Disease severity associated with cystic fibrosis mutations ΔF508 and S549R(T→G)

K.P. Dawson,[1] P.M. Frossard[2] and B. Al-Awar[3]

وخامة الأمراض المترافقة مع الطفرتين ΔF508 و S549 (T→ G) للتليُّف الكيسي

كينيث ب. داوسون، وفيليب م. فروسارد وبدرية الأعور

خلاصة: تهدف هذه الدراسة للمقارنة بين الوخامة السريرية (الإكلينيكية) التي ترافق كلا من الطفرة ΔF508 والطفرة S 549 (T → G) للتليف الكيسي. وقد تمت مقارنة متغيرات التليف الكيسي في مجموعات متشابهة من الأطفال المصابين بالتليف الكيسي في الإمارات العربية المتحدة. وقد أظهرت المعطيات التي توافرت لدينا نماذج يمكن مقارنتها مع قيمة متدنية جداً لأحراز شفاشمان ومستويات مرتفعة للكلوريد في العرق. وقد استنتجنا أن المرضى المتماثلي الزيجوت بالنسبة لطفرتي التليف الكيسي ΔF508 و S549R (T → G). تتجلى بصور سريرية متعددة وبأمراض مختلفة ولا يمكن تمييزها سريرياً.

ABSTRACT We compared the clinical severity associated with the two cystic fibrosis (CF) mutations S549R(T→G) and ΔF508. Clinical and biochemical variables of CF were compared in two age- and sex-matched groups of CF children in the United Arab Emirates (UAE). The clinical severity of mutations S549R(T→G) and ΔF508 showed comparable patterns, with very low Shwachman scores and high sweat chloride levels. We conclude that patients homozygous for the CF mutations ΔF508 and S549R(T→G) have a severe clinical presentation and illness and are indistinguishable on clinical grounds.

Gravité de la maladie associée aux mutations ΔF508 et S549R(T→G) de la mucoviscidose
RESUME Nous avons comparé la sévérité clinique associée à chacune des deux mutations S549R(T→G) et ΔF508 de la mucoviscidose. Les variables cliniques et biochimiques de la mucoviscidose ont été comparees dans deux groupes d'enfants atteints de mucoviscidose, appariés selon l'âge et le sexe, aux Emirats arabes unis. La sévérité clinique des mutations S549R(T→G) et ΔF508 avait des caractéristiques comparables, avec des scores de Shwachman très bas et de fortes concentrations de chlorures dans la sueur. Nous concluons que les patients homozygotes aux mutations ΔF508 et S549R(T→G) de la mucoviscidose présentent un tableau clinique et une maladie sévères et qu'aucune distinction entre eux ne peut être établie sur le plan clinique.

[1]*Department of Paediatrics;* [2]*Department of Pathology, Faculty of Medicine and Health Science, UAE University, Al-Ain, United Arab Emirates.*
[3]*Dubai Hospital, Dubai, United Arab Emirates.*
Received: 25/06/00; accepted: 29/03/01

Introduction

Cystic fibrosis (CF) is a disease characterized by a marked clinical variability as a result of the existence of allelic heterogeneity [1]. Other than mutation ΔF508, there are over 1000 mutations which are potentially disease-producing [2]. We have previously described the clinical presentation associated with the S549R(T→G) CF mutation as it presents in the Bedouin population of the United Arab Emirates (UAE) [3,4]. In the homozygous state, the disease produced is severe and is characterized by extreme lung disease and malabsorption. The mutation ΔF508 is also present in a homozygous state in nationals of the UAE who are of Baluch descent [5].

The purpose of this study was to compare the disease severity associated with each of these two mutations as they present in the UAE, where environmental factors and health care access are uniform and controlled and the patients are homozygous for their respective mutations.

Methods

Patients

The study subjects consisted of two groups of 5 children who were age- and sex-matched and were homozygous for the mutation S549R(T→G) or ΔF508 (Table 1). There were 2 males and 3 females in each group. We also compared the group of 5 CF, ΔF508 homozygote patients with a non-matched group of 15 CF children who were S549R(T→G) homozygotes. This latter group, which we have described before [3], included the 5 patients constituting the matched S549R(T→G) group.

Although the overall number of CF patients in this study is relatively small, we have shown previously that these subjects constitute the pool of most of the CF patients found in the UAE [6], and the pool of 20 patients investigated here has been actively recruited over the past 7 years. The patients were referred to the CF Clinic, Tawam Hospital (Al-Ain, UAE) from all parts of the country by different physicians. All children, as UAE citizens, have free access to hospital care and the costs of their drugs and investigations are met by the State.

Clinical investigation

Data on age, age at diagnosis, current height and weight percentiles were collected for the matched children. A Shwachman score was calculated for each child [7]. Data were expressed as means plus one standard deviation for the separate CF mutation groups. Information was also collected on the following: history of meconium ileus, pulmonary infection, evidence of pancreatic insufficiency (determined on the basis of enzyme replacement and/or chymotryptic activity in the stool) and associated problems such as nasal polyps, sinusitis, pancreatitis, diabetes mellitus or rectal prolapse.

The presence of respiratory tract colonization was noted and specific organisms were identified from positive sputum cultures or tracheal aspirate results.

CF transmenbrane conductance regulator (CFTR) mutation analyses

DNA of the patients was extracted from leukocytes in 2 to 5 mL of venous blood collected in EDTA tubes. The detection of mutation S549R localized in exon 11 (T→G at nucleotide 1779) was carried out 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 [4,6,8]. ΔF508 was detected by PCR analysis according to conditions that have been previously described, and mutant alleles were also confirmed by sequence analysis [4,6,8].

Results

The comparative pooled data for both groups of children are listed in Table 1. Included for comparison are the findings from fifteen children with mutation S549R(T→G) previously published [3]. In the matched groups the main difference is the earlier diagnosis in the ΔF508 group, a difference, however, that was not statistically significant ($P = 0.57$). Shwachman scores showed no difference and sweat electrolyte levels were marginally higher in the S549R(T→G) group. The mean sweat chloride levels were higher in both the matched and unmatched S549R(T→G) groups. This was not statistically significant ($P = 0.34$).

Discussion

Our findings support the view that the S549R(T→G) CF mutation is associated with a clinically severe form of the disease. Further, the results are in keeping with our contention that the clinical disease associated with the mutation in the homozygous state is as severe as that produced by the ΔF508 mutation. The major difference found between the age- and sex-matched patients was that the diagnosis was established earlier in the group of ΔF508 homozygotes. However, despite this, there was no difference in the Shwachman scores between these groups.

One of the unique features of this study is that the patients are all homozygous for

Table 1 **Clinical data associated with CFTR mutations S549R(T→G) and ΔF508**

Variable	Matched ΔF508 ($n = 5$)	Matched S549R ($n = 5$)	Non-matched S549R ($n = 15$)
Current age range (months)	36–120	36–120	1–144
Mean age at diagnosis ± *s* (months)	6.8 ± 4.2 (range 2–12)	22.0 ± 25.6 (range 1–60)	12.0 ± 13.0 (range 2–36)
Mean sweat chloride level ± *s* (mmol/L)	97.8 ± 19.1	113.0 ± 23.3	120.0 ± 21.0
Shwachman score ± *s*	45.0 ± 5.7	45.4 ± 5.1	45.5 ± 7.0
Pancreatic insufficiency	4/5	5/5	13/15
Pseudomonas colonization	4/5	2/5	9/15
Meconium ileus	0/5	0/5	0/15

s = *standard deviation.*

their particular mutation, lived in the same harsh climatic and physical environment, and had free access to health care. We included another group of patients with the S549R(T→G) mutation in Table 1 whose details have been published before [*3*]. The clinical details from this larger group suggest that our age- and sex-matched sample reflect the general pattern of disease resulting from the mutation found in our society. However, it is of interest to note that in general the diagnosis was made earlier than in the matched group and was nearer to that of the group of ΔF508 homozygotes.

CF results from modifications in the sequence, structure and/or expression of CFTR. More than 1000 CF-causing mutations in the CFTR gene have been identified worldwide [*2*]. The gene mutations of the gene have been divided into five classes (I–V). Most of the CFTR mutations, however, are rare. Interest has centred on the relationship of phenotype to genotype. Kerem and Kerem [*9*] have classified phenotypes into severe, milder and variable. The severity of the effect of CF mutations has usually been based upon whether there is pancreatic sufficiency or not. ΔF508 is regarded as a severe mutation conferring marked pancreatic insufficiency and the presence of meconium ileus in 10%–15% of affected neonates [*10*]. In addition, the mutation is usually associated with lung disease. This mutation remains the commonest severe mutation worldwide, resulting in major CFTR chloride channel disruption and producing little channel activity. It has been labelled as a class II mutation (defective protein processing) which results in the failure of the protein to reach the epithelial membrane [*11*]. The S549R mutant has also been shown to yield an inadequately glycosylated form of the protein, which in turn results in its failure to reach its correct cellular location (as occurs with ΔF508); the S549R(T→G) mutation is thus also a class II mutation [*12,13*]. Our clinical findings are therefore in keeping with the molecular changes.

The patients we have described match the classical homozygote pattern for ΔF508, with chronic obstruction and infection of the respiratory tract, pancreatic insufficiency and elevated levels of sweat electrolytes [*14*]. However, we have not seen a patient in the UAE, homozygous for either of the studied mutations, who presented with meconium ileus. As the patients studied here all showed pancreatic insufficiency, and if we take into account the estimated frequency of 10%–15% [*10*], we would have expected to observe 2 to 3 neonates with meconium ileus in the overall cohort of 20 patients. Whether the relatively small sample size accounts for the absence of meconium ileus in UAE patients or whether additional factors play a role remains to be elucidated. Similarly, the higher sweat chloride levels in the S549R(T→G) patients may reach significance if and when additional ΔF508 patients are found. The apparent, but statistically insignificant, earlier diagnostic age of ΔF508 patients is not likely to be due to a referral bias.

It has been proposed that some patients with severe mutations show a milder phenotype, implying the action of modifier genes or the presence of additional CFTR mutations that temper the severity of the disease [*11,13*]. It is of interest in this context to compare our patients with severe S549R(T→G) who present with severe disease with a group of patients from Europe with the same mutation and mild disease. The unexpected finding was that the mildly affected patients also had a novel complex allele in the CFTR, combining a sequence change in the minimal promoter (−102T→A) with the mutation S549R(T→G) [*13*]. Romey et al. [*15*] have

since shown that the –102T→A mutation creates a Ying Yang 1 core element and thus increases significantly the expression of the CFTR gene. All the UAE patients studied here lacked the promoter sequence change, as described elsewhere [*12*].

CF mutations vary from Arab country to Arab country. Some 17 different mutations have been identified in Saudi Arabia, with ΔF508 forming only 12% of those identified. In Lebanon, 10 different mutations occur, with ΔF508 representing 34% of the total. Oman appears to be similar to the UAE with the predominance of the S549R(T→G) mutation. In Qatar, all patients identified to date have the mutation I1234V. These geographic variations may reflect population movements within the Arab world from pre-Islamic times [*16,17*].

We conclude from this study that S549R(T→G) homozygous patients have severe clinical disease and that this disease severity is equivalent to the clinical severity of ΔF508 homozygous patients. These clinical findings are in keeping with the known molecular modifications affecting the CFTR gene.

Note from the authors

We fully appreciate the limitations of the paper and its descriptive nature and that no strong statisitical analysis can be performed because of the small numbers. However, it is based on all known patients with DFS08 mutation in the United Arab Emirates.

References

1. Tsui L-C. Mutations and sequence variations detected in the cystic fibrosis transmembrane conductance regulator (CF TR) gene: A report from the Cystic Fibrosis Genetic Analysis Consortium. *Human mutation*, 1992, 1:197–203.
2. Cystic Fibrosis Genetic Analysis Consortium, CFGAC, 17 November 1999. http://genet.sickkids.on.ca
3. Frossard PM et al. Genotype-phenotype correlations in cystic fibrosis: clinical severity of mutation S549R (T→G). *European respiratory journal*, 1999, 13:100–2.
4. Frossard PM et al. Radiological analysis of children with cystic fibrosis who are homozygous for cystic fibrosis transmembrane conductance regulator mutation S549R (T→G). *Journal of tropical pediatrics*, 1999, 45:158–60.
5. Frossard PM et al. Identification of cystic fibrosis mutations in the United Arab Emirates. *Human mutation*, 1998, 11: 412–3.
6. Frossard PM et al. Determination of the prevalence of cystic fibrosis in the United Arab Emirates by genetic carrier screening. *Clinical genetics*, 1999, 55:496–7.
7. Shwachman H, Kulczycki LL. Long-term study of one hundred five patients with cystic fibrosis: studies made over a five- to fourteen-year period. *American journal of disease in childhood*, 1958, 96:6–15.
8. Costes B et al. A rapid, efficient and sensitive assay for simultaneous analysis of multiple cystic fibrosis mutations. *Human mutation*, 1993. 2:185–91.
9. Kerem E, Kerem B. Genotype-phenotype correlations in cystic fibrosis. *Pediatric pulmonology*, 1996, 22:387–95.
10. Davidson DJ, Porteous DJ. The genetics of cystic fibrosis lung disease. *Thorax*, 1998, 53:389–97.

11. Estivill X. Complexity in a monogenic disease. *Nature genetics*, 1996, 12:348–50.

12. Welsh MJ, Smith AE. Molecular mechanisms of CFTR channel dysfunction in cystic fibrosis. *Cell*, 1993, 73:1251–4.

13. Romey M-C et al. Complex allele [-102 (T→A)+S549R(T→G)] is associated with milder forms of cystic fibrosis than allele S549R(T→G) alone. *Human genetics*, 1999, 105:145–50.

14. The Cystic Fibrosis Genotype-Phenotype Consortium. Correlation between genotype and phenotype in patients with cystic fibrosis. *New England journal of medicine*, 1993, 329:1308–13.

15. Romey M-C et al. A naturally occurring sequence variation that creates a YY1 element is associated with increased cystic fibrosis transmembrane conductance regulator gene expression. *Journal of biological chemistry*, 2000, 275: 3561–7.

16. El-Harith EA et al. Novel and characteristic CFTR mutations in Saudi Arabian children with severe cystic fibrosis. *Journal of medical genetics*, 1997, 34:996–9.

17. Wahib AA et al. *Heterogeneity of the cystic fibrosis phenotype in a large kindred family in Qatar with cystic fibrosis mutation I1234V*. Paper presented at the 2nd Qatari International Pediatric Conference, Doha, April 2000.

Classification of Cystic Fibrosis and Related Disorders: Report of a joint WHO/ICF(M)A/ECFTN meeting, Stockolm, Sweden, 3 June 2000

Making a diagnosis of cystic fibrosis (CF) is not always simple. In the current edition of the *International Classification of Diseases* (ICD 10), the classification for cystic fibrosis is subdivided into four parts: CF with pulmonary manifestations, CF with intestinal manifestations, CF with other manifestations and CF unspecified. Because it would be of benefit to patients, families, health care providers, health insurers, medico legal interests and clinicians if this classification were revised to take account of current knowledge and diagnostic problems, and to separately designate those related conditions which are not CF, and whose diagnosis has different implications, WHO, the International Cystic Fibrosis (Muscoviscidosis) Association, the European Cystic Fibrosis Thematic Network and the European Cystic Fibrosis Society convened a joint working group in June 2000 to produce a new classification of CF suitable for inclusion in the next edition of the *International Classification of Diseases* (ICD). This document reports on the outcome of the meeting held to consider CF classification. The document can be obtained from: Noncommunicable Diseases and Mental Health Cluster, World Health Organization, Avenue Appia 20, CH-1211 Geneva 27, Switzerland. It is also available free on the Internet at: http://www.who.int/ncd/hgn/Classification_Cystic_Fibrosis.htm