Letters to the Editor

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Cystic Fibrosis Mutations in Heterozygous Newborns with Hypertrypsinemia and Low Sweat Chloride

To the Editor:

Measurement of immunoreactive trypsinogen concentration (IRT) in dried blood spots is the most common technique for cystic fibrosis (CF) neonatal screening. Since a considerable number of newborns show raised IRT levels, the screening specificity is often improved by determining whether infants with hypertrypsinemia have the most common CF mutations: diagnosis is established in neonates carrying two mutations, but a sweat test is required if only one mutation is found, to distinguish between affected individuals—who would have a second, unrecognized mutation—and heterozygotes. Infants with raised IRT, one CF mutation, and normal sweat electrolyte concentrations are usually considered to be carriers only. However, the carrier frequency among nonaffected IRT-positive babies is almost three times higher than that in the general population (Laroche et al. 1991; Castellani et al. 1997); this could be partially explained if some of these babies carry on the other chromosome a mild mutation, associated with scarce symptoms and normal sweat chloride values. A DNA polymorphic sequence of five thymines (TTTTT) in intron 8 of the CF transmembrane conductance regulator (CFTR) gene, which is very common in men with a primarily genital CF form called "congenital bilateral absence of the vas deferens" (CBAVD) (Chillon et al. 1995), has been found to occur more frequently in newborns with raised IRT values than in controls (Castellani et al. 1997; Chin et al. 1997). To look further into the hypothesis that, in at least some babies, raised trypsin levels at birth could be a phenotypic expression of a compound heterozygosity, we investigated a subset of 18 newborns, using the following selection criteria: IRT >99.5 percentile; one identified CFTR mutation among a panel of 15 mutations that are present in 85% of the CF chromosomes in our area; normal sweat chloride, as determined by pilocarpine iontophoresis (mean 16.9 mEq/liter; maximum 32 mEq/liter; minimum 6 mEq/liter). In these neonates and in a control group of 15 healthy subjects (Pignatti et al. 1995), novel and rare mutations of the CFTR gene were sought by use of a complete gene search, with denaturing gradient-gel–electrophoresis analysis of all 27 exons and intronic flanking regions. PCR products that displayed an altered behavior in the gel were sequenced after cloning. Seven CFTR gene mutations were found in eight IRT-positive newborns, compared with one mutation (L997F) in the control group ($P = .02$ by Fisher's exact test; see table 1). Three of these mutations (R117H, Y301C, and E527G) are thought to be disease causing in CF or in CBAVD, since they determine the substitution of an amino acid in evolutionarily conserved residues and therefore are tentatively classified, on the basis of the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) database, as "mutations"; the other four mutations (1716 G/A, 2622+14 G/A, 3041-71 G/C, and 4002 A/G) are not believed to be disease causing in CF and CBAVD, either because they do not determine any amino acid substitutions (in the case of 1716 G/A and 4002 A/G) or because they occur in noncoding regions that, as determined by sequence-analysis software, produce no apparent alteration (in the case of 2622+14 G/A and 3041-71 G/C) and therefore are tentatively classified, on the basis of the CFGAC database, as CF "polymorphisms." Mutations E527G and 2622+14G/A are described here for the first time. It is problematic to understand the clinical significance of the detected "mutations not supposed to cause CF," but, as far as "mutations supposed to cause CF" are concerned, in the 3 (16.6%) of 18 newborns who were compound heterozygous, the raised IRT probably was not a casual finding but was a biochemical sign of an only partially functioning CFTR protein. Whether these neonates ought to be diagnosed as affected with CF is a moot point. In ~2% of patients with CF, there is an "atypical" phenotype, which consists of chronic sinopulmonary disease, pancreatic sufficiency, and either borderline or normal sweat chloride concentrations (Rosenstein et al. 1998). Unfortunately, it is not possible at present to predict the clinical outcome of our newborns, nor is it possible to provide satisfactory genetic counseling for the family. A close clinical follow-up should help in clarifying the extent of the disease in these subjects.

Table 1

Sweat Chloride Concentration and CFTR Genotypes

Case	Sweat Chloride (mEq/liter)	Mutation	
		Allele 1[a]	Allele 2[b]
1	10	R1162X	3041-71G/C,[c] 4002A/G[c]
2	14	ΔF508	
3	30	R1162X	R117H
4	21	ΔF508	E527G
5	8	ΔF508	
6	12	N1303K, 2622+14G/A[d]	
7	6	ΔF508	
8	20	ΔF508	1716G/A[c]
9	16	ΔF508	
10	10	ΔF508	
11	19	R1162X	
12	19	N1303K	
13	12	G542X	1716G/A[c]
14	32	ΔF508	
15	14	ΔF508	
16	26	N1303K	2622+14G/A[c]
17	18	ΔF508	Y301C
18	18	2183AA→G	

[a] First mutation found, assigned to one gene.

[b] Second mutation found, assigned to the gene other than that to which the first mutation found was assigned.

[c] Mutation located in allele 1 or allele 2 (no segregation analysis was possible, since the parents were not available for testing).

[d] Mutation located in the same gene.

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C. Castellani[1], M. G. Benetazzo,[2] A. Bonizzato,[1] P. F. Pignatti,[2] and G. Mastella[1]
[1]*Cystic Fibrosis Centre, Ospedale Civile Maggiore, and* [2]*Institute of Biology and Genetics, University of Verona, Verona*

Electronic-Database Information

URL for data in this article is as follows:

Cystic Fibrosis Genetic Analysis Consortium database, http://www.genet.sickkids.on.ca/cftr/ (for mutations in CF and CBAVD)

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Address for correspondence and reprints: Dr. Carlo Castellani, Cystic Fibrosis Centre, Ospedale Civile Maggiore, Piazzale Stefani 1, 37126 Verona, Italy. E-mail: cfc@linus.univr.it

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