March/April 2000 · Vol. 2 · No. 2

The interactivity between the CFTR gene and cystic fibrosis would be limited to the initial phase of the disease

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Purpose: Cystic fibrosis (CF) is a lethal genetic disorder affecting secretory epithelia, caused by mutations on the CFTR gene. In this paper we study the interactivity between the CFTR gene and CF disease over the time course of CF. **Method:** Cross-sectional analysis of CF patient population data from Latin-America, Canada, and The Netherlands, under the assumption that they represent stationary populations, was used to determined and correlates hazard rates, average cores and CF progression rates. **Results:** Results suggests the existence of two phases throughout the course of CF. **Conclusion:** While the initial phase was related to the CFTR genotype, the kinetics of the second phase seems to be common to all groups considered. The hypothesis that the interactivity between the CFTR gene and CF disease would be limited in time is presented, suggesting that mutant CFTR would trigger a disease that evolves to become independent from the CFTR gene itself. ***Genetics in Medicine*, 2000:2(2):124–130.**

Key Words: *cystic fibrosis, epidemiology, genetics, health care*

Cystic fibrosis (CF) is a frequent genetic disorder affecting the secretory epithelia (airways, digestive tract, gonads, and sweat glands) and leading to early death.[1] Respiratory insufficiency causes over 90% of deaths among CF patients. The primary defect is a deficient Cl^- transport through the plasma membrane, which leads to the production of viscous mucus and, in the airways, causes obstruction of airway conducts and favors infection by pathogenic bacteria.[2-8] Colonization of the airways by bacteria triggers a recurrent inflammatory reaction that progressively destroys the lung.

Based on the long-term follow-up of a group of CF patients, a model has previously been proposed to describe the course of the respiratory obstructive disease, measured by the ratio %FEV1/FVC.[9] Patients went through the CF disease following two phases, separated by a breakpoint: During the initial phase there was little change in %FEV1/FVC; while after the onset of the breakpoint, there was a linear decline in %FEV1/FVC. The slope of the decline is independent of the age of onset of the breakpoint. The nature of the breakpoint, as well as the factors determining its onset, are unknown.

Since 1989, when the linkage between the CFTR gene and CF was first reported,[5,6] substantial evidence has been obtained involving the mutant CFTR at the genetic cause of CF. More than 800 mutations on the CFTR gene have been associated with the CF-related failure in Cl^- transport.[10]

In addition to the fact that the CFTR gene is involved in the pathogenesis of CF, it is usually assumed that the CF phenotype is steadily and actively maintained by the absence of CFTR activity all along the course of the disease. To this hypothesis of lasting interaction between CFTR and CF phenotype, it follows that replacement of the mutant CFTR function should lead to the reversion of the CF phenotype. Current therapeutic approaches to CF rely on the assumption of a lasting interaction between CFTR and CF. However, no evidence has been shown so far in support of that assumption. Mainly based on cross-sectional population data, we suggest that the course of CF might be divided in two phases separated by a *nonreturn point*: that the decline after the *nonreturn point* is independent of the age of its onset and that the second phase would be noninteractive with CFTR activity.

MATERIALS AND METHODS

Age distribution of CF patients

Cross-sectional CF patient population data from Latin-America (N = 872 patients), Canada (N = 240 patients), and The Netherlands (N = 162 patients), were obtained from the literature.[11-13] Data are represented as distributions by age, for age intervals of 3 and 1 years for Canada and The Netherlands and for Latin-America, respectively. Newborn CF patient population data from Latin-America (N = 420 patients) were obtained from the literature[14]; they represent a defined group of CF patients observed from birth over three years.

The work presented in this paper has been supported by CONICET and ALFIQ, Argentina.

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Received: September 7, 1999.

Accepted: November 19, 1999.

Hazard rates

According to Fisher *et al.*,[15] the estimated *hazard rate* (λ), or probability of dying within 3 year intervals, is defined as follows:

$$\lambda = N/n_o \times t = (n_o - n_t)/n_o \times t$$

where N is the number of observed deaths in the age interval t; n_o and n_t are the number of patients alive at the beginning and at the end of the age interval, respectively. Hazard rates were calculated from the age distribution data in Figure 1. For every peak and starting at the corresponding *age at peak*, λ was obtained for every 3 year interval.

Average score NIH

The *score NIH* has previously been defined as a prognostic parameter scoring the health status of *individual* CF patients and shown to be related to the probability of dying within three years.[16] The probability of dying for an individual patient can be estimated from its *score NIH*, using the population-based standard curve described by Taussig *et al.*[16] Inversely, if the estimated probability of dying for an individual patient were known, then its expected *score NIH* value could be estimated from the curve.

We introduce the idea of *average score NIH* (*sNp*) for a given group of patients as the average of the individual *score NIH* values

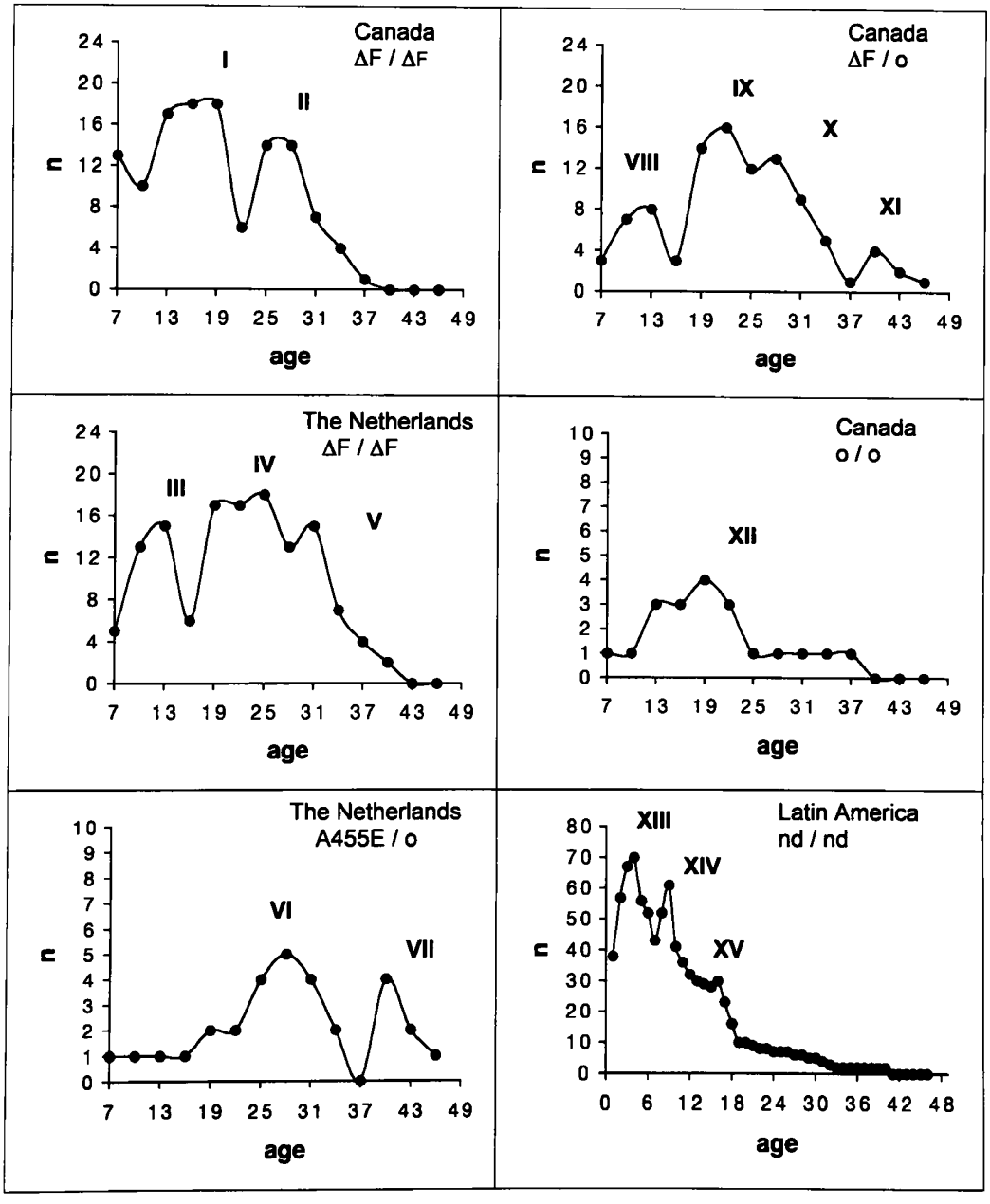

Fig. 1 Age distributions of CF patients in Latin-America, Canada, and The Netherlands. Cross-sectional CF patient population data from Latin-America (N = 872), Canada (N = 240), and The Netherlands (N = 162) were obtained from the literature.[11-13] Numbers I-XV indicate the peaks referred to in the text. nd, nondefined genotype; ΔF, ΔF508 genotype; o, (other) non-ΔF508 genotype; A455E, A455E genotype.

for the patients constituting the group. The *average score NIH* scores the health status of the patient group. More healthy groups would display higher *sNp* values, while the progression of the disease in a group would lead to a decrease in its *sNp* over time.

The distribution of *sNp* by age was obtained for every population shown in Figure 1 (Canada ΔF508/ΔF508; The Netherlands ΔF508/ΔF508, Canada ΔF508/other, etc). For that, the *average score NIH* (*sNp*) corresponding to patients grouped by age (3 year intervals) was obtained by entering the corresponding hazard rates λ, calculated as indicated in the previous section, into the curve reported by Taussig *et al.*[16]

Progression rate

The progression rate of the disease is estimated by the rate of decline of *sNp*, (r_s), or the variation of *sNp* over time. r_s was calculated from data in Figure 2, according to:

$$rs = \Delta sNp/\Delta t$$

Student's *t*-test

The Student's *t*-test[17] was used to test for the hypothesis that the slope of the straight line obtained by linear regression be-

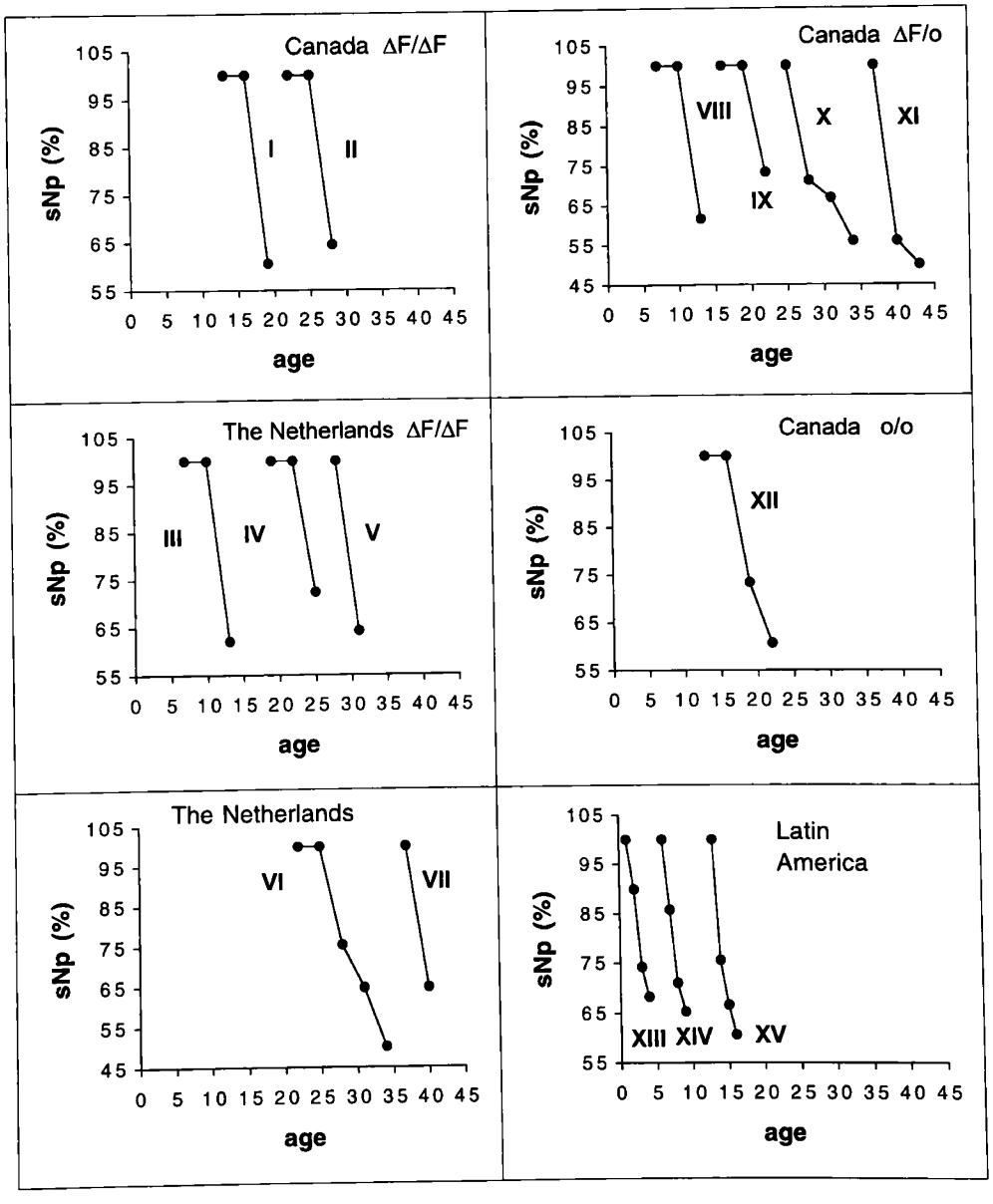

Fig. 2 Average score NIH corresponding to the peaks in Figure 1. The average score NIH (*sNp*) was calculated as described in the "Materials and Methods" section. *sNp* is expressed as percent of the maximal *sNp* for each peak (I-XV) in Figure 1. nd, nondefined genotype; ΔF, ΔF508 genotype; o, (other) non-ΔF508 genotype; A455E, A455E genotype.

tween r_s and *ap* (Fig. 3) is equal to 0, with N = 15 (number of peaks considered).

RESULTS

Figure 1 shows the *age* distributions of CF patient populations from Latin-America, Canada, and The Netherlands, obtained from published cross-sectional data.[11-13] Data from Canada and The Netherlands include patients genotypically characterized as ΔF508/ΔF508, ΔF508/other, A455E/other, and other/other as well as uncharacterized. Most patients from REGLAFQ are not genotypically characterized. Twenty to forty percent of alleles identified as "other" are expected to correspond to the 5-6 most frequent non-ΔF508 severe mutations.[18,19] In all cases shown in Figure 1, the appearance of clinical symptoms preceded CF diagnosis. A number of peaks (I-XV) can be distinguished in the *age* distribution profiles in Figure 1. The age corresponding to the top of a peak is named *ap* or *age at peak*.

Despite the rather small sample numbers, the assumption that data in Figure 1 are representative of stationary populations[20] was made for the analysis that follows. The relative numerical distribution of the populations in the various age groups, *e.g.*, the distribution profile, is assumed not to change with time. When an individual patient leaves an age group, either by death or growing older, their place is assumed to be immediately taken by another patient either from the next lower age group or entering the CF population by diagnosis. Thus, the number of patients would rise (and generate peaks) at age intervals when recruitment of new patients by diagnosis is more prevalent over death of existing patients, and it would drop in the inverse situation.

In the statistical analysis of survival, the estimated hazard rate, λ, is defined as the probability for individuals in a given population of dying per unit time given survival to the time point in question.[15] From the distributions by age in Figure 1, we calculated the estimated hazard rates for age intervals of 3 years, as described in the "Materials and Methods" section. λ was obtained as the fraction of patients leaving every age group (death is assumed) in 3 year age intervals.

The probability of an *individual* CF patient of dying within 3 years has been shown to be related to, and can be estimated from, its *score NIH*[16], one of the parameters widely used to score for the clinical status of individual patients. For the analysis that follows, we made the reasoning that, inversely, if the average probability of dying for patients within a given patient group were known, then, the average *score NIH* corresponding to that group and representing the average health status of that group could be estimated. Using the hazard rates λ obtained as described above and the standard curve reported by Taussig *et al.*,[16] the average *score NIH* corresponding to the patient groups represented by the peaks in Figure 1 was obtained for every time point and for every peak, and is shown in Figure 2. The change with time in the *sNp* corresponding to a given patient group estimates the rate of progression of the disease in that group. The rate of change of *sNp* with age, r_s (see "Materials and Methods"), was calculated from data in Figure 2 as $\Delta sNp/\Delta t$.

In addition to data in Figure 1, newborn CF patient population data from Latin-America,[14] consisting of 420 newborns clinically diagnosed at birth and observed over a period of 3 years, were analyzed as described for data in Figure 1. The number of survivors at the end of the first, second, and third years of follow up was 358, 345, and 330 individuals, respectively. The estimated hazard rate λ (for the 3 year interval) and the corresponding *sNp* and r_s were calculated as described above.

Figure 3 shows the rate of change of *sNp* with time (r_s) for the patient groups considered in Figure 1, distributed by their respective *age at peak*. r_s corresponding to the newborn population is also shown in Figure 3. As seen in the figure, r_s takes the same value for all groups considered and is, then, independent from *ap* (slope = 0; $p < 0.0005$).

Data in Figure 3 indicate that independently from the time necessary for every CF patient group to reach the point given by *ap*, the progression of the CF disease after *ap* would follow a common and constant rate estimated by r_s. Thus, *ap* might represent a time point beyond which the further progression of the disease follows a common and universal kinetics.

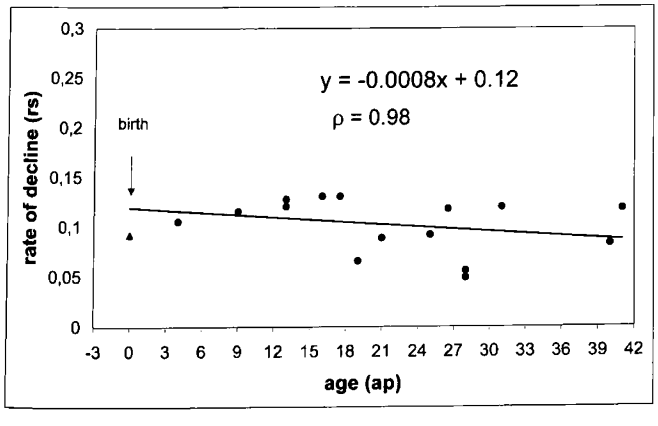

Fig. 3 Relationship between the rate of decline of sNp (r_s) and the age at peak (ap). r_s was calculated, as described in the "Materials and Methods" section, as the variation in *sNp* over time (slopes in Fig. 2) for each of the peaks (I-XV) in Fig. 1 (●) and for the newborn CF patient population (nCF) data discussed in the text (▲). $y = -0.0008 \times 0.12$ is the equation of the linear regression between r_s and *ap*, and $\rho = 0.98$ is the corresponding correlation coefficient.

DISCUSSION

The hypothesis presented in this article is strictly based on the assumption that the cross-sectional CF patient population data considered represent stationary populations. The assumption may be suspect, particularly because CF epidemiology (rigorously speaking, life expectancy) has changed in North-America, Europe, and Latin-America in the last decades due to improvements in health-care.[21-23] However, a constant rate of change of *sNp* (r_s), common to patient groups of different age, genotype, and origin, would be difficult to explain if the peaks observed in Figure 1 were due to small sample sizes or

to other bias either in the populations or in the samples. In addition, data from the follow-up of a defined population of 420 newborn CF patients over 3 years, gives a rate of decline of *sNp* (r_s) equivalent to those estimated from cross-sectional data (Fig. 3).

A universal value for r_s suggests the existence of a common law that determines the course of the CF disease, following arrival to a time point that may be called here the *nonreturn point*, and which is somehow related to *ap*. Beyond the *nonreturn point*, all CF patients included in the analysis progress through CF into death according to a common kinetics, given by r_s. Although the time necessary to reach the *nonreturn point* (estimated by *ap*), would change from patient to patient, the kinetics of progression of the disease beyond the *nonreturn point* would be common for all patients. Regardless of genotype, environmental factors, and the time necessary for every individual patient to reach the *nonreturn point*, this would be a confluent end-point beyond which all CF patients would follow a common kinetics and fate.

The A455E mutation is known to lead to a mild form of the CF disease and to be related to late ages at diagnosis, while the severe ΔF508 mutation is related to earlier age at diagnosis.[12,13] In agreement with that, patients carrying the A455E mutation show higher *ap* values compared with those carrying more severe genotypes (Fig. 1). According to the extremes, ΔF508 versus A455E alleles, the more severe the disease, the earlier the appearance of the clinical symptoms and the age at diagnosis and smaller *ap* (Fig. 1). The level of activity of the CFTR gene, at least for the ΔF508 and A455E mutations, would be correlated to the age at diagnosis and to the time point given by *ap*, and therefore, to the kinetics of progression of the disease *before* that point. Factors like socio-economic level, health-care, and/or genetic background[24-26] would introduce interindividual variation in the kinetics of arrival to *ap*, in addition to the CFTR gene, as suggested by the presence of diverse peaks in populations genotypically homogeneous for the CF gene (Fig. 1).

In non-CF individuals, the wild-type CFTR activity prevents the lung from bacterial infection[8] and the individual from reaching any sort of *nonreturn point*. Although the mild mutant A455E-CFTR, somewhere in the middle between wild-type and ΔF508 CFTR, cannot prevent the patient from reaching the *nonreturn point*, it significantly delays the arrival toward it.[12,27,28] Most interestingly, however, once the *nonreturn point* has been reached, the A455E-CFTR does not decrease the rate of progression into death any longer, compared with the ΔF508 mutation, and the disease takes an autonomous and common kinetics.

Colonization of the lung by pathogenic bacteria, like *Pseudomonas sp* and *Staphylococcus sp*, leads to cycles of bacterial proliferation and inflammation. Colonization, and the consequent increase in the local levels of cytokines and immune cells, is considered to be the major causes of the progressive CF lung destruction[28] and is, then, a strong candidate to be the trigger of the *nonreturn point*. The initial phase of CF would probably extend beyond the colonization of the lung. DNAse, a drug currently used for CF treatment is aimed at decreasing mucus viscosity by digestion of DNA liberated from (patient and bacterial) cells destroyed along the cycles of proliferation-inflammation.[29-31] Some CF patients show a positive response to DNAse treatment (*e.g.*, to the decrease in mucus viscosity) suggesting that the onset of the *nonreturn point* might not have occurred, although colonization is already present. Patients that are nonresponsive to DNAse, on the contrary, probably have already passed over the *nonreturn point* into the second phase of the disease, which would no longer be interactive with mucus viscosity. Given the relevance of the *nonreturn point*, efforts should be made to predict the time of its onset and to elucidate its precise nature and relationship with the bacterial proliferation-inflammation cycles.

Newborn CF patients (nCF; Fig. 3), clinically diagnosed at birth because of severe affectation and dying within the 3 first years of life, can be considered to be born "at" of "after" the *nonreturn point*. In that case, for this group of severely affected patients, the *nonreturn point* would have been reached in utero. Despite the very early time of arrival to the *nonreturn point*, these patients follow the common kinetics into death given by r_s.

Based on the precedent analysis, we support the hypothesis that the time course of CF would be characterized by two phases separated by a breakpoint (Fig. 4). The first phase extends from the initiation of the symptoms (in utero, after birth, etc.) up to the *nonreturn point*. The phase would be characterized by an abnormal function of the CFTR protein that would cause a number of CF-related initial respiratory and digestive symptoms, would make the lung more susceptible to bacterial infection and colonization, and would lead to the *nonreturn point*. This phase would be interactive with those factors affecting the fluidity of the luminal mucus, the salt concentration, and the CFTR activity in the respiratory tract. The second phase would extend from the *nonreturn point* up to death. It would be characterized by severe symptoms principally affecting the lung and not exclusively related to CF. This phase has an autonomous kinetics (measured by r_s; Figs. 2 and 3), which would not be dependent on the CF genotype (or CFTR activity). Environmental factors, healthcare, and the genetic background would introduce interindividual variation (vertical dispersion) in the main course of the phases. Diagnosis, the *nonreturn point*, and death would occur at defined intervals on the health status scale.

The existence of two phases in the course of the respiratory disease has been previously reported by others.[9] Menendez *et al.*[9] measured the decline in the ratio %FEV1/FVC in patients followed over 12 years and established the pattern of deterioration in pulmonary function. They found that the model describing the pattern would consist of a period of constant tracking followed by a linear decline phase, both separated by a breakpoint. They used the slope of the straight line equation after the breakpoint to obtain the annual rate of decline and arrived to the conclusion that "the magnitude of the rate of decline appears to be independent of the age at which the decline begins." Patient genotypes were unknown. The observation and analysis made by Menendez *et al.*[9] on 49 patients over

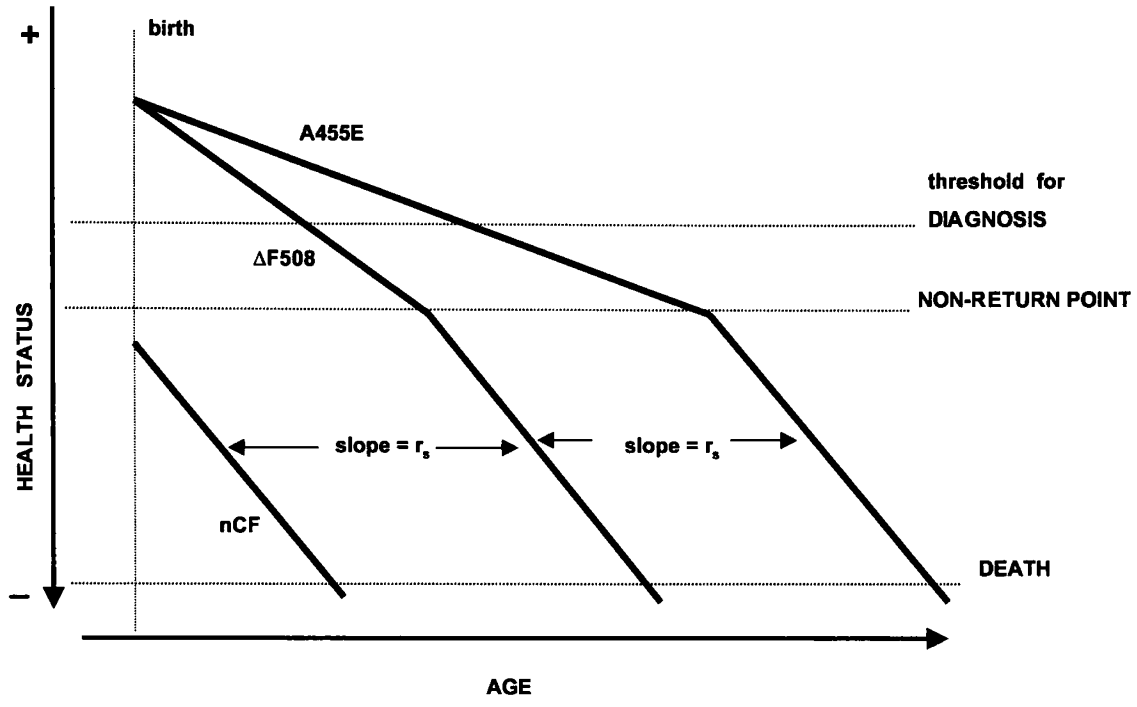

Fig. 4 Hypothetic model for the time course of the CF disease. Two phases, separated by a *nonreturn point*, would characterize the course of the CF disease. The pathogenesis and kinetics of the initial phase would be dependent on several factors, including the CFTR activity. Severe mutations (ΔF508) would lead the patient toward the *nonreturn point* faster than milder mutations (A455E). The kinetics of the second phase (measured by r_s) would be common to all populations and independent from the time necessary to reach the *nonreturn point* and from the CFTR genotype. In the nCF patients, the *nonreturn point* would presumably be either *in utero* or soon after birth. Environmental factors, healthcare, and genetic background would introduce interindividual variation (vertical dispersion) in the main course of the phases. Diagnosis, the *nonreturn point*, and death would occur at defined intervals on the health status scale.

12 years strongly supports the hypothesis proposed in this paper.

The existence of two phases in the course of CF, with the rate of decline during the second phase being independent from the CFTR, would have strong implications on CF therapeutics and diagnosis. If the course of the second phase of the CF disease is not interactive with the CFTR activity, then therapeutic approaches based on: 1) the complementation of mutant CFTR by gene transfer[32-36]; 2) the activation of mutant CFTR by pharmaceutical drugs[37,38]; or 3) the fluidification of the luminal mucus[29-31] would not be expected to be clinically effective once the *nonreturn point* has been reached.

If the hypothesis presented in this paper is true, then the identification of the nature of the *nonreturn point* and the prevention to reach the second phase by delaying the arrival to the *nonreturn point* would become key actions in the fight against CF. In that context, it can be expected that population based newborn screening will have a major impact on both the effectiveness of therapeutic interventions as well as the timing of the *nonreturn point*. Preventive healthcare on newborn screening-diagnosed patients could strongly delay the arrival to the *nonreturn point*, thus improving the quality of life of the patients and giving longer times for treatments before the *nonreturn point* is reached. For most patients, it is possible that if the CF genetic status of the newborn were known early enough, the preventive health care could delay the *nonreturn point* long enough to prevent the early entry of the patient into the final phase of the disease and, then delay early death. A confirmation of the hypothesis would need extensive long-term follow-up of genotypically different CF patient populations.

Acknowledgments

M. V. Masciovecchio was sponsored by a research fellowship from ALFIQ (Association against Cystic fibrosis, Bahia Blanca, Argentina). The work was partially supported by the National Research Council of Argentina (CONICET).

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Erratum:

The title for the CME/Invited Review Self-Assessment Exam, in the September/October 1999 issue of *Genetics in Medicine*, should have read "A new age in the genetics of deafness."