Original articles

Genotype-phenotype relationship in 12 patients carrying cystic fibrosis mutation R334W

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Abstract

We present a phenotype-genotype correlation analysis in 12 patients with cystic fibrosis (CF) carrying the mutation R334W in the CFTR gene. The clinical data obtained for this group were compared with the clinical data of $\Delta F508/\Delta F508$ patients. Current age and age at diagnosis were significantly higher in the R334W mutation group ($p=0.028$ and $p=0.0001$). We found a lower rate of *Pseudomonas aeruginosa* colonisation in patients carrying the R334W mutation, although the difference was not found to be statistically significant. However, we found a statistically significant higher age of onset of *Pseudomonas aeruginosa* colonisation ($p=0.0036$) in the group of patients with the R334W mutation. Thirty three percent of R334W patients were pancreatic insufficient, significantly lower than the $\Delta F508/\Delta F508$ patients ($p=0.004$). We also found that the weight expressed as a percentage of ideal weight for height was significantly higher in patients with the R334W mutation ($p=0.0028$).

(J Med Genet 1997;34:89-91)

Keywords: cystic fibrosis; R334W mutation; genotype-phenotype correlation.

Cystic fibrosis (CF) is an exocrine disorder predominantly affecting the pancreas, gut, lung, sweat ducts, and vas deferens. The cloning of the gene responsible for CF led to the identification of its product, the cystic fibrosis transmembrane conductance regulator (CFTR), and has led to the determination of the molecular defects of the gene¹ and the correlation of gene defects and clinical expression of CF.²

The CFTR, a protein composed of 1480 amino acids, belongs to a family of P glucoprotein membrane transport proteins, and acts as a phosphorylation regulated chloride channel.³⁻⁵ Since the CFTR gene was cloned, more than 400 mutations have been identified, the commonest being $\Delta F508$. Mutation $\Delta F508$ has a significant variation in prevalence among populations.⁶ In addition to $\Delta F508$, different

nonsense, missense, splicing, and frameshift mutations have been identified (Cystic Fibrosis Analysis Consortium, CFGAC, personal communication).

Information about the clinical characteristics of specific genotypes may have significant implications for prognosis and patient care and is also important for genetic counselling.

We present an analysis of the phenotype-genotype correlation in 12 patients with the mutation R334W, an arginine to tryptophan change at codon 334 of the CFTR gene.⁷

Materials and methods

PATIENTS

A total of 102 unrelated families originating from the south of Spain, with at least one affected person with a confirmed diagnosis of CF, were included in this study. The diagnosis of CF was made on the basis of a chloride concentration >60 mmol/l. The clinical data collected were current age, sex, age at diagnosis, sweat test value, weight, height, weight expressed as a percentage of ideal weight for height, forced expiratory volume in one second (FEV1), forced vital capacity (FVC), Chrispin-Norman score, presence of meconium ileus, presence of dehydration, *Pseudomonas aeruginosa* (PA) colonisation, age at PA colonisation, Schwachman score, and presence of other clinical complications.

METHODS

Genomic DNA was isolated from peripheral blood lymphocytes according to standard protocols. CFTR gene mutations were investigated as described elsewhere.^{7,8} Microsatellite haplotypes within the CFTR gene were studied in the patients and their parents as previously described.⁹ Sputum was considered positive if colonised by PA for more than six months/year. The sputum was washed and then homogenised in 2% N-acetylcysteine at pH 7.2. Serial tenfold dilutions were prepared and inoculated onto blood agar, bile salt agar, phenylethyl agar (PEA), PEA with 0.5 $\mu\text{g/ml}$ of penicillin, PEA with 4 $\mu\text{g/ml}$ methicillin, and Levinthal's agar. Organisms were identified and enumerated by standard bacteriological methods. Patients were diagnosed as either pancreatic sufficient

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Received 8 July 1996
Revised version accepted for publication
4 September 1996

Table 1 Clinical characteristics of 12 patients with R334W mutation

	Patients											
	1	2	3	4	5	6	7	8	9	10	11	12
Genotype	R334W/ ΔF508	R334W/ ΔF508	R334W/ ΔF508	R334W/ ΔF508	R334W/ ΔF508	R334W/ ΔF508	R334W/ G542X	R334W/ R1162X	R334W/ del84	R334W/ G542X	R334W/ G542X	R334W/ G542X
Sex	F	M	F	M	F	M	F	F	F	F	F	F
Current age	3 y 7 mth	27 y	2 y 5 mth	25 y	17 y	59 y	10 y 9 mth	23 y	3 y 3 mth	27 y	25 y	23 y
Age at diagnosis	5 mth	15 y	5 mth	20 y	2 y	49 y	5 y 6 mth	15 y	15 mth	25 y	24 y	21 y
Age at first clinical symptoms	3 mth	1 y 4 mth	5 mth	4 y	3 mth	35 y	8 y	1 y	4 mth	5 y	17 y	—
First clinical symptoms	Pulmonary/ pancreatic	Pulmonary	Dehydration	Dehydration	Pulmonary	Pancreatic	Pulmonary	Dehydration	Dehydration	Pulmonary	Pulmonary	Asymptomatic
Sweat Cl ⁻ mEq/l	90	90	80	100	105	110	110	100	85	100	100	100
Weight* (%)	100	100	100	115	90	89	95	76	89	116	115	121
Chrispin-Norman	4	20	2	—	—	—	—	23	—	—	—	—
Schwachman-Kulczycki	90	65	100	95	65	70	95	50	95	85	90	95
FEV1 % predicted	—	24	—	82	41	44	—	27	—	74	85	91
FVC % predicted	—	43	—	91	51	60	—	43	—	82	84	97
Lung colonisation (LC)	No	Yes	No	No	Yes	Yes	No	Yes	No	No	No	No
Age of onset of LC	—	15 y	—	—	17 y	58 y	—	14 y	—	—	—	—
Meconium ileus	No	No	No	No	Yes	No	No	No	No	No	No	No
Dehydration	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No
Pancreatic insufficiency (PI)	Yes	Yes	No	No	No	Yes	No	No	No	No	Yes	No
Age of onset of PI	3 mth	26 y	—	—	—	?	—	—	—	—	24 y	—
Sterility	—	Yes	—	Yes	—	Yes	—	—	—	No	No	No

* Weight expressed as a percentage of ideal weight for height.

or insufficient on the basis of fecal fat balance studies according to the method of Van der Kamer *et al.*¹⁰

STATISTICAL ANALYSIS
The difference in mean values between the groups was analysed by Student’s unpaired *t* test (two tailed) or Newman-Keules *t* test (for unequal variances) for continuous variables with normal distribution, and by the Mann-Whitney test for continuous variables with non-normal distribution. Categorical variables were analysed by the chi-square test or Fisher’s exact test. All statistical analyses were performed with BMPD Dynamic statistical software.¹¹

Results
The 12 patients with R334W originated from Andalusia (south of Spain). Six patients were compound heterozygotes for R334W and ΔF508, four for R334W and G542X, one for R334W and 1949del84, and one for R334W and R1162X. All the R334W chromosomes showed the intragenic microsatellite haplotype 17-46-13 (IVS8CA-IVS17BTA-IVS17BCA).

Detailed clinical data for these 12 patients are shown in table 1. The clinical data for these 12 patients were compared with those of 28 ΔF508 homozygous patients (table 2). Patients 10, 11, and 12 are sisters.

No significant differences were found when comparing sweat test values, presence of meconium ileus, dehydration, lung function, and Shwachman score. The current age and age at diagnosis were significantly higher in the R334W mutation group (*p*=0.028 and *p*=0.0001, respectively). A lower rate of PA colonisation was found in the group of patients with the R334W mutation, although the difference was not statistically significant. However, a statistically significant higher age of onset of PA colonisation (*p*=0.0036) was found in the group of patients with the R334W mutation. Thirty three percent of R334W patients were pancreatic insufficient, a figure significantly lower than the ΔF508/ΔF508 patients (*p*=0.004). We also found that the weight expressed as a percentage of ideal weight for height was significantly higher in patients with the R334W mutation (*p*=0.0028).

Table 2 Clinical characteristics of 12 patients with R334W mutation, compared to ΔF508/ΔF508 patients

	Genotype		
	ΔF508/ΔF508 (n=28)	R334W (n=12)	
	Mean (SD)		
Current age	9.6 (6.0)	20.4 (15.6)	<i>p</i> = 0.028
Age at diagnosis	2.4 (3.3)	14.7 (14.6)	<i>p</i> = 0.0001
Sweat test	99.4 (6.4)	95.8 (9.0)	NS
FEV1 % predicted	54.1 (21.6)	58.5 (27.4)	NS
FVC % predicted	59.9 (17.3)	68.9 (22.1)	NS
Weight*	0.9 (0.06)	1.0 (0.1)	<i>p</i> = 0.0028
Chrispin-Norman	7.9 (4.3)	9.2 (5.1)	NS
Age of onset of LC	7.1 (4.8)	26.0 (21.4)	<i>p</i> = 0.0036
Age of onset of PI	0.3 (1.2)	24.8 (20.0)	
Schwachman-Kulczycki	71.2 (23.2)	82.9 (16.2)	NS
	No positive/No studied (%)		
Meconium ileus	3/28 (10.7)	1/12 (8.3)	NS
Dehydration	9/28 (32.1)	7/12 (58.3)	NS
Pancreatic insufficiency	28/28 (100.0)	4/12 (33.3)	<i>p</i> = 0.004
Lung colonisation	17/28 (60.7)	4/12 (33.3)	NS

* Weight expressed as a percentage of ideal weight for height.

Discussion

The R334W mutation of the CFTR gene is a missense mutation, first identified in 1991,⁷ that corresponds to the substitution of an arginine by a tryptophan at position 334 in exon 7 of the CFTR gene. R334W is a class IV (defective conductance) mutation.¹² Class IV mutations (for example, R117H, R334W, and R347P) occur in the membrane spanning domains and are predicted to cause a mild CF phenotype.¹²

The R334W mutation has been described in a number of patients in different populations, although the worldwide prevalence is less than 0.1%. In our CF patients, R334W has a prevalence of 4.9% (10/204 CF chromosomes). The association of intragenic microsatellite haplotypes 17-46-13 (IVS8CA-IVS17BTA-IVS17BCA) with the different R334W chromosomes points to a single origin in the Spanish families described previously.¹³

Our results provide new data to support that patients with the R334W mutation suffer less severe expression of the disease and that the disease can be diagnosed later, as reported by Estivill *et al.*¹⁴ The proportion of pancreatic insufficient patients with the R334W mutation in our study is lower (33%) when compared to the proportion of pancreatic insufficient patients in the paper by Estivill *et al.*¹⁴ (60%). In our study the number of pancreatic sufficient patients with R334W varied according to the age at which pancreatic status was assessed, the later the age the smaller the number of pancreatic sufficient patients, with most patients being pancreatic sufficient at 20 years of age. It may suggest that pancreatic function in this group of patients declines with age. This fact is supported by the pancreatic status found in patients 10, 11, and 12, belonging to the same family. While patients 10 and 12 are at the lower limit of pancreatic sufficiency, patient 11 has minimal pancreatic insufficiency.

Chronic infection with PA is the main cause of morbidity and mortality in CF patients. We did not find statistically significant differences with regard to lung colonisation by this pathogenic bacteria, although a lower rate of colonisation was found in R334W patients. A slightly higher proportion of lung colonisation in R334W patients compared to $\Delta F508/\Delta F508$ patients has previously been reported,¹⁴ although the lung colonisation was referred to as bacterial pathogens in that report. On the other

hand, a significant higher age at colonisation was found in our group of patients suggesting that R334W may be a lower risk allele than $\Delta F508$ for the acquisition of PA.

Interfamilial and intrafamilial clinical diversity for pancreatic insufficiency and the variation in pulmonary disease indicates that other factors, either genetic or environmental, must affect the severity and progression of the disease. In fact, it has been recently shown that the severity of CF in mice can be modified by at least one unlinked genetic locus.¹⁵ Environmental factors, such as age at diagnosis, a previous affected sib, medical care, or degree of compliance with treatment regimens, may further contribute to the variation of the clinical spectrum of the disease in CF patients.

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