for the family-based samples. Similarly for *rs498055,* the difference in frequencies between the cases and controls is greater for the unrelated samples than for the linkage families (table 1). Thus, the failure of Bertram et al. to replicate our results does not necessarily indicate that the original association was a false-positive result. We concur with Bertram et al. that the significant association of *rs498055* in four of six samples "may be unlikely to occur by chance"[1(p181)] (in this issue). However, it is possible that our initial study provided an overestimate of the allelic OR for *rs498055*. If this were true and the OR were <1.3, then the study by Bertram et al. would clearly be underpowered. Further replication in well-characterized sample sets is required to assess whether the association is genuine. Ideally, this should be done with large case-control sample sets, to achieve maximum power. For this particular marker, we estimate that 360 cases and 360 controls are needed to achieve 80% power in a replication study (one-sided $\alpha = .05$), assuming an allelic OR of 1.3 and a risk-allele frequency of 45.6%. A meta-analysis of all studies should then be performed to determine whether *rs498055* is associated with late-onset AD. In addition, it might be interesting to test the other reported significant markers from this region in additional sample sets.

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Web Resource

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for AD and *APOE*)

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The *SERPINE2* Gene and Chronic Obstructive Pulmonary Disease

To the Editor: In the February 2006 issue of the *Journal,* DeMeo et al.[1] identified *SERPINE2* as a positional candidate gene for susceptibility to chronic obstructive pulmonary disease (COPD [MIM 606963]) and reported on the association of polymorphic variants of this gene with early-onset disease in a family-based study and with severe disease in a case-control study. With early prior information provided by the authors, we have independently tested for an association of the *SERPINE2* gene with COPD in the largest case-control study reported to date. Our study consists of 1,018 COPD cases and 911 controls prospectively recruited from six European centers. We have provided details about the patients elsewhere.[2] The study population was screened for genotypes at the Medical Research Council (United Kingdom) Gene Services Unit for five SNPs (table 1) in the *SERPINE2* gene. All the SNPs evaluated were reported in the study by DeMeo et al. as associated with disease, with three of the five associated with disease in both the family and case-control study cohorts they assessed.

Table 1. LD between *SERPINE2* SNPs Expressed as r^2

	r^2 for SNP			
SNP	*rs1438831*	*rs920251*	*rs6747096*[a]	*rs3795879*
rs920251	.952 (1.0)	…	…	…
rs6747096[a]	.140	.148	…	…
rs3795879[a]	.140 (.145)	.145 (.145)	.964	…
ss49785625[a]	.020	.023	.054	.055

NOTE.—The r^2 values in parentheses are values obtained from HapMap and compared with our own data in controls. *ss49785625* and *rs6747096* are not in HapMap.

[a] SNP reported by DeMeo et al.[1] to be associated with disease in both family and case-control cohorts.

We examined linkage disequilibrium (LD) between the SNPs (table 1) and evaluated SNP and haplotype associations as described elsewhere.[2] DeMeo et al. did not report specific LD values between SNPs or noncontiguous SNPs contributing to haplotypes. SNPs and genotype frequencies in the study population are shown in table 2. We found no significant deviation from Hardy-Weinberg equilibrium in frequencies for any of the SNPs.

We found no association between any of the *SERPINE2* SNPs and disease, in examining both the allelic and genotype distributions, although our study was well powered to detect associations of the magnitude observed by DeMeo et al., and we would have expected to see these frequency differences with the SNPs that we studied. We also failed to find a relationship between any haplotypes of these SNPs and disease (data not shown). It was of interest that the allele and genotype frequencies observed in our control and patient groups were virtually identical to those observed in control subjects by DeMeo et al., indicating a common distribution of *SERPINE2* variants in the European and North American populations studied. Our previous study has also shown that there is no evidence of population stratification in our sample.

Patients evaluated in both the family-based and case-control studies reported by DeMeo et al. represent a severe subset of the disease spectrum. To determine whether the association with *SERPINE2* noted by DeMeo et al. was related to disease severity, we also analyzed SNP allele and genotype frequencies in the subgroup of our patients with forced expiratory volume at 1 s $\leqslant$45% (n = 388), a group that represents severe disease, but we failed to observe any association.

Our inability to replicate the observations of DeMeo et al. in a more highly powered case-control study may be related to differences in the disease phenotype of the patients studied, because our patients included those with and without emphysema. The possibility, however, that the associations reported by DeMeo et al. represent false-positive results must also be considered. In this respect, it is of note that, in the study by DeMeo et al., different associations were reported for SNPs that are in linkage disequilibrium with one another. For example, *rs3795879* and *rs3795877* have an r^2 value of 1 in HapMap, yet different associations with quantitative spirometric phenotypes were reported for the family study. Similarly, *rs1438831* and *rs920251* are in complete LD, with an r^2 value of 1 in HapMap and 0.95 in our study; however, in DeMeo et al.'s case-control study, the allele and genotype frequencies of *rs920251* were found to be significantly associated with disease (P values of 0.015 and 0.011, respectively), whereas no similar association was observed for *rs1438831*. In both instances, the almost complete linkage between these pairs of SNPs would be expected to result in similar associations.

These results underline the importance of replication in other large independent studies before *SERPINE2* can be unequivocally assigned as a candidate gene for COPD. It is becoming apparent that, to detect modest genetic effects for complex diseases, several independent studies may be required and the data may need to be subjected to meta-analysis. For example, this approach has been used to study Alzheimer disease (see Alzheimer's Association Web site). Similar approaches need to be adopted for COPD. It would also be helpful to have similar criteria adapted for phenotypic selection and to plan prospective studies on this basis.

Table 2. *SERPINE2* Genotype and Allele Frequencies in Controls and COPD Cases

SNP and Sample	Frequency of Allele		Frequency of Genotype		
	C	T	CC	CT	TT
rs1438831:					
COPD case	.66	.34	.43	.45	.12
Control	.66	.34	.43	.46	.11
	A	G	AA	AG	GG
rs920251:					
COPD case	.35	.65	.13	.45	.42
Control	.35	.65	.12	.46	.42
	A	G	AA	AG	GG
rs6747096:					
COPD case	.79	.21	.61	.35	.04
Control	.79	.21	.63	.33	.04
	C	T	CC	CT	TT
rs3795879:					
COPD case	.78	.22	.60	.36	.05
Control	.79	.21	.62	.33	.05
	A	G	AA	AG	GG
ss49785625:					
COPD case	.54	.46	.30	.48	.22
Control	.53	.47	.29	.48	.23

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Web Resources

The URLs for data presented herein are as follows:

Alzheimer's Association, http://www.alz.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for COPD)

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Reply to Chappell et al.

To The Editor: We appreciate the efforts of Chappell and colleagues[1] to replicate our *SERPINE2* findings. We identified *SERPINE2* as a candidate gene for chronic obstructive pulmonary disease (COPD [MIM 606963]) on the basis of our gene-expression results (in both murine and human lung) and our genetic association analysis results in two study populations. Chappell et al. found no evidence for association of five *SERPINE2* SNPs with COPD in their case-control study. As in many complex-disease genetic association studies in general, and in previous COPD genetic association studies in particular,[2] the results are inconsistent.

There are many potential explanations for these inconsistent results, including population stratification, genetic heterogeneity, false-positive and/or false-negative results, differences in the number of SNPs genotyped, and phenotypic heterogeneity.[3] In comparing the results of our two research groups for association analysis of *SERPINE2* SNPs with COPD, phenotypic heterogeneity is of particular importance. COPD is a syndrome composed of both emphysema and airway disease, with variable contributions of these processes in different individuals with COPD. Review of chest CT scans of probands from the Boston Early-Onset COPD Study—the population in which we performed family-based association analysis of COPD-related phenotypes—revealed that the vast majority of these probands had emphysema.[4] Moreover, the COPD cases in our case-control replication population were clearly selected for emphysema as part of the National Emphysema Treatment Trial (NETT). In addition, the Boston Early-Onset COPD Study probands and the NETT cases had very severe COPD. Thus, our test and replication populations were severely affected with COPD, typically with a substantial degree of emphysema. As noted by Chappell et al., our cases represent "a severe subset of the disease spectrum,"[1(p185)] and their cases represent a broader spectrum of severity, including individuals with and without emphysema. The differences in disease severity and emphysema may be important contributors to their nonreplication of our association findings. Also of note, although Chappell et al. genotyped five SNPs in *SERPINE2,* they did not genotype several other SNPs for which we observed replicated associations and LOD score reduction in conditional linkage models.

Chappell et al. also comment about apparently inconsistent association results in our family-based and case-control association analyses among SNPs in tight linkage disequilibrium (LD). Modest differences in the statistical significance of the association analysis results were noted for several SNPs that are in strong but not complete LD in our study populations. There are reasonable explanations for these modest differences. (1) The SNP pairs mentioned are not in complete LD; in our combined case-control cohort, the r^2 values were 0.93 for *rs3795879* and *rs3795877* and 0.91 for *rs1438831* and *rs920251*. (2) Despite excellent genotype completion rates, there were slight differences in missing data between these SNP pairs. Of note, these were not the only *SERPINE2* SNPs significantly associated with COPD-related phenotypes in our study; we observed 18 significantly associated *SERPINE2* SNPs in the family-based association analysis and 7 significantly associated SNPs in the case-control analysis.

We fully agree with Chappell et al. that replication of significant associations is essential—which is why we included in our article the replication of our family-based association analysis results in a separate case-control study. This is also the reason why we provided early access to significantly associated SNPs to the Chappell and Kalsheker group.

Is *SERPINE2* a *confirmed* COPD susceptibility gene? Certainly not. Before the impact of *SERPINE2* on COPD susceptibility is fully known, more genetic association studies as well as functional studies will be needed. However, we contend that *SERPINE2* remains a valid COPD candidate gene. Finally, we agree with Chappell et al. that agreement on phenotypic definitions and collaboration between re-