoking stratification provided stable to increased evidence for linkage on chromosomes 2q, 12p, and 19q.

Of interest, Malhotra and colleagues also performed genome-scan linkage analysis of spirometric measurements in a sample of 264 individuals from 26 extended pedigrees from the Centre d'Etude du Polymorphisme Humain (CEPH) project who were not selected for any respiratory disease (28). They found suggestive evidence for linkage of FEV_1/FVC to chromosome 2q, in a similar region to the significant FEV_1/FVC linkage reported in the Boston Early-Onset COPD Study.

The initial approach to susceptibility gene identification within these linked regions involved inspecting the regions for candidate genes previously implicated in COPD pathophysiology. TGFB1 (transforming growth factor β1), a reasonable pathophysiologic candidate gene for COPD, which has been associated with COPD in one previously reported case-control genetic association study (29), is located within the region of suggestive linkage to FEV_1 on chromosome 19q. Initially, five TGFB1 SNPs were genotyped in 949 individuals from 127 pedigrees in the Boston Early-Onset COPD Study. Using family-based association analysis with the pedigree-based association test (PBAT) program, one SNP in the promoter region of TGFB1 and two SNPs in the 3′ genomic region of TGFB1 were significantly associated with pre- and post-bronchodilator FEV_1 ($p < 0.05$). Among 304 COPD cases from NETT and 441 smoking control subjects from the NAS, two SNPs in the promoter region of TGFB1 and one SNP in exon 1 of TGFB1 were significantly associated with COPD ($p \leqslant 0.02$ in all cases) (27). Only one promoter SNP replicated across both study designs and a functional variant has not been confirmed in every population—although the exon 10 SNP remains a viable candidate variant. Thus, genetic variants in the TGFB1 gene may influence the pathogenesis of COPD among cigarette smokers.

LINKAGE ANALYSIS FOLLOWED BY ASSOCIATION ANALYSIS OF GENE EXPRESSION CANDIDATES

An alternative strategy for selecting candidate genes within a linkage region is to use gene expression information in relevant tissues; our research group has used this approach to prioritize candidate genes within the chromosome 2q linkage region identified in the Boston Early-Onset COPD Study (30). Assessment of gene expression in murine lungs during development identified three genes syntenic to the chromosome 2q linkage region that had markedly increased expression; one of these genes, SERPINE2 (also known as serine proteinase inhibitor clade E, member 2 or protease nexin 1), also showed significant negative correlations with diffusing capacity of carbon monoxide ($D_{L_{CO}}$) and FEV_1 and positive correlations with total lung capacity (consistent with COPD), in a set of emphysematous and normal lung tissues assayed by Dr. A. Spira and colleagues. Therefore, we investigated the genetic association of a panel of 48 SERPINE2 SNPs with COPD-related phenotypes in 949 individuals from 127 pedigrees in the Boston Early-Onset COPD Study under an additive mode of inheritance. In models that included an interaction term to capture SNP-by-smoking (gene-by-environment) effects in the PBAT program, 18 SNPs in SERPINE2 demonstrated significant association with quantitative and/or qualitative spirometric phenotypes ($p < 0.05$).

We attempted to replicate the family-based associations for SERPINE2 in a case-control analysis using 304 NETT cases and 441 NAS smoking control subjects, as for TGFB1. In the case-control association analysis, seven SERPINE2 SNPs demonstrated significant association with $p < 0.05$, including five of the SNPs that were significantly associated in the family-based analysis. Using a sliding window approach, we analyzed adjacent 6, 4, and 2 SNP haplotypes, and localized the most significant association ($p < 0.01$) to a haplotype of two SNPs (rs6747096 and rs3795879), which are located in exon 3 and at the boundary of exon 3, respectively. None of the significantly associated SERPINE2 SNPs has an obvious potentially functional effect, such as an amino acid sequence change or an exon/intron boundary disruption. Thus, it is likely that the functional variant or variants in or near SERPINE2 are in linkage disequilibrium with the significantly associated SNPs. Although additional studies will be required to determine whether SERPINE2 can be confirmed as a COPD susceptibility gene, the approach of intersecting gene expression and genetic linkage results to select candidate genes for further study does appear to be promising.

RARE VARIANT ANALYSIS

Although linkage and association analysis approaches can be very useful tools in the identification of common genetic determinants of complex diseases, they are typically unable to identify rare genetic determinants of such conditions. The relative importance of common versus rare genetic variants in complex diseases remains unclear; however, it is certainly possible that rare genetic variants could play a major role in the genetic architecture of a complex disease like COPD.

One approach to identify rare genetic determinants of a common disease is to select a rare monogenic syndrome that has the complex disease as a component of its syndrome constellation. In COPD, cutis laxa, a rare genetic syndrome related to improper elastic fiber processing, may serve this role: emphysema in childhood and adolescence often occurs in cutis laxa (31). Several cases of cutis laxa relate to frameshift mutations in the distal part of the elastin gene (32, 33). Therefore, our research group, in collaboration with Drs. Kelleher and Mecham, resequenced the distal six exons of the elastin gene in 116 severe, early-onset COPD cases from the Boston Early-Onset COPD Study (34).

A single early-onset COPD proband with a mutation in the first base of the last exon of elastin was identified. This variant, which led to a substitution of aspartic acid for glycine in a highly conserved amino acid residue of elastin, showed reasonable but not complete concordance with airflow obstruction in the extended pedigree of this early-onset COPD individual. This variant was found in 1.2% of NETT COPD cases, confirming that it is not a private mutation to that one early-onset COPD pedigree. However, this variant was also found in 0.6% of control subjects, so the importance of this variant for COPD susceptibility could not be confirmed by genetic association studies. Functional analysis did confirm that this variant interferes with normal elastic fiber assembly, demonstrating the importance of functional analysis of rare variants identified by genetic approaches.

An alternative approach for identifying rare variants in a complex disease is to identify the total number of rare variants found within a particular candidate gene in case versus control subjects; if rare deleterious mutations can cause the condition of interest, a higher frequency of rare variants will be observed in case subjects (35). This approach has not yet been reported in COPD.

FUTURE DIRECTIONS

Although some progress has been made in COPD genetics, there are many areas that require more investigation. Additional linkage studies will be required to identify genomic regions that likely contain COPD susceptibility genes, and association analysis of densely genotyped SNPs within linkage regions will likely be required to identify novel COPD susceptibility genes within those regions. Novel phenotypes will likely also be essential in the identification of COPD genetic determinants, including biomarkers of the pathophysiologic processes involved in COPD and radiologic assessment to separate the components of emphysema and airway disease.

Animal models of COPD have been essential in identifying key pathways for the pathogenesis of COPD. The use of gene-targeted ("knockout") and transgenic mice has been especially powerful. For example, matrix metalloproteinase-12 (MMP12) knockout mice are protected from emphysema development in a cigarette smoke model (36), and transgenic mice overexpressing the cytokine interleukin 13 (IL-13) in the adult lung developed emphysema, mucus metaplasia, and inflammation, a phenotype similar to human COPD (37).

However, animal model genetics, which attempts to identify the genetic variants responsible for differential susceptibility between murine strains, has not been widely studied in COPD. Such approaches have the potential to lead to progress in COPD genetics. Guerrasimov and colleagues have demonstrated that there are substantial differences among murine strains in emphysema susceptibility in response to cigarette smoke exposure (38). These differences could be exploited in crosses among these strains in classical quantitative trait locus analysis, or by bioinformatic comparisons of SNP maps between susceptible and resistant strains.

There is currently a great deal of excitement within the genetics community regarding the potential of genomewide association analysis (39). Rather than limiting association analysis to a candidate gene or linked region, such genomewide approaches will involve genotyping 300,000 to 500,000 SNPs across the genome to identify genomic regions in linkage disequilibrium with the disease of interest. Multiple levels of replication will likely be required to overcome the multiple statistical testing challenge of genomewide association, but this approach may lead to important new insights into COPD pathogenesis.

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References

1. Burrows B, Knudson RJ, Cline MG, Lebowitz MD. Quantitative relationships between cigarette smoking and ventilatory function. *Am Rev Respir Dis* 1977;115:195–205.
2. Fletcher C, Peto R, Tinker C, Speizer FE. Factors related to the development of airflow obstruction. In: The natural history of chronic bronchitis and emphysema. Oxford, UK: Oxford University Press; 1976. pp. 70–105.
3. Redline S, Tishler PV, Rosner B, Lewitter FI, Vandenburgh M, Weiss ST, Speizer FE. Genotypic and phenotypic similarities in pulmonary function among family members of adult monozygotic and dizygotic twins. *Am J Epidemiol* 1989;129:827–836.
4. Lewitter FI, Tager IB, McGue M, Tishler PV, Speizer FE. Genetic and environmental determinants of level of pulmonary function. *Am J Epidemiol* 1984;120:518–529.
5. Kueppers F, Miller RD, Gordon H, Hepper NG, Offord K. Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. *Am J Med* 1977;63:336–342.
6. Cohen BH. Chronic obstructive pulmonary disease: a challenge in genetic epidemiology. *Am J Epidemiol* 1980;112:274–288.
7. Hersh CP, Demeo DL, Lange C, Litonjua AA, Reilly JJ, Kwiatkowski D, Laird N, Sylvia JS, Sparrow D, Speizer FE, *et al.* Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. *Am J Respir Cell Mol Biol* 2005;33:71–78.
8. Hersh CP, Dahl M, Ly NP, Berkey CS, Nordestgaard BG, Silverman EK. Chronic obstructive pulmonary disease in alpha 1-antitrypsin PI MZ heterozygotes: a meta-analysis. *Thorax* 2004;59:843–849.
9. He JQ, Connett JE, Anthonisen NR, Pare PD, Sandford AJ. Glutathione S-transferase variants and their interaction with smoking on lung function. *Am J Respir Crit Care Med* 2004;170:388–394.
10. Ishii T, Matsuse T, Teramoto S, Matsui H, Miyao M, Hosoi T, Takahashi H, Fukuchi Y, Ouchi Y. Glutathione S-transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease. *Thorax* 1999;54:693–696.
11. Harrison DJ, Cantlay AM, Rae F, Lamb D, Smith CA. Frequency of glutathione S-transferase M1 deletion in smokers with emphysema and lung cancer. *Hum Exp Toxicol* 1997;16:356–360.
12. Baranova H, Perriot J, Albuisson E, Ivaschenko T, Baranov VS, Hemery B, Mouraire P, Riol N, Malet P. Peculiarities of the GSTM1 0/0 genotype in French heavy smokers with various types of chronic bronchitis. *Hum Genet* 1997;99:822–826.
13. Poller W, Faber JP, Weidinger S, Tief K, Scholz S, Fischer M, Olek K, Kirchgesser M, Heidtmann HH. A leucine-to-proline substitution causes a defective alpha 1-antichymotrypsin allele associated with familial obstructive lung disease. *Genomics* 1993;17:740–743.
14. Ishii T, Matsuse T, Teramoto S, Matsui H, Hosoi T, Fukuchi Y, Ouchi Y. Association between alpha-1-antichymotrypsin polymorphism and susceptibility to chronic obstructive pulmonary disease. *Eur J Clin Invest* 2000;30:543–548.
15. Guo X, Lin HM, Lin Z, Montano M, Sansores R, Wang G, DiAngelo S, Pardo A, Selman M, Floros J. Surfactant protein gene A, B, and D marker alleles in chronic obstructive pulmonary disease of a Mexican population. *Eur Respir J* 2001;18:482–490.
16. Huang S-L, Su C-H, Chang S-C. Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. *Am J Respir Crit Care Med* 1997;156: 1436–1439.
17. Sakao S, Tatsumi K, Igari H, Shino Y, Shirasawa H, Kuriyama T. Association of tumor necrosis factor alpha gene promoter polymorphism with the presence of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;163:420–422.
18. Smith CAD, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997;350:630–633.
19. Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Pare PD. Susceptibility genes for rapid decline of lung function in the Lung Health Study. *Am J Respir Crit Care Med* 2001;163:469–473.
20. Horne SL, Cockcroft DW, Dosman JA. Possible protective effect against chronic obstructive airways disease by the GC2 allele. *Hum Hered* 1990;40:173–176.
21. Ito I, Nagai S, Hoshino Y, Muro S, Hirai T, Tsukino M, Mishima M. Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. *Chest* 2004;125:63–70.
22. Silverman EK, Palmer LJ. Case-control association studies for the genetics of complex respiratory diseases. *Am J Respir Cell Mol Biol* 2000;22: 645–648.
23. Silverman EK, Mosley JD, Palmer LJ, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, *et al.* Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes. *Hum Mol Genet* 2002;11:623–632.
24. Silverman EK, Palmer LJ, Mosley JD, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, *et al.* Genomewide linkage analysis of quantitative spirometric phenotypes in severe early-onset chronic obstructive pulmonary disease. *Am J Hum Genet* 2002;70:1229–1239.
25. Palmer LJ, Celedon JC, Chapman HA, Speizer FE, Weiss ST, Silverman EK. Genome-wide linkage analysis of bronchodilator responsiveness and post-bronchodilator spirometric phenotypes in chronic obstructive pulmonary disease. *Hum Mol Genet* 2003;12:1199–1210.
26. DeMeo DL, Celedon JC, Lange C, Reilly JJ, Chapman HA, Sylvia JS, Speizer FE, Weiss ST, Silverman EK. Genome-wide linkage of forced mid-expiratory flow in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004;170:1294–1301.
27. Celedon JC, Lange C, Raby BA, Litonjua AA, Palmer LJ, DeMeo DL, Reilly JJ, Kwiatkowski DJ, Chapman HA, Laird N, *et al.* The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). *Hum Mol Genet* 2004;13: 1649–1656.
28. Malhotra A, Peiffer AP, Ryujin DT, Elsner T, Kanner RE, Leppert MF, Hasstedt SJ. Further evidence for the role of genes on chromosome 2 and chromosome 5 in the inheritance of pulmonary function. *Am J Respir Crit Care Med* 2003;168:556–561.
29. Wu L, Chau J, Young RP, Pokorny V, Mills GD, Hopkins R, McLean L, Black PN. Transforming growth factor-beta1 genotype and susceptibility to chronic obstructive pulmonary disease. *Thorax* 2004;59:126–129.
30. DeMeo D, Mariani TJ, Lange C, Srisuma SS, Litonjua AA, Celedon JC, Lake SL, Reilly JJ, Chapman HA, Mecham BH, *et al.* The SERPINE2 gene is associated with chronic obstructive pulmonary disease. *Am J Hum Genet* 2006;178:253–264.
31. Corbett E, Glaisyer H, Chan C, Madden B, Khaghani A, Yacoub M. Congenital cutis laxa with a dominant inheritance and early onset emphysema. *Thorax* 1994;49:836–837.
32. Zhang MC, He L, Giro M, Yong SL, Tiller GE, Davidson JM. Cutis laxa arising from frameshift mutations in exon 30 of the elastin gene (ELN). *J Biol Chem* 1999;274:981–986.
33. Tassabehji M, Metcalfe K, Hurst J, Ashcroft GS, Kielty C, Wilmot C, Donnai D, Read AP, Jones CJ. An elastin gene mutation producing abnormal tropoelastin and abnormal elastic fibres in a patient with autosomal dominant cutis laxa. *Hum Mol Genet* 1998;7:1021–1028.
34. Kelleher CM, Silverman EK, Broekelmann T, Litonjua AA, Hernandez M, Sylvia JS, Stoler J, Reilly JJ, Chapman HA, Speizer FE, *et al.* A functional mutation in the terminal exon of elastin in severe, early-onset chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2005;33:355–362.
35. Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* 2004;305:869–872.
36. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science* 1997;277:2002–2004.
37. Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ, Chapman HA, Shapiro SD, Elias JA. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 2000;106:1081–1093.
38. Guerassimov A, Hoshino Y, Takubo Y, Turcotte A, Yamamoto M, Ghezzo H, Triantafillopoulos A, Whittaker K, Hoidal JR, Cosio MG. The development of emphysema in cigarette smoke-exposed mice is strain dependent. *Am J Respir Crit Care Med* 2004;170:974–980.
39. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 2005;6:95–108.
40. Shapiro SD, Demeo DL, Silverman EK. Smoke and mirrors: mouse models as a reflection of human chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004;170:929–931.