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Cystic Fibrosis Patients Bearing Both the Common Missense Mutation Gly→Asp at Codon 551 and the ΔF508 Mutation Are Clinically Indistinguishable from ΔF508 Homozygotes, Except for Decreased Risk of Meconium Ileus

Ada Hamosh,*,†Terri M. King,‡ Beryl J. Rosenstein,† Mary Corey,§ Henry Levison,‖,†† Peter Durie,** Lap-Chee Tsui,#,** Iain McIntosh,*,‡‡ Marion Keston,‡‡ David J. H. Brock,‡‡ Milan Macek, Jr.,§§,**** Dana Zemková,‖‖ Hana Krásničanová,‖‖ Věra Vávrová,‖‖ Milan Macek, Sr.,## Neil Golder,*** Martin J. Schwarz,*** Maurice Super,*** Eila K. Watson,††† Carolyn Williams,††† Andrew Bush,‡‡‡ Sinead M. O'Mahoney,§§§ Peter Humphries,‖‖‖ Miguel A. DeArce,‖‖‖ André Reis,**** Joachim Bürger,**** Manfred Stuhrmann,****,[1] Jörg Schmidtke,****,[1] Ulrich Wulbrand,†††† Thilo Dörk,†††† Burkhard Tümmler,†††† and Garry R. Cutting*,†

*Center for Medical Genetics, †Department of Pediatrics, School of Medicine, and ‡Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore; Divisions of §Cystic Fibrosis Research and ‖Respiratory Diseases, the Research Institute, and #Department of Genetics, Hospital for Sick Children, and, **Department of Medical Genetics and Medical Biophysics and ††Department of Pediatrics, University of Toronto, Toronto; ‡‡Human Genetics Unit, Western General Hospital, Edinburgh; §§Department of Clinical Biochemistry, First Medical Faculty and ‖‖Second Medical Faculty, Charles University, and ##Center of Medical Genetics, Children's Faculty Hospital, Prague; ***Pediatric Genetics Unit, Royal Manchester Children's Hospital, Manchester; †††Regional DNA Laboratory, St. Mary's Hospital Medical Center, and ‡‡‡Royal Brompton and National Heart Hospital, London; §§§Medical Professorial Unit, University College and St. Vincent's Hospital, and ‖‖‖Department of Genetics, Trinity College, Dublin; ****Institute of Human Genetics, Free University Berlin, Berlin; and ††††Abteilung für Biophysikalische Chemie, Medizinische Hochschule, Hannover

Summary

The glycine-to-aspartic acid missense mutation at codon 551 (G551D), which is within the first nucleotide-binding fold of the cystic fibrosis transmembrane conductance regulator (CFTR), is the third most common cystic fibrosis (CF) mutation, with a worldwide frequency of 3.1% among CF chromosomes. Regions with a high frequency correspond to areas with large populations of Celtic descent. To determine whether G551D confers a different phenotype than does ΔF508, the most common CF mutation, we studied 79 compound heterozygotes for G551D/ΔF508, from nine centers in Europe and North America. Each subject was matched, by age and sex, with a ΔF508 homozygote from the same center. A retrospective cohort analysis was performed on the following outcome parameters: age at diagnosis, sweat chloride, meconium ileus at birth, height, weight, weight for height, FVC, FEV_1, chest X-ray score, pseudomonas colonization, pancreatic sufficiency, and Shwachman clinical score. There was less meconium ileus among the G551D/ΔF508 compound heterozygotes (relative risk 0.33; 95% confidence interval .13–.86), as well as a trend toward later age at diagnosis of pancreatic insufficiency. No statistically significant difference was found between the groups for any other parameter. These results suggest that the CF genotype can be a predictor of pancreatic and intestinal phenotype. Prenatal counseling for the two genotype groups should differ only with respect to probability of meconium ileus. Clinical outcome (after survival of meconium ileus) for G551D/ΔF508 compound heterozygotes and ΔF508 homozygotes is indistinguishable; therefore, prognostic counseling should not differ.

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Address for correspondence and reprints: Ada Hamosh, M.D., Blalock 1008, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21205.

1. Present address: Abteilung für Humangenetik, Medizinische Hochschule, Hannover, Germany.

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Introduction

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder affecting the Caucasian population, with a frequency of 1 in 2,500 live births (Boat et al. 1989). The most common mutation is the deletion of a phenylalanine residue at codon 508 (ΔF508) of the 1,480-amino-acid CF transmembrane conductance regulator (CFTR) protein (B.-S. Kerem et al. 1989). Worldwide, ΔF508 is present on 70% of CF chromosomes, and 50% of CF patients are homozygous for this mutation (Cystic Fibrosis Genetic Analysis Consortium 1990). The first nucleotide-binding fold of CFTR is the site of the ΔF508 mutation and a cluster of other mutations (Cutting et al. 1990; B.-S. Kerem et al. 1990), which suggests that it is functionally significant. Among these is the missense mutation, G551D, a glycine-to-aspartic acid substitution at codon 551 (Cutting et al. 1990), which is the third most common CF mutation, with a worldwide frequency of 3.1% (Cystic Fibrosis Genetic Analysis Consortium, unpublished data). To determine whether G551D confers a different phenotype than does ΔF508, we studied 79 compound heterozygotes for G551D/ΔF508 and 79 age- and sex-matched ΔF508 homozygotes.

Subjects and Methods

Nine centers from Europe and North America contributed patient information for this study (Johns Hopkins Hospital, Baltimore; Hospital for Sick Children, Toronto; Royal Manchester Children's Hospital, Manchester; St. Mary's Hospital, London; Trinity College, Dublin; Medical Faculty, Charles University, Prague; The Free University of Berlin, Berlin; Medizinische Hochschule, Hannover; and Royal Hospital for Sick Children and Western General Hospital, Edinburgh). Each G551D/ΔF508 individual was matched, for age (±6 mo) and sex, with a ΔF508 homozygous individual from the same center. Since age, sex, and genotype are determined at conception, a retrospective cohort analysis of several outcome variables was possible. Patient matching within a center controlled for differences in medical management among centers.

Nutritional parameters including height percentile, weight percentile, and percent ideal body weight predicted for height and sex were requested. Data concerning clinical presentation included age at diagnosis, sweat chloride concentration, and history of meconium ileus. Information on pulmonary status included percent predicted forced vital capacity (FVC), percent predicted forced expiratory volume in 1 s (FEV_1), pseudomonas colonization and age at onset, if applicable, and chest X-ray score, which was rated on either the Brasfield scale (0–25, 25 = best possible) (Brasfield et al. 1979) or the Chrispin-Norman scale (0–38, 0 = best possible) (Chrispin and Norman 1974). Centers were asked to submit whichever chest X-ray score they routinely used. The two sets of chest X-ray scores were sufficiently large to allow separate analysis, obviating the need for data transformation.

Categorical (yes/no) data regarding pancreatic sufficiency were requested along with initial age at diagnosis of pancreatic insufficiency, if applicable. The diagnosis of pancreatic insufficiency was not supported by objective evidence such as 72-h fecal fat determination. This may have resulted in excess reporting of pancreatic insufficiency, on the basis of subjective assessment of clinical improvement with pancreatic enzyme supplementation. This bias should affect both groups equally, since, in the vast majority of cases, the assignment of pancreatic insufficiency was made before the availability of genotype analysis, but it might decrease differences between the groups if pancreatic enzyme supplementation is excessive or is administered without determining status.

The Shwachman-Kulczycki clinical score was recorded for each patient (Shwachman and Kulczycki 1958). This is a subjective assessment of activity, physical examination, growth and nutrition, and the chest X-ray, each of which has a best possible score of 25, for an overall best possible score of 100.

Relative risk and 95% confidence intervals were calculated for each of the categorical variables (Kahn and Sempos 1989, pp 62–64). Means of continuous variables were compared by two-tailed *t* tests (Systat, Inc. 1990). Data regarding the population frequency of the G551D mutation among CF chromosomes were obtained from the Cystic Fibrosis Genetic Analysis Consortium (unpublished results).

In addition to the 79 G551D/ΔF508 compound heterozygotes and the 79 matched ΔF508 homozygotes, data were submitted on 3 G551D homozygotes and on 6 compound heterozygotes with both the G551D mutation and another, unknown CF mutation. Although the small sample size precluded valid statistical analysis, the categorical data for these patients are included.

Results

Country-specific European population frequencies for the G551D mutation among CF chromosomes are shown in figure 1 (Cystic Fibrosis Genetic Analysis Consortium, unpublished data). The population frequency among U.S. Caucasians is 4.2% but varies considerably from center to center (range 1.1%–17.1%) (Cystic Fibrosis Genetic Analysis Consortium, unpublished data). The frequency is 3.0% in the Canadian population but is only 1.2% among French Canadians.

The age, sex, and genotype of the study population are shown, by center, in table 1. There was one case (Baltimore) where sex matching was not possible because a higher priority was placed on age matching. All patients were Caucasian. No further specification of ethnic origin was possible for 94 patients from Baltimore, Toronto, Manchester, and London. All patients from Prague, Dublin, Berlin, and Hannover were Czech, Irish, and German, respectively. Thirteen of the patients from Edinburgh were Scottish; an additional two were Irish.

The comparison between clinical parameters for G551D/ΔF508 compound heterozygotes and those for ΔF508 homozygotes is shown in tables 2 and 3. The mean age of the two groups was similar, confirming appropriate matching. There were no statistically significant differences between the groups for any of the parameters, although a trend toward later age at diagnosis of pancreatic insufficiency is seen among the G551D/ΔF508 compound heterozygotes. Data for each of the categorical outcome variables are shown in table 3. There were no statistically significant differences between the groups with regard to pseudomonas colonization or pancreatic sufficiency (95% confidence interval included unity in each case). The relative risk for meconium ileus for the G551D/ΔF508 compound heterozygotes was only one-third that of the ΔF508 homozygotes ($P < .05$). A difference

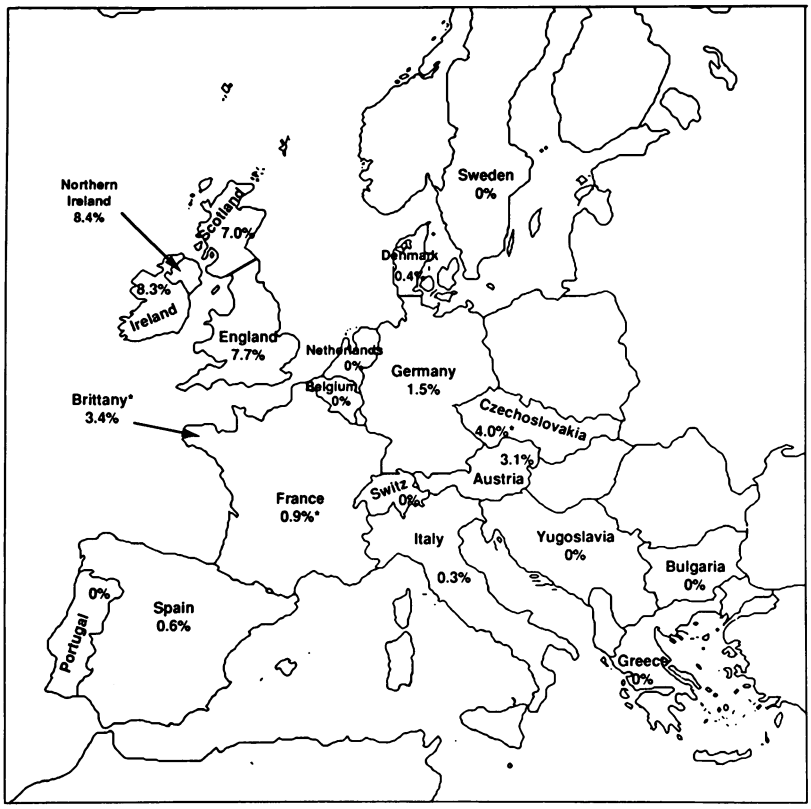

Figure 1 European population distribution of the G551D mutation on CF chromosomes (Cystic Fibrosis Genetic Analysis Consortium, unpublished data). Data for French Brittany (marked by an asterisk) is only for the Celtic population. Data for France (marked by an asterisk) excludes the Brittany data. Data for Czechoslovakia (marked by an asterisk) is only for the ethnic Czech (not Slovak) population.

Table 1

Characteristics of the Population, by Center

	No. of Cases Reported by Center in								
	Baltimore (mean age ± SD [in years] 13.5 ± 5.3)	Manchester (mean age ± SD [in years] 17.2 ± 5.5)	London (mean age ± SD [in years] 12.1 ± 4.8)	Prague (mean age ± SD [in years] 11.5 ± 5.6)	Dublin (mean age ± SD [in years] 19.1 ± 3.1)	Berlin (mean age ± SD [in years] 22.6 ± 3.7)	Hannover (mean age ± SD [in years] 14.2 ± 1.2)	Edinburgh (mean age ± SD [in years] 10.7 ± 7.6)	Toronto (mean age ± SD [in years] 14.8 ± 9.6)
Genotype:									
G551D/ΔF508	6	12	7	12	7	3	2	12	18
ΔF508/ΔF508	6	15	8	16	7	4	2	12	18
G551D/other	0	0	1	4	0	1	0	0	0
G551D/G551D ...	0	3	0	0	0	0	0	0	0
Sex:									
Male	7	12	8	20	4	4	0	10	18
Female	5	18	8	12	10	4	4	14	18

Table 2

Comparison of G551D/ΔF508 with ΔF508/ΔF508, using Continuous Variables

Variable	Value for Characteristic			
	G551D/ΔF508 (*n*) (mean ± SD)	ΔF508/ΔF508 (*n*) (mean ± SD)	*t* Statistic	*P* Value
Age (years)	14.1 ± 7.3 (79)	14.1 ± 7.3 (79)	−.042	.966
Height (%-ile)	45.5 ± 31.8 (73)	47.9 ± 32.9 (74)	−.439	.661
Weight (%-ile)	40.2 ± 30.8 (73)	38.6 ± 32.0 (74)	.316	.752
Ideal weight for height (%-ile)	95.9 ± 14.0 (41)	93.4 ± 12.9 (41)	.846	.400
Age at diagnosis (years)	1.9 ± 2.8 (79)	1.9 ± 3.3 (79)	.019	.985
Sweat chloride (mM)	100.7 ± 15.6 (58)	103.5 ± 21.8 (58)	−.802	.424
FEV_1 (% predicted)	65.1 ± 23.8 (61)	66.7 ± 22.4 (61)	−.396	.693
FVC (% predicted)	75.2 ± 22.3 (61)	76.1 ± 20.9 (61)	−.222	.825
Brasfield chest X-ray score	18.3 ± 3.8 (29)	17.5 ± 3.2 (30)	.889	.378
Chrispin-Norman chest X-ray score	9.8 ± 8.1 (26)	8.7 ± 6.1 (24)	.566	.574
Age at first pseudomonas colonization (years)	9.9 ± 5.9 (52)	8.7 ± 6.1 (57)	1.021	.309
Age at pancreatic insufficiency (years)	2.7 ± 4.0 (51)	1.7 ± 2.8 (51)	1.450	.151
Shwachman-Kulczycki clinical score	71.5 ± 17.1 (62)	71.3 ± 17.2 (64)	.087	.930

Table 3

Comparison of G551D/ΔF508 with ΔF508/ΔF508 using Categorical Variables

Condition	No. of Individuals with		Relative Risk	95% Confidence Interval
	G551D/ΔF508	ΔF508/ΔF508		
Meconium ileus:				
Present	5	15	. . .	. . .
Absent	73	62	.33	.13–.86
Pseudomonas colonization:				
Present	52	61	. . .	. . .
Absent	27	18	.85	.70–1.04
Pancreatic sufficiency:				
Present	1	3	. . .	. . .
Absent	72	70	.33	.04–3.13

in survival may have been missed because only living patients were selected for analysis.

Of the three patients homozygous for G551D and the six patients who are compound heterozygotes for G551D paired with an unspecified (non-ΔF508) mutation, only one (G551D/other) was pancreatic sufficient; two-thirds of each group had been colonized with pseudomonas, and none presented with meconium ileus. Although the numbers are too small to permit statistical analysis, they support the observation of less meconium ileus seen among the G551D/ΔF508 compound heterozygotes.

Discussion

The high frequency of G551D among the Czechs and Austrians, the Celts of Brittany, and the Celtic population of England, Scotland, and Ireland is consistent with a Celtic origin for the G551D mutation, as proposed by Macek et al. (1991). Comparison of phenotypic effects of different CF genotypes should allow further determination of functionally significant regions of the CFTR protein, with implications for prenatal and prognostic counseling. To date, studies of genotype/phenotype correlations in CF have examined single-clinic populations (E. Kerem et al. 1990; Santis et al. 1990; Campbell et al. 1991; Johansen et al. 1991). Because of limited numbers of patients, ΔF508 homozygotes have been compared with individuals heterozygous for ΔF508 and other CF mutations or with CF patients lacking the ΔF508 mutation. Since more than 100 different CF mutations have been described (Cystic Fibrosis Genetic Analysis Consortium, unpublished data), this grouping of dissimilar mutations has not allowed clear distinction of genotype-specific effects on clinical outcome. The present study, a retrospective cohort analysis of 14 outcome parameters, revealed a statistically significant reduction in the risk of meconium ileus among G551D/ΔF508 compound heterozygotes relative to ΔF508 homozygotes.

Meconium ileus, a form of neonatal intestinal obstruction caused by inspissated meconium, occurs in 5%–15% of CF patients (di Sant'Agnese and Hubbard 1984; Olsen et al. 1987; Boat et al. 1989). The condition is thought to be caused by a combination of severe pancreatic insufficiency leading to inadequate enzymatic digestion of intestinal contents and intrinsic intestinal dysfunction, which results in less hydrated meconium. The prevalence of meconium ileus in this group of living CF patients was 6.4% among the G551D/ΔF508 compound heterozygotes and 19.5% among the ΔF508 homozygotes. While the difference could be due to increased mortality from meconium ileus in G551D/ΔF508 patients, this seems unlikely in light of the other similarities between the groups. Mortality from meconium ileus is currently 5%–15% (Olsen et al. 1987; E. Kerem et al. 1989). Long-term (>1 year) survival of CF patients with meconium ileus is not different from that in CF patients without meconium ileus. Prenatal counseling for the two groups should differ only with respect to meconium ileus risk; prognostic counseling should not differ.

Kerem and co-workers (E. Kerem et al. 1990) found statistically significant differences, in age at diagnosis and status of pancreatic function, between ΔF508 homozygotes and compound heterozygotes for ΔF508 and other, unspecified mutations. Our results indicate that G551D/ΔF508 compound heterozygotes have less meconium ileus and may have later age at onset of pancreatic insufficiency than ΔF508 homozygotes, which supports an earlier observation of increased frequency of meconium ileus among ΔF508 homozygotes relative to ΔF508/other or non-ΔF508 CF patients (European Working Group on CF Genetics 1990). These findings confirm that the CF genotype can be a predictor of pancreatic disease. The tremendous range of pulmonary function values—including many in the normal range—in each genotype group suggests that these CF genotypes are not predictive of pulmonary phenotype. The low frequency of the remaining CF mutations necessitates collaborative studies such as the one presented here, to reliably assess the effect of genotype on phenotype.

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References

Boat TF, Welsh MJ, Beaudet AL (1989) Cystic fibrosis. In: Scriver CL, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 2649–2680

Brasfield D, Hicks G, Soong S, Tiller RE (1979) The chest

roentgenogram in cystic fibrosis: a new scoring system. Pediatrics 63:24–29

Campbell PW III, Phillips JA III, Krishnamani MRS, Maness KJ, Hazinski TA (1991) Cystic fibrosis: relationship between clinical status and F508 mutation. J Pediatr 118: 239–241

Chrispin AR, Norman AP (1974) The systematic evaluation of the chest radiograph in cystic fibrosis. Pediatr Radiol 2:101–106

Cutting GR, Kasch LM, Rosenstein BJ, Zielenski J, Tsui L-C, Antonarakis SE, Kazazian HH Jr (1990) A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein. Nature 346:366–369

Cystic Fibrosis Genetic Analysis Consortium (1990) Worldwide survey of the ΔF508 mutation—report from the Cystic Fibrosis Genetic Analysis Consortium. Am J Hum Genet 47:354–359

di Sant'Agnese PA, Hubbard VS (1984) The gastrointestinal tract. In: Taussig LM (ed) Cystic fibrosis. Thieme Stratton, New York, pp 212–229

European Working Group on CF Genetics (1990) Gradient of distribution in Europe of the major CF mutation and of its associated haplotype. Hum Genet 85:436–441

Johansen HK, Nir M, Hoiby N, Koch C, Schwartz M (1991) Severity of cystic fibrosis in patients homozygous and heterozygous for the ΔF508 mutation. Lancet 337:631–634

Kahn HA, Sempos CT (1989) Statistical methods in epidemiology. Oxford University Press, New York

Kerem B-S, Rommens JM, Buchanan JA, Markiewicz DA, Cox TK, Chakravarti A, Buchwald M, et al (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073–1080

Kerem B-S, Zielenski J, Markiewicz D, Bozon D, Gazit E, Yahav J, Kennedy D, et al (1990) Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. Proc Natl Acad Sci USA 87:8447–8451

Kerem E, Corey M, Kerem B-S, Durie P, Tsui L-C, Levison H (1989) Clinical and genetic comparisons of patients with cystic fibrosis, with or without meconium ileus. J Pediatr 114:767–773

Kerem E, Corey M, Kerem B-S, Rommens J, Markiewicz D, Levison H, Tsui L-C, et al (1990) The relation between genotype and phenotype in cystic fibrosis: analysis of the most common mutation (ΔF508). N Engl J Med 323: 1517–1522

Macek M Jr, Macek M, Serre J-L, Vavrova V, Burger J, Reis A, Schmidtke J, et al (1991) Population study of CFTR gene mutations in Bohemia and Moravia: hypothesis on the historical spread of G551D and ΔF508 in Europe. Am J Hum Genet 49 [Suppl]: A474

Olsen MM, Gauderer MW, Girz MK, Izant RJ Jr (1987) Surgery in patients with cystic fibrosis. J Pediatr Surg 22: 613–618

Santis G, Osborne L, Knight RA, Hodson ME (1990) Independent genetic determinants of pancreatic and pulmonary status in cystic fibrosis. Lancet 336:1081–1084

Shwachman H, Kulczycki LL (1958) Long-term study of one hundred five patients with cystic fibrosis. Am J Dis Child 96:6–15

Systat, Inc. (1990) Systat 5.0 for the Macintosh. Systat, Evanston, IL