that the polymorphism at codon 129 has on clinical and pathological expression in Japanese (Miyazono et al. 1992) and Caucasian CJD patients (Salvatore et al. 1994).

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CFTR Gene Variant IVS8-5T in Disseminated Bronchiectasis

To the Editor:

Obstructive pulmonary disease includes asthma, chronic obstructive pulmonary disease (COPD; i.e., pulmonary emphysema and chronic bronchitis), bronchiectasis, and cystic fibrosis (CF) (Nadel 1994). It represents a leading cause of death in developed countries. Both familial clustering of non-CF obstructive pulmonary disease and familial aggregation of impaired lung function have been described (Kueppers 1992). This suggests that genetic factors contribute to non-CF obstructive pulmonary disease, even if it is difficult to determine the relative contribution of environmental factors.

Some clinical commonalities between CF and other obstructive pulmonary disease have been observed (Welsh et al. 1995); therefore the CF gene may be a candidate locus for a role in the etiology or the patho-

genesis of the disease. The CF gene has been identified, and ~500 different mutations have been reported to the Cystic Fibrosis Genetic Analysis Consortium (authors' unpublished observation); it is therefore possible to test the hypothesis that the CF transmembrane regulator (CFTR) gene is involved in obstructive pulmonary disease, by screening for mutations in the gene itself.

In particular, a CFTR gene variant, the intron 8 polypyrimidine-tract length, was investigated. The 5T allele has been shown to influence the phenotype when found in cis with the R117H mutation: the phenotype is pancreatic-sufficient CF, with 5T; congenital bilateral absence of the vas deferens (CBAVD) or asymptomatic, with 7T (Kiesewetter at al. 1993). A high frequency of IVS8-5T has been more recently described in infertile men with either CBAVD (Chillon et al. 1995; Zielenski et al. 1995) or obstructive azoospermia (i.e., the vas deferens and/or epididymis is present but obstructed) (Jarvi et al. 1995). The 5T allele results in less efficient (compared with the more common 7T and 9T variants) splicing of CFTR exon 9, which leads to reduced CFTR expression (Chu et al. 1993). We therefore investigated the possibility that changes in the expression of CFTR may be associated with a spectrum of pulmonary defects possibly related to CF. We here report on the frequency of the IVS8-5T allele and of other CFTR gene mutations in patients affected by obstructive pulmonary diseases.

The study included 16 patients affected by disseminated bronchiectasis of unknown origin, 33 affected by COPD, 36 affected by several nonobstructive pulmonary diseases (Pignatti et al. 1995), 85 atopic asthmatic children (Martinati et al., in press), and 66 normal individuals. None of them had clinical or laboratory manifestations of CF, CBAVD, or obstructive azoospermia. Clinical data on the disseminated bronchiectasis patients have been reported (Pignatti et al. 1995); some clinical data useful to dismiss the possibility that a significant fraction of these patients have an atypical form of CF follow. Sweat tests were negative in 14/16 disseminated bronchiectasis patients. In two of them no sweat test was performed; however, a complete genomic screening for CFTR gene mutations by denaturing gradient gel electrophoresis (DGGE) analysis was negative, thus practically (i.e., with 99% confidence) ruling out the occurrence of CF. Sinus disease was absent, whereas nasal polyposis was observed in two patients (one had genotype 5T and R1066C and 2736 A→G, and one was 7T/7T). None of the subjects investigated had malabsorption. Eight of the 16 disseminated bronchiectasis patients were positive, by culture, for *Pseudomonas* colonization (distribution of genotypes was as follows: one DF508/−, one M1137V/−, one 3667ins4 and 5T, one 5T/5T, one 5T/7T, and three 7T/7T), 7 were negative (distribution of genotypes was as follows: one R1066C and 2736A→G and 5T, one R75Q/−, one G576A-R668C-7T/L997F-9T, one 5T/7T, and three 7T/7T), and 1 was not tested (genotype 7T/7T). Males were 7/16 of disseminated bronchiectasis patients: 4 of them had children; 1 was a boy; 1 was not married, and for 1 no data were available.

The polypyrimidine stretch was analyzed with the nested-PCR method (Chillon et al. 1995), and known genotype controls were coelectrophoresed. The frequency of the IVS8-5T allele was found to be significantly elevated in the disseminated bronchiectasis patients. As detailed in table 1, IVS8-5T was detected in 5/16 (31%) disseminated bronchiectasis patients, in 5/33 (15%) COPD patients, in 5/36 (14%) nonobstructive pulmonary disease patients, in 9/85 (11%) atopic asthmatic patients, and in 4/66 (6%) normal controls. The difference between 5T frequency in the disseminated bronchiectasis group compared with controls is significant ($P = .028$; Fisher's exact test). The frequency of 5T in normal individuals has been similarly reported in other studies to be ~5% (Kiesewetter et al. 1993; Chillon et al. 1995; Jarvi et al. 1995). When CFTR gene alleles are counted, IVS8-5T was present in 6/32 (19%) alleles in disseminated bronchiectasis patients, one of whom was a homozygote, compared with 4/132 (3%) alleles in normal individuals ($P = .008$; Fisher's exact test). The high frequency of IVS8-5T observed in this group of patients indicates that the 5T allele has to be considered as a disease-predisposing mutation in disseminated bronchiectasis of unknown origin. The proportion of disseminated bronchiectasis patients with IVS8-5T (i.e., 31%) was lower than that reported for CBAVD (40% [Chillon et al. 1995] or 51% [Zielenski et al. 1995]) and was very similar to that reported for obstructive azoospermia (29% [Jarvi et al. 1995]). No significant association was found with COPD, nonobstructive pulmonary disease, or asthma, although the frequency of the 5T allele was slightly greater than expected: 15%, 14%, and 11%, respectively, compared with 6% in normal controls.

When the data from the now completed CFTR gene screening of all the 27 gene exons and flanking intronic sequences by the DGGE technique as described (Pignatti et al. 1995) are added, a total of 9/16 (56%) disseminated bronchiectasis patients had an IVS8-5T allele and/or a CFTR gene mutation (table 1). In the remaining 7/16 (44%) patients, in which neither a CFTR mutation nor an IVS8-5T allele has been detected, other genetic or environmental factors could be responsible for the disease. Our results therefore confirm, at the molecular genetic level, the clinical connection between CF and one obstructive pulmonary disease, disseminated bronchiectasis of unknown origin, and extend the broad spectrum of diseases (Pig-

Table 1

CFTR Genotypes of Pulmonary Disease Patients

Clinical Status and CFTR Genotype[a]	No. of Cases	Poly-T Genotype[b]
Disseminated bronchiectasis:		
ΔF508*/–	1	9T/7T
R1066C*, 2736A→G*	1	7T/5T
M1137V*/–	1	7T/7T
3667ins4*/–	1	7T/5T
R75Q*/–	1	7T/7T
G576A–R668C*/L997F*	1	7T/9T
–/–	1	5T/5T
–/–	2	7T/5T
–/–	7	7T/7T
Total	16	
COPD:		
3271+18C→T*/–	1	9T/7T
R117H*/–	1	7T/7T
R668C*/–	1	7T/7T
–/–	1	7T/5T
–/–	4	7T/7T
–/–	4	9T/7T
?/?	3	5T/7T
?/?	1	5T/9T
?/?	13	7T/7T
?/?	3	9T/7T
?/?	1	9T/9T
Total	33	
Nonobstructive pulmonary diseases:		
ΔF508*/–	1	9T/7T
L997F*/–	1	9T/7T
–/–	5	5T/7T
–/–	23	7T/7T
–/–	6	9T/7T
Total	36	
Asthma:		
?/?	1	5T/5T
?/?	8	7T/5T
?/?	59	7T/7T
?/?	17	9T/7T
Total	85	
Normal controls:		
3690A→G/–	1	7T/7T
L997F*/–	1	9T/7T
–/–	1	5T/7T
–/–	10	7T/7T
–/–	1	9T/7T
–/–	1	9T/9T
?/?	3	5T/7T
?/?	34	7T/7T
?/?	14	9T/7T
Total	66	

SOURCE.—Pignatti et al. (1995).

[a] A minus sign (–) denotes absence of CFTR mutation after DGGE analysis of all 27 gene exons and adjoining intronic sequences; and a question mark (?) denotes that no DGGE analysis of the gene was done.

[b] The 5T allele has been underlined, for greater visibility. The 5T phase relative to the mutations is unknown.

natti 1994) that are associated with abnormal CFTR production.

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Affecteds-Only Linkage Methods Are Not a Panacea

To the Editor:

Affected-sib-pair (ASP) methods and their variations, such as the affected-pedigree-member (APM) method (Weeks and Lange 1988), have become preferred methods of analysis. Three reasons are usually given as the advantages of ASP/APM methods over LOD-score analysis (likelihood-maximization techniques). First, the most frequently cited is that ASP/APM analysis is nonparametric, whereas LOD-score analysis requires specification of a genetic model. A related consideration is that penetrance is not a confounding factor in ASP/APM analysis, as it is with LOD scores. Second, ASP/APM methods require the collection of data from only a limited number of family members—and, presumably, from those most motivated to help research in the disease, i.e., those affected. A third reason concerns the ease and intuitive appeal of the theory and calculations. Sib-pair analysis is easily understood and can be done on the "back of the envelope." Except for the more sophisticated ASP methods (Haseman and Elston 1972), one need only count the numbers of sibs sharing no, one, or two alleles in common and apply conventional statistics, to get an answer (although APM methods do require more sophisticated analysis). LOD-score analysis, on the other hand, appears more complex, requiring likelihood maximization, computers and sophisticated programs, and the assumption of a mode of inheritance.

These considerations have led some investigators to believe that only ASP/APM-type methods can provide the ultimate authoritative linkage-analysis results. Some investigators suggest that the LOD-score method has low statistical power, compared with ASP methods (e.g., see Zara et al. 1995). Other investigators, failing to find linkage by using LOD scores, either turn to ASP methods in the hope that these methods will be more successful (e.g., see Byerley et al. 1995; Nair et al. 1995) or suggest that APM methods can be used to confirm a LOD score–derived result (e.g., see Weeks and Harby 1995). Other authors assert that the need to assume a mode of inheritance is a major disadvantage of LOD scores (e.g., see Curtis and Sham 1995; Rice et al. 1995; Rogus and Haines 1995). We mention these examples not to criticize any one group but to document that these ideas need to be discussed.

The purpose of this letter is to clarify some points and raise some questions about ASP/APM methods versus likelihood maximization. We will address several issues that we consider important for linkage analysis: (1) use of parametric versus nonparametric approaches, (2) parameter estimation, and (3) study design and data collection.

1. How nonparametric is ASP/APM analysis?—Knapp et al. (1994) recently showed that one ASP method—one that tests the mean number of alleles shared in common—is statistically *identical* (or "equivalent," in Knapp et al.'s terminology) to a LOD-score analysis assuming a recessive model. In this case "equivalent" means that the rejection regions are identical and that the distributions of the test statistics are the same. In other words, by using this particular method, one is, without being aware of it, applying a test that will give the *same results* as if one performed a LOD-score analysis assuming recessive inheritance. This powerful and counterintuitive result calls into question the advantages of ASP/APM tests. This particular result does not apply to all ASP/APM tests, but it does illustrate that seemingly uncomplicated, "intuitively" appealing tests can harbor surprising characteristics. Blangero (1995) has also pointed out that the label "model-free," which is often applied to ASP methods, is misleading.

The corresponding *dis*advantage of LOD-score analysis is the necessity of specifying a genetic model. For many common diseases, not only is the genetic model unknown, but it may be unknowable by segregation analysis. Thus, the argument is that it is better to use nonparametric techniques, because no model need be specified. But what *is* the danger of assuming an incorrect mode of inheritance? It is not that one will get a false indication of linkage. As long as LOD scores are calculated under a *single* genetic model, the probability of type I error (getting a high LOD-score value by chance alone) is not increased asymptotically, provided that the genetic parameters of either the disease or marker locus are known (Williamson and Amos 1990). The criticism of LOD-score analysis has been that calculating LOD scores under the assumption of an arbitrarily chosen genetic model is an unjustifiable procedure. At least, if we calculate LOD scores under a single genetic model, we are explicitly choosing what that model is, and we know that we are making that choice.