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Genotype–phenotype correlations in cystic fibrosis: clinical severity of mutation S549R(T→G)

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Genotype–phenotype correlations in cystic fibrosis: clinical severity of mutation S549R(T→G). P.M. Frossard, J. Hertecant, Y. Bossaert, K.P. Dawson. ©ERS Journals Ltd 1999.

ABSTRACT: With a view to assessing genotype-to-phenotype correlations in cystic fibrosis (CF), the clinical presentation of CF children from the United Arab Emirates (UAE) who were homozygous for cystic fibrosis transmembrane conductance regulator (CFTR) mutation S549R(T→G) was investigated. This mutation is localized in intron 11 (nucleotide binding domain 1 of the CFTR protein) and had so far been described as a private mutation only.

The associations between the R549/R549 genotype and 20 outcome variables, including age at diagnosis, sweat chloride concentrations, growth percentiles, meconium ileus, pancreatic sufficiency, pulmonary disease, associated complications and micro-organism colonization were examined in a group of 15 CF children (9 females and 6 males).

Mean current age and age at diagnosis were both low (5.4±3.5 and 1.0±1.1 yrs, respectively). Although none of the 15 CF patients had presented with meconium ileus at birth, all were pancreatic insufficient and had very severe lung disease, with a high rate of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Two patients died during the course of this investigation (one was 5 months and the other, 6 yrs old).

The clinical presentation associated with S549R(T→G) homozygosity in the United Arab Emirates is quite homogeneous and shows an extreme degree and course of cystic fibrosis severity.

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Cystic fibrosis (CF) is an inherited multisystem disorder characterized by abnormalities in exocrine gland function. The clinical expression of the disease is heterogeneous, and the spectrum of clinical manifestations of CF includes varied degrees of chronic, obstructive, pulmonary disease, pancreatic dysfunction (exocrine or endocrine), hepatobiliary disease, and of abnormalities in eccrine sweat gland function [1].

Following the identification of the cystic fibrosis transmembrane conductance regulator (CFTR) gene [1], the founding of the CF Genetic Analysis Consortium in 1989 has fostered extraordinary advances in the molecular genetics of CF. Besides ΔF508, more than 750 other putative disease-causing mutations have been reported so far, scattered throughout the CFTR gene [2].

CF is characterized by remarkable clinical heterogeneity that is due to allelic heterogeneity [2, 3]. Specific mutations, however, have also been reported to be associated with a wide spectrum of clinical manifestations [4–8], and this is true of ΔF508 [4–11]. Genotype–phenotype correlation analyses thus contribute to providing unique information on optimal patient management, prognostic evaluation, and unravelling of the CFTR function [7, 12, 13].

It has previously been found by this group that a very severe form of CF amongst nationals from the United Arab Emirates (UAE) is due to the mutation S549R(T→G) [14]. A guanine (G)-to-thymine (T) transversion occurs at CFTR nucleotide 1779, which is located in exon 11. This mutation results in the replacement of a serine with an arginine in the first nucleotide binding domain of the protein, and is thus expected to cause a severe clinical presentation [15]. In this report, the clinical characteristics that are associated with this mutation in a group of 15 CF patients who are homozygous for S549R are presented.

Subjects and methods

Patients

The study group consisted of 15 CF patients (9 females and 6 males) who were all UAE nationals of Bedouin origin. These patients were referred to the CF Clinic of Tawam Hospital (Al Ain, Abu Dhabi Emirate, UAE) between 1994 and 1996. The protocol for this CF programme was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences, UAE University, Al Ain, UAE.

All the families studied were urbanized and members of one of the world's most affluent societies. There is universal free medical care for nationals of the UAE and thus drug and investigation costs are covered by the State. The CF Clinic is run by the staff of the Faculty of Medicine and regular follow-up care is provided to all patients. Initial monthly follow-up appointments are given to all newly diagnosed patients. Compliance with treatment was regarded as good.

Clinical investigations

General outcome variables that were evaluated in this investigation (table 1) included current age, age at diagnosis, sweat chloride values (measured by quantitative

pilocarpine iontophoresis), height, weight, and weight for height percentiles (from most recent clinic visits), and Shwachman–Kulczycki scores [16]. All results are presented as means±SD.

The following presenting clinical problems were recorded (table 1): meconium ileus, pulmonary problems, failure to thrive, and diarrhoea. Presence of pancreatic insufficiency was determined on the basis of requirement for pancreatic enzyme supplementation to control steatorrhea and the measurement of stool chymotrypsin activity. Associated complications included nasal polyps, sinusitis, pancreatitis, diabetes mellitus, and rectal prolapse (table 1).

Respiratory bacterial infections (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) were determined as the first positive culture on record, and cultures of tracheal aspirates were performed in the case of children who were too young to provide sputum samples (table 1).

Due to the young age of the subjects, regular pulmonary function tests could not be performed. Radiological analyses, however, were carried out in 12 of the 15 children by a single observer in order to evaluate both the extent and the severity of lung disease.

CFTR S549R mutation analysis

Deoxyribonucleic acid (DNA) was extracted from leukocytes isolated from 2–5 mL of the subject′s venous blood collected in ethylenediaminetetraacetic acid (EDTA) tubes according to standard protocols [17].

The detection of mutation S549R localized in exon 11 (T→G at nucleotide 1779) was performed routinely by *Dra*III restriction endonuclease analysis of exon 11 polymerase chain reaction (PCR) products, and the mutation was confirmed by sequencing analysis according to protocols and conditions that have been described elsewhere [14].

Table 1. – Clinical description of 15 cystic fibrosis patients with cystic fibrosis transmembrane conductance regulator mutation S549R(T→G)

Variable	R549/R549
Patients n	15 (6 M, 9 F)
Mean current age yrs	5.4±3.5 (Range: 1–12)
Mean age at diagnosis yrs	1.0±1.1 (Range: 0.1–3)
Sweat chloride mmol·L^{-1}	120.0±21.0
Schwachman score	45.5±7.0
Height percentile	<3rd–10th
Weight percentile	<3rd–5th
Presentation n	
Meconium ileus	0
Pulmonary	14
Failure to thrive	13
Diarrhoea	10
Chymotrypsin in stool	7
Pancreatic insufficiency	13
Associated complications (nasal polyps, sinusitis, pancreatitis, diabetes mellitus, rectal prolapse)	0
Pseudomonas colonization	9
Staphylococcus colonization	7

Values are mean±SD; range or number of subjects. M: male; F: female.

Results

The clinical presentation of 15 CF children (6 males and 9 females) who were homozygous for mutation S549R was determined. The clinical description of the pooled group of patients is presented in table 1. Mean current age was 5.4±3.5 yrs, mean age at diagnosis was 1.0±1.1 yr. Sweat chloride concentrations were 120±21 mmol·L^{-1} (range 105–155 mmol·L^{-1}). Shwachman scores (45.5±7.0) indicated an extremely severe overall clinical manifestation.

No child had presented with meconium ileus at birth, but all were pancreatic insufficient and had severe pulmonary disease. None of the 15 CF patients had associated complications (table 1). *P. aeruginosa* and *S. aureus* colonization were frequently observed (9 and 7 cases out of 15, respectively).

The severity of lung disease was examined by radiological investigations in 12 of the 15 CF patients (two patients died and a third was lost to follow-up). All 12 children included in this group presented with hyperinflation that was mainly due to inflammatory mucosal oedema and accumulation of mucopurulent secretions.

Discussion

S549R is a missense mutation that was first reported in 1989 by KEREM *et al.* [18] in a Moroccan Jewish individual who was a compound heterozygote S549R/ΔF508. As it was associated with the pancreatic insufficiency phenotype, it was labelled as severe. In a subsequent study, CHEVALIER-PORST *et al.* [19] screened 105 mutations in 600 CF patients from France and identified one S549R homozygote. This individual was a French settler from Algeria who was affected by a mild form of CF (F. Chevalier-Porst, personal communication). Similar genotype–phenotype variations have been reported for other mutations as well and several hypotheses have been put forward. For example, KIESEWETTER *et al.* [20] showed that a close association exists between chromosome background of the R117H mutation and clinical phenotype, which means that the genetic context in which a mutation occurs can play a significant role in determining the type of illness produced. BOWLER *et al.* [21] reported a more severe clinical course of ΔF508 in Asians than in matched Europeans, and concluded that genetic and environmental factors may be contributory.

The UAE is a federation of seven Emirates, the largest being Abu Dhabi (the six others include Ajman, Dubai, Fujaira, Ras Al Khaimah, Sharjah, and Umm Al Quwain). The indigenous population is principally composed of Emirati who have been registered into tribes of Bedouin origin. Genetic flow has also come from Oman, Yemen, Iran and Baluchistan. Previous work [14] by the authors indicated that all CF patients of Bedouin origin that have been investigated so far were homozygous for S549R (T→G), which led to the hypothesis that the mutation is the result of a founder effect that occurred in an ancestral Bedouin tribe. Although S549R has been described elsewhere as a private mutation only, the uniquely high number of homozygotes in the UAE allowed us to evaluate the overall clinical presentation that is associated with S549R.

This data indicates that S549R is a very severe mutational allele. First of all, the age at diagnosis was low (1.0±1.1 yr, see table 1), despite the fact that CF has been

under- and misdiagnosed in this part of the world until recently [22]. Sweat chloride concentrations associated with S549R homozygosity were high, even at very young ages (mean sweat chloride levels in the group of 15 patients were 120±21 $mEq \cdot L^{-1}$). This in itself does not indicate severity. All 15 patients, however, exhibited the pancreatic insufficiency phenotype. Chronic infections with *P. aeruginosa* and *S. aureus* were present in 9 and 7 patients, respectively, and were found at very young ages. Overall Shwachman–Kulczycki scores, as well as height and weight percentiles, were poor (see table 1).

An interesting feature of the clinical presentation associated with S549R in the UAE is that none of the 15 homozygous patients had presented with meconium ileus at birth. This would contrast with mutation ΔF508, which is associated with meconium ileus in 15% of cases [4] although, given the relatively small number involved, these findings may not be statistically significant. Furthermore, ΔF508 subjects who present with meconium ileus are more likely to be colonized earlier with *P. aeruginosa* [23]. In R549 homozygotes, the rate of serious chronic infections (including *P. aeruginosa*) is high and severe pathogenic colonization occurs early in life. Early colonization by *P. aeruginosa* has also been reported in CF patients from Saudi Arabia [24], which suggests that genetic as well as nongenetic factors play an aggravating role in the course of CF in this part of the world.

It is also interesting to note that, besides meconium ileus, none of the 15 patients has shown evidence of classically associated complications (table 1). All 15 patients, however, have had severe pulmonary complications. Even though pulmonary function tests on the patients in this study could not be performed, the severity of radiological findings on 12 of these patients led us to conclude that the pulmonary phenotype was extremely severe in all 15 R549 homozygous patients.

Although there is usually good correlation between CFTR genotype and pancreatic function status, genotypes have failed to predict pulmonary severity and consequently the pathophysiology of lung complications remains elusive [4–8, 11–13]. The results of this study also point to the fact that the pulmonary phenotype associated with R549 mutations is indeed fairly heterogeneous, although it indicates a marked overall severity. Of the 15 CF patients of this investigation, two died at ages 5 months and 6 yrs of pulmonary failure. The oldest patient, however, who has complied well with therapeutic interventions, is now 12 yrs old.

In conclusion, genotype-phenotype analysis in cystic fibrosis patients from the United Arab Emirater shows that the R549 allele is associated with an extremely severe clinical presentation. Detection of the S549R mutation is thus imperative in these cystic fibrosis children, as aggressive treatment and management modalities should be instigated as soon as possible in order to increase the patient′s chances of survival.

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