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Categories of ΔF508 homozygous cystic fibrosis twin and sibling pairs with distinct phenotypic characteristics

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Cystic fibrosis (CF), the most common severe autosomal recessive trait among Caucasians, is caused by molecular lesions in the cystic fibrosis transmembrane conductance regulator gene (CFTR). The course of the multi-organ disease CF is highly variable, suggesting the influence of environmental factors and/or modulating genes other than CFTR on the disease phenotype. To evaluate the cause of CF disease variability, the European CF Twin and Sibling Study collected data on two clinical parameters most sensitive for the course and prognosis of CF, ie weight predicted for height (wfh)% (representative for the nutritional status) and FEVPerc (representative for the pulmonary status) for a cohort of 277 sibling pairs, 12 pairs of dizygous twins and 29 pairs of monozygous twins. Of these 318 CF twin and sib pairs, 114 were reported to be homozygous for the most frequent CF disease-causing lesion, ΔF508. Intra-pair discordance was assessed by the intra-pair differences with wfh% and FEVPerc and by DELTA, a composite parameter defined by linear combination of wfh% and FEVPerc in order to describe discordance with respect to the overall disease severity. Monozygous twins had a significantly lower DELTA than dizygous twins ($P = 0.05$) indicating that CF disease severity is modulated by an inherited component in addition to the CFTR gene itself. Extreme phenotypes are considered to be more informative for the analysis of any quantitative trait. Thus, we aimed to quantify disease severity and intra-pair discordance in order to select pairs with the extreme phenotypes DIS (discordant patient pairs), CON^+ (concordant and mildy affected patient pairs) and CON^- (concordant and severely affected patient pairs). The algorithm reliably discriminated between pairs DIS, CON^+ and CON^- among the cohort of ΔF508 homozygotes. The selected pairs from these categories demonstrated non-overlapping properties for wfh%, FEVPerc and the intra-pair difference of both parameters. Twin Research (2000) 3, 277–293.

Keywords: cystic fibrosis disease severity, affected relative pair, twin and sibling study, extreme phenotypes, algorithm based selection

Introduction

Cystic fibrosis (CF) is known as the most common severe autosomal recessive disease within the Caucasian population, exhibiting an incidence of 1 in 2500 births.[1] The symptoms of the disorder are caused by an impaired function of exocrine glands in many organs, but major manifestations involve the respiratory and the gastrointestinal tracts.[1] The disease is caused by mutations in both chromosomal copies of the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[2] The course of CF is highly variable when comparing unrelated patients with identical CFTR mutation genotypes,[3,4] or even CF siblings who carry the same CFTR alleles and share several environmental factors, such as socio-economic status, general living conditions and therapeutic measures. This indicates the impact of factors other than the CFTR genotype on the CF disease phenotype. By studying affected patient pairs, the European CF Twin and Sibling Study pursues a classic approach to address the relative impact of the CFTR gene, other inherited factors and environmental effects on CF disease.

Approximately 70% of CF alleles in central European populations bear the same CFTR mutation ΔF508.[5] Consequently, half of all CF patients are homozygous for the same CFTR lesion which enables analysis of the disease severity in a group with a homogeneous mutation genotype in the major disease-causing gene. Due to the prevalence of one mutation genotype in a so-called monogenic disease that follows an autosomal recessive trait, CF is the only inherited disorder in which a relatively large number of patient pairs can be selected who carry the same mutation genotype in the disease-causing gene.

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The search for disease-modulating factors of CF equals the assessment of CF disease severity as a quantitative trait, whereby the phenotype under investigation – the CF disease severity – assumes a continuous distribution. Under this condition, individuals with extreme phenotypes are likely to have a large number of functional alleles at most loci determining the quantitative trait, and therefore extreme phenotypes are generally considered to be most informative.[6–9] Based on the phenotype of an individual, three categories of patient pairs with extreme phenotypes can be distinguished: concordant/mildly affected patient pairs (CON^+) composed of two siblings with mild disease, concordant/severely affected patient pairs (CON^-) comprised of two severely diseased siblings, and discordant pairs (DIS) wherein one sibling is mildly affected and the other is severely affected. With the aim of identifying these most informative pairs, we looked for quantitative description of disease severity and intra-pair discordance for CF patients. The evaluation was based on two clinical parameters most sensitive to course and prognosis of CF disease, ie weight expressed as weight predicted for height (wfh%) – so as to assess the nutritional status of the CF patient – and values of forced expiratory volume in 1 s (FEV1) expressed as age and gender normalised parameter, so as to assess the pulmonary status of the CF patient.[10] As a result, the CF disease phenotype was rated accounting for both major afflicted organs, ie the respiratory and the gastro-intestinal tracts.

Methods

Patients and clinical parameters

CF patient pairs were enrolled from 158 CF clinics in 14 European countries. Using a one-page evaluation form, information on gender, CFTR genotype, actual weight, height and forced expiratory volume in 1 s (FEV1) and the zygosity status of twin pairs was requested. From these data, two clinical parameters most sensitive to course and prognosis[10] were calculated: nutritional status was assessed by wfh% on the basis of age and gender corrected centiles for weight and height by Prader et al.[11] Pulmonary status was assessed by FEV1%pred which are predicted values referring to the non-CF population based on the data by Knudson et al.[12]

Among CF patients, FEV1%pred declines with age[13] (Figure 1b) as expected for this progressive lung disease. To correct for the CF-specific age decline of FEV1%pred, age-corrected centiles for the CF population for FEV1%pred, called FEVPerc, were calculated based on the European CF registry (ERCF) report of 1996[14] which compiles lung function data of FEV1%pred from 25 667 CF patients from Austria, Canada, Denmark, France, Germany,

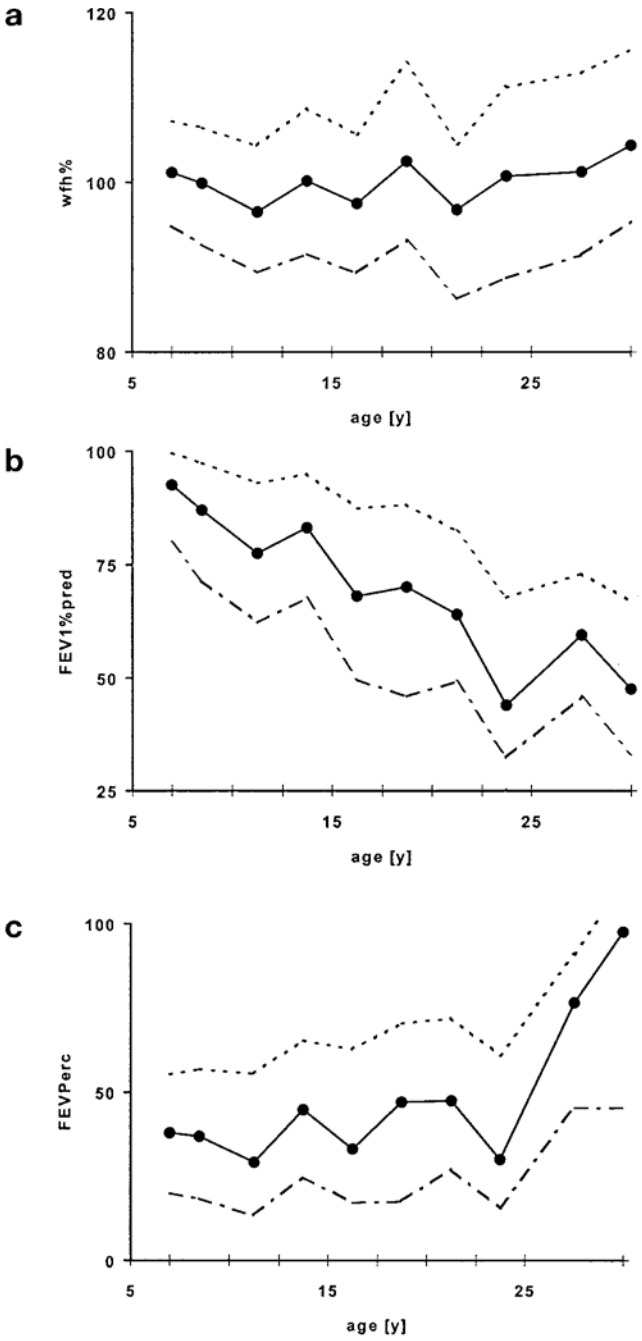

Figure 1 Age dependence of wfh% (a), FEV1%pred (b) and FEVPerc (c). The solid line indicates the median, the dotted lines the inner quartiles. The number of patients within each age group are: < 7 y: 36, 7–10 y: 75, 10–12.5 y: 66, 12.5–15 y: 75, 15–17.5 y; 84, 17.5–20 y: 76, 20–22.5 y: 48, 22.5–25 y: 50, 25–30 y: 58, > 30 y: 68

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Ireland, The Netherlands, Sweden, the United Kingdom and USA.

Consistent with data from other cross-sectional studies, the centiles were age-independent for wfh%[15] (Figure 1a) and for FEVPerc (Figure 1c) in the cohort of CF twin and sibling pairs.

Evaluation of mono and dizygosity status of CF twins

Where DNA was available, the zygosity status of twin pairs was assessed to confirm the information provided by the CF centre using the AmpFLSTR Profiler Plus™ typing kit on an ABI Prism 377 (Perkin Elmer Applied Biosystems, Foster City, CA, USA)[16] or by oligonucleotide fingerprinting of simple repeats applying in situ gel hybridisation of MboI or HinfI genomic digests.[17]

Definition of composite parameters

To assess the overall CF disease severity and the intra-pair discordance, the two clinical parameters describing a patient's nutritional and pulmonary status, ie wfh% and FEVPerc, were combined. Rank numbers x_i for wfh% and y_i for FEVPerc were assigned to the complete patient cohort, whereby a rank number of 1 indicated the most severely affected state. The disease severity of patient i was characterised by the distance form origin (DfO) in the plot of x_i versus y_i (Figure 2). The intra-pair discordance was quantified by the distance between two data points representing two patients i and j of a pair within the same diagram (DELTA). Thus disease severity and intra-pair discordance were defined by:

$$DfO = \sqrt{x_i^2 + y_i^2} \qquad (1)$$

$$DELTA = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2} \qquad (2)$$

Analysis of intra-pair rank number difference (IRND) distributions

Intra-pair similarity of CF twins and siblings was characterised by comparison of the patient pair cohort with a set of unrelated couples. To assess the intra-pair similarity of the complete cohort, the distribution of intra-pair rank number differences (IRND) was analysed.

The IRND distribution expected for unrelated couples was derived as follows. For a cohort of n individuals, or n/2 pairs, IRNDs between 1 and (n – 1) are possible. The minimal IRND of m = 1 is obtained if two individuals from a couple occupy rank numbers (n – 1) and n. (n – 1) rank number combinations of two individuals result in an IRND of 1, but there is only one possibility to obtain the maximal IRND of m = (n – 1), by occupying rank numbers 1 and n, respectively. In general, the probability f_m for any IRND m in a cohort of n individuals is given by the normalised expression

$$f_m = \frac{n-m}{\sum_{m=1}^{n-1} n-m} = \frac{2}{n}\left(\frac{n-m}{n-1}\right); \sum_{m=1}^{n-1} f_m = 1 \qquad (3)$$

To test whether the IRND distribution observed among the CF twins and siblings differed from a random IRND distribution, classes of IRNDs $\sum_{m=i}^{j} f_m$ were defined whereby the boundaries were chosen such that each class was occupied with the same probability in a random IRND distribution: $\sum_{m=i}^{j} f_m$ = const. The size of the classes was set to an expectancy value $E = n\,(\sum_{m=i}^{j} f_m)$, where E = 20, 30 or 50 couples per class.

For the analysis of the cohort of all CF twins and sibs, rank numbers were assigned to wfh% for 467 pairs (n = 934, corresponding to 24 (E = 20), 16 (E = 30) and 9 (E = 50) IRND classes) and to FEVPerc

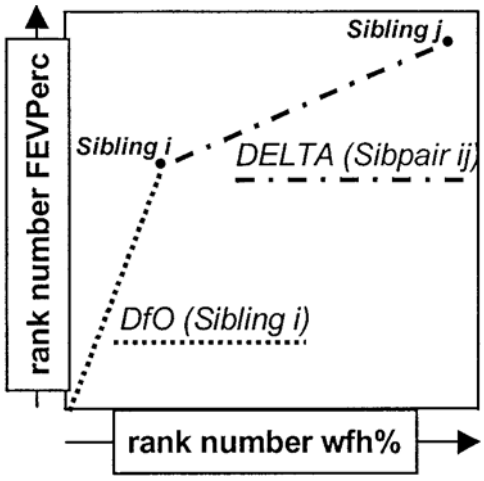

Figure 2 Definition of composite parameters. The two clinical parameters, wfh% and FEVPerc, describing the patient's nutritional and pulmonary status, were combined as a measure of the patient's overall disease severity. Rank numbers for wfh% and for FEVPerc were assigned to all patients. The disease severity of a patient was characterised as distance form origin (DfO) in the plot of the patient's rank number for FEVPerc vs the rank number for wfh%. The intra-pair discordance was quantified through the distance between two data points representing two patients i and j of a pair within the same diagram (DELTA). For the set of 318 pairs, maximal values of DfO and DELTA as defined by equations (1) and (2) are 899 and 898, respectively

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for 318 pairs (n = 648, corresponding to 16 (E = 20), 11 (E = 30) and 7 (E = 50) IRND classes). Within the cohort of ΔF508 homozygous twins and siblings, rank numbers for wfh% and FEVPerc were assigned to 114 pairs (n = 228, corresponding to 6 (E = 20), 4 (E = 30) and 2 (E = 50) IRND classes). Observed occupancy of IRND classes was compared with expectancy values by χ^2 statistics.[18]

Comparison of disease severity and intra-pair discordance

Unless stated otherwise in the results section, all comparisons were carried out using the non-parametric Mann-Whitney rank test.[19]

Results

Clinical data on 318 CF twin and sibling pairs

Data on wfh% were obtained for both patients in 467 pairs. Complete clinical data, ie wfh% and FEVPerc, could be calculated for 318 CF patient pairs (Tables 1, 2, 5), of which 114 pairs were reported to be ΔF508 homozygous. FEVPerc was lower in our patient pair cohort than expected from the ERCF report (Table 5a, Figure 1c). This systematic shift reflects different modes of data collection and coincides with the well known difference between best and average annual values of FEV1%pred which was also demonstrated by the average 8.2% difference between best and mean annual FEV1%pred value for the patient population at the CF clinic in Hannover (646 entries). The EUCFR registry recorded the best FEV1%pred within a 2 year period, whereas in our study the questionnaire asked for the most recent lung function data.

Clinical data on monozygous and dizygous CF twin pairs

Zygosity status was determined or reliably reported by the CF centre for 41 twin pairs with wfh% and FEVPerc available (Tables 1, 2, 4), ΔF508 allele frequency was 0.67, consistent with population genetic data for central Europe.[5] The average age of DZ twins was slightly lower than that of MZ twin pairs, and ΔF508 homozygous twins were younger on the day of evaluation compared with ΔF508 heterozygous twins, but the differences in age were not significant (Table 2). There was no bias between MZ and DZ twins in respect of country of origin (Table 3). However, whereas ΔF508 homozygous twins were recruited from several European countries, pairs from Italy were over-represented among ΔF508 heterozygous pairs, reflecting the lower ΔF508 frequency in southern European countries[5] (Table 3). Comparing MZ and DZ twins, the groups were indistinguishable in wfh% but FEVPerc was significantly lower for DZ twins than for MZ twins (P = 0.02; Table 4).

Intra-pair discordance was assessed by the intra-pair difference in wfh% (representative of nutritional status), the intra-pair difference in FEVPerc (representative of pulmonary status) and DELTA (composite parameter describing discordance in

Table 1 Genotype and gender of 318 CF twin and sibling pairs

	Sib pairs	Twins	
		DZ	MZ
mm	75	3	14
ff	72	2	15
mf	130	7	0
Non-ΔF508/non-ΔF508	88	3	2
Non-ΔF508/ΔF508	94	5	12
ΔF508/ΔF508	95	4	15
Total	277	12	29

Non-ΔF508: all CFTR alleles other than ΔF508, including CFTR alleles with unknown mutation; m: male; f: female.

Table 2 Distribution of age at day of evaluation of CF

	Median	Inner quartiles	Range
Twin pairs			
Monozygous (29 pairs)	14.9 years	8.8–21.8	6.8–37.2
Dizygous (12 pairs) n.s.	14.6 years	11.0–17.9	6.1–31.3
Non-ΔF508/non-ΔF508 (17 pairs)	15.1 years	12.2–22.9	6.8–37.2
ΔF508/ΔF508 (19 pairs) n.s.	12.7 years	8.8–17.3	6.1–30.3
Sibling pairs			
All siblings (277 pairs)	17.2 years	12.1–23.5	5.9–59.1
ΔF508/ΔF508 (95 pairs) P=0.005	16.9 years	11.2–20.3	6.0–38.1

Table 3 Country of origin of CF twin pairs

	Number of pairs (%) recruited from:				
	France	UK & Eire	Germany	Italy	Other[a]
Monozygous	4 (14%)	5 (17%)	7 (24%)	7 (24%)	6 (17%)
Dizygous	2 (17%)	2 (17%)	3 (25%)	3 (25%)	2 (17%)
Non-ΔF508/ΔF508	3 (18%)	4 (24%)	2 (12%)	6 (35%)	2 (12%)
ΔF508/ΔF508	3 (16%)	3 (16%)	5 (26%)	2 (11%)	6 (32%)

[a]Total number of pairs recruited from The Netherlands, Sweden, Poland, Austria and Switzerland.

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respect of overall disease severity, Figure 2). Regarding CF twin pairs with all CFTR genotypes, MZ patient pairs had a significantly lower DELTA than DZ twin pairs, but intra-pair differences in wfh% and FEVPerc were comparable for MZ and DZ twins (Table 4).

Intra-pair rank number difference distribution in a cohort of CF twins and siblings

To characterise the cohort of CF twins and sibs in terms of intra-pair similarity, the distribution of intra-pair rank number differences (IRND) of the patient pair cohort for wfh% and FEVPerc was compared with the IRND distribution of a set of randomly assigned couples (see eqn 3). The IRND distribution of the CF patient pairs differed significantly from a random IRND distribution (Table 6 and Figure 3), ie the average IRND was significantly lower in CF twin and sib pairs than in unrelated couples. Likewise, the sub-group of ΔF508 homozygous twins and sib pairs was significantly more concordant in IRND distribution of the nutritional parameter wfh% (Table 6 and Figure 3). In contrast, the IRND distribution of FEVPerc in the ΔF508 homozygous pairs was indistinguishable from that of randomly assigned couples. The range of intra-pair differences in wfh% or FEVPerc was similar in the whole cohort of CF patient pairs and in the ΔF508 homozygous sub-group (Table 5).

Properties of discordant CF patient pairs

The age-independent clinical parameters wfh% and FEVPerc were linearly combined to define the composite parameters DfO (eqn 1 and Figure 2), as a measure of the overall disease severity based on equal weight of both anthropometric and lung function parameters. The parameter DELTA, defined as the absolute distance between the DfO values of a twin or sib pair (eqn 2)was taken as the indicator of intra-pair differences of disease severity (Figure 2).

Table 4 Disease manifestation and intra-pair discordance of CF twins

	Monozygous (58 patients)			Dizygous (24 patients)			
	Median	Inner quartiles	Range	Median	Inner quartiles	Range	P
Disease manifestation							
wfh%	98.9	91.9–109.4	72.0–136.7	98.7	92.6–109.6	84.2–125.7	0.43
FEVPerc	49.6	30.6– 74.6	0.5–111	28.4	16.0– 55.0	0.1–114	0.02
Intrapair discordance							
wfh%	5.8	3.0– 9.3	0.4– 23.9	6.6	3.8– 11.7	0.7– 21.0	0.48
FEVPerc	13.8	6.0– 23.9	0.0– 69.8	27.8	5.6– 49.8	1.7– 92.6	0.14
Composite parameter							
DELTA	145.1	78.2–213.6	17.1–366.0	179.1	135.6–215.3	70.4–510.1	0.04

Table 5 Disease manifestation and intra-pair discordance in CF siblings

	All CF siblings (277 pairs)			ΔF508/ΔF508 (95 pairs)			
	Median	Inner quartiles	Range	Median	Inner quartiles	Range	P
Disease manifestation							
wfh%	100.1	91.0–109.2	54.5–175.8	98.7	89.5–105.5	54.5–145.2	0.002
FEVPerc	43.8	21.2– 74.7	–3.0–120	34.6	16.5– 60.6	–3.1–115	<0.0001
Intrapair discordance							
wfh%	11.3	5.7– 18.5	0.1– 61.4	10.2	6.1– 15.6	0.3– 53.4	0.21
FEVPerc	23.4	11.8– 41.7	0.0– 96.9	24.1	11.9– 42.7	0.0– 96.9	0.41
Composite parameter							
DELTA	244.8	145.0–349.1	8.1–771.6	253.3	179.0–347.9	46.1–694.8	0.13

Table 6 P values of χ^2 test comparisons of IRND distributions of CF twin and sibling pair cohorts with expected IRND distributions for a cohort of random couples

		E=20	E=30	E=50
All pairs				
wfh	(647 pairs)	P<0.001	P<0.001	P<0.001
FEVPerc	(318 pairs)	P<0.001	P<0.001	P<0.001
ΔF508 homozygotes				
wfh	(114 pairs)	0.025<P<0.05	0.025<P<0.05	0.025<P<0.05
FEVPerc	(114 pairs)	0.9 <P<0.95	0.7 <P<0.9	0.9 <P<0.95

E: number of pairs expected within each IRND class, see Methods for details.

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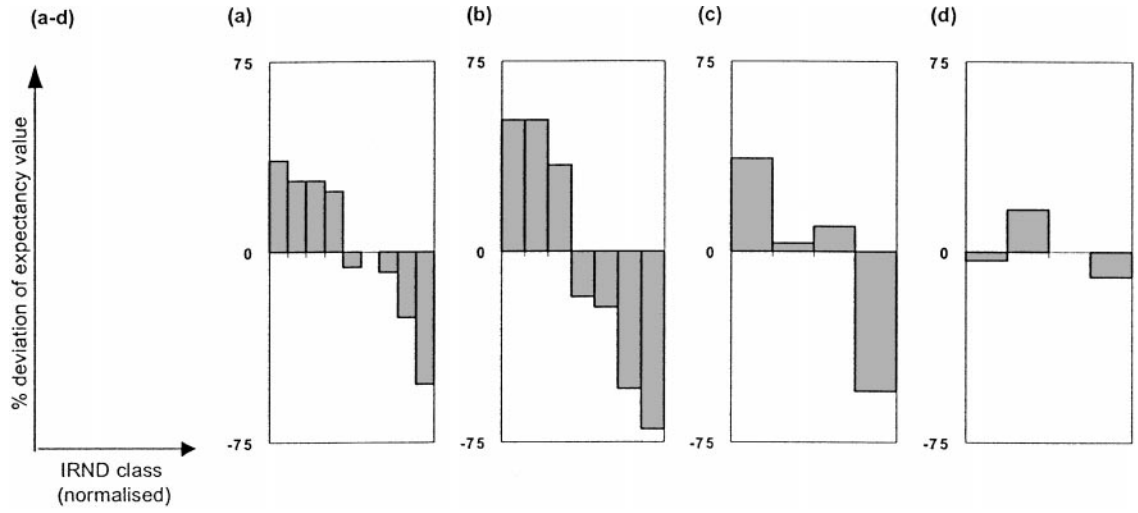

Figure 3 Differences of intra-pair rank number difference (IRND) distributions comparing a cohort of CF twins and siblings with a cohort of random couples of similar size. (a) for wfh% for patient pairs with various CFTR genotypes (467 pairs, 9 IRND classes, 50 pairs expected within each IRND class; E = 50); (b) for FEVPerc and patient pairs with various other CFTR genotypes (318 pairs, 7 IRND classes, 50 pairs expected within each IRND class; E = 50); (c) for wfh% and (d) FEVPerc of ΔF508 homozygous pairs (114 pairs, 4 IRND classes, 30 pairs expected within each IRND class; E = 30). Bars representing the 9, 7, 4 and 4 IRND classes in (a)–(d) are ordered according to the magnitude of the IRNDs from low IRND to high IRND. To allow comparison of data, the scale within plots (a)–(d) is normalised so as to display a 75% deviation from the expectancy value E with E = 50 (a) and (b) and E = 30 (c) and (d). In plots (a) and (b), +75 corresponds to an occupation of an IRND class by 88 pairs and –75 corresponds to an occupation of an IRND class by 13 pairs in contrast to the expected 50 pairs. By analogy, in plots (c) and (d) +75 corresponds to an occupation of an IRND class with 52 pairs and –75 corresponds to an occupation of an IRND class with 8 pairs in contrast to the expected 30 pairs

As shown in Figure 4, the value of DELTA did not correlate with the intra-pair age difference of sib pairs. Discordant pairs, indicated by high values of DELTA, were observed at a similar frequency in sib-pairs with high and low age differences.

For 318 patient pairs carrying various CFTR genotypes, the maximum rank number that can be assigned to wfh% or FEVPerc values is, by definition, 636. There was no correlation between the intra-pair rank number differences for wfh% and FEVPerc (data not shown): patient pairs were observed to be discordant for both parameters, or only discordant for wfh%, but concordant for FEVPerc and vice versa. Among monozygous twins, the highest value for the composite parameter DELTA was 366; 64 dizygous patient pairs had values for DELTA > 366. These extremely discordant pairs could be grouped in three cohorts as indicated in Figure 5: 15 pairs were concordant in wfh% but discordant in FEVPerc (cohort I), 25 pairs were concordant in FEVPerc but discordant in wfh% (cohort II) and 24 pairs were discordant for both parameters (cohort III). These three phenotypes were distinguished neither by the patient's absolute values for age, wfh% or FEVPerc, nor by the intra-pair age difference (see legend and table to Figure 5. There was a trend towards over-representation of ΔF508 homozygotes in cohort I compared with cohorts II and III (P = 0.15; Fisher's exact test).[20]

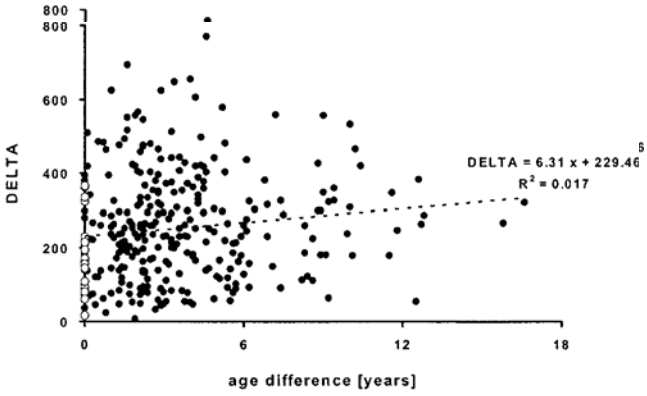

Figure 4 Composite parameter DELTA and intra-pair age difference. DELTA was defined as indicated in Figure 2 based on rank numbers for wfh% and FEVPerc to quantify intra-pair discordance. Closed circles: dizygous CF patient pairs. Open circles: monozygous CF twin pairs

The average value for DELTA was highest in cohort III and average values for intra-pair differences in DfO were lower for cohort I (Figure 5) than for cohort II and III. The intra-pair difference in DfO (DiffDfO) differentiated between pairs who are discordant (II, III) and not discordant (I) in wfh%. By defining a discordant pair (category DIS) as one composed of a sibling with low DfO and a sibling with high DfO, pairs from cohort I could be distinguished from pairs belonging to the category DIS by taking into account the intra-pair difference in DfO (DiffDfO). Table 7 shows clinical data from two

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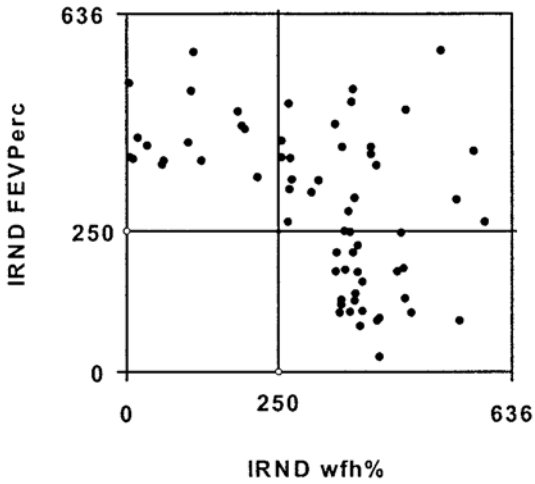

Figure 5 Characteristics of the most discordant patient pairs. Within this plot, 64 pairs with values of DELTA = 366 or higher are divided into three cohorts with the following characteristics:

	I *Upper left*	*II* *Lower right*	*III* *Upper right*
IRND wfh%	<250	>250	>250
IRND FEVPerc	>250	<250	>250
No of pairs, of which:	15	25	24
ΔF508 homozygous	7 (47%)	7 (28%)	7 (29%)
ΔF508 heterozygous	5	9	12
Average values for:			
age (years)	20.2	17.7	21.5
wfh%	104	102	99
FEVPerc	45	50	41
DELTA	441	429	537
Average values for intrapair differences in:			
age (years)	2.7	2.8	2.6
wfh%	7	28	29
FEVPerc	71	25	67
DfO	278	556	625

ΔF508 homozygous patient pairs (Examples I and II) with similar high DELTA but different DiffDfO to illustrate their phenotypic differences.

Properties of concordant CF patient pairs

To identify concordant pairs, two characteristics, ie concordance and disease severity, had to be combined to distinguish between concordant pairs with mild phenotype and concordant pairs with severe phenotype. In Table 7, data from three ΔF508 homozygous patient pairs (Examples III, IV, and V) representative of the phenotypes 'concordant/mildly affected' (category CON^+, example III), 'concordant/moderately affected' (example IV) and 'concordant/severely affected' (category CON^- example V) are shown. These three pairs all have similar low values of DELTA and intra-pair difference in DfO (DiffDfO), indicating their concordance. In concordant pairs, the intra-pair sum of DfO (ΣDfO) is a measure of disease severity: mild: high ΣDfO with both siblings displaying wfh% and FEVPerc values above the 75th centile (example III), moderate: intermediate ΣDfO with both siblings displaying wfh% and FEVPerc values close to the 50th centile (example IV) and severe: low ΣDfO with both siblings displaying wfh% and FEVPerc values below the 25th centile (example V).

Definition of rank numbers

Based on DELTA, the intra-pair sum of DfO and the intra-pair difference in DfO (DiffDfO), 5 rank numbers were calculated (Table 8): $DISC_{DELTA}$ defined the pair's position in the sequence of discordant pairs, whereby the discordance was quantified solely on the basis of DELTA. The most discordant pairs were recognised by low $DISC_{DELTA}$. Rank numbers within the sequence of concordant pairs were assigned by linearly combining a parameter describing the disease severity of a pair with a parameter describing the pair's discordance. For instance, in a diagram where the rank number for DELTA was assigned to the x axis (whereby rank number 1 corresponded to the lowest DELTA, ie the most concordant pair) and the rank number for ΣDfO was assigned to the y axis (whereby rank number 1 corresponded to the highest value for ΣDfO, ie the most mildly affected pair), the data set closest to the origin defined the most concordant/mildly diseased pair, under these criteria. Accordingly, the rank number for the distance from origin in this diagram was used to define CON^+_{DELTA}. By analogy, CON^-_{DELTA}, $CON^+_{DiffDfO}$ and $CON^-_{DiffDfo}$, were defined as in Table 8. Thus the four rank numbers for CON^+_{DELTA}, CON^-_{DELTA}, $CON^+_{DiffDfO}$ and $CON^-_{DiffDfO}$ defined a pair's position in the sequences CON^+ and CON^-, wherein discordance was defined via the composite parameter DELTA and the pair's position in the sequence CON^+ and CON^- when discordance was defined via DiffDfO.

In Figure 6, rank numbers $DISC_{DELTA}$ (Figure 6e), CON^+_{DELTA} (Figure 6f), CON^-_{DELTA} (Figure 6g), $CON^+_{DiffDfO}$ (Figure 6h) and $CON^-_{DiffDfO}$ (Figure 6i) are graphically displayed for monozygous twins. Intra-pair differences of rank numbers for wfh% and FEVPerc were lower for ΔF508 homozygous twins (Figure 6a, b) and monozygous twins with other genotypes (Figure 6c, d) ($P = 0.0005$ for wfh% and $P = 0.01$ for FEVPerc). Rank numbers for $DISC_{DELTA}$ were significantly lower for ΔF508 homozygous monozygous twins than for dizygous ΔF508 homozygotes (Figure 6e, $P = 0.05$, Mann-Whitney rank test). In contrast, rank numbers for CON^+_{DELTA}, CON^-_{DELTA}, $CON^+_{DiffDfO}$ and $CON^-_{DiffDfO}$ which were defined by a linear combination of a parameter describing the disease severity and a parameter describing the

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Table 7 Examples of CF patient pairs

	Sibling A				Sibling B			
	age (years)	wfh%	FEVPerc	DfO	age (years)	wfh%	FEVPerc	DfO
Similar high DELTA, different intra-pair differences in DfO								
Example I	25	119.5 [578]	1 [12]	578 [502]	21	120.0 [582]	82 [526]	784 [588]
DELTA = 514								
DiffDfDO = 206								
Example II	20	103.1 [389]	75 [490]	625 [432]	16	94.2 [206]	1 [27]	207 [115]
DELTA = 497								
DiffDfO = 418								
Similar DELTA, different intra-pair sum of DfO								
Example III	8	134.3 [618]	100 [624]	878 [634]	9	115.9 [550]	87 [543]	772 [574]
DELTA = 105								
DiffDfDO = 106								
ΣDfO = 1650								
Example IV	18	94.9 [222]	66 [446]	498 [273]	12	98.4 [284]	79 [515]	588 [360]
DELTA = 92								
DiffDfDO = 90								
ΣDfO = 1086								
Example V	14	92.2 [176]	14 [121]	281 [107]	6	93.9 [197]	2 [33]	199 [112]
DELTA = 90								
DiffDfDO = 14								
ΣDfO = 412								

Graphic representation of disease severity and intra-pair discordance of these pairs appears in Figure 9.
Example I; 9l, pair 1; example II: 9h, pair 7; example III: 9d, pair 1; example IV: 9m, pair 3; example V: 9a, pair 5.
DELTA and DfO: composite parameters as defined in Figure 2; DiffDfO: intra-pair difference in DfO; ΣDfO: intra-pair sum of DfO.
* Figures in brackets represent Rank numbers assigned to wfh%, FEVPerc and DfO in the cohort of 318 patient pairs.

Table 8 Definition of rank numbers $DISC_{DELTA}$, CON^{+}_{DELTA}, $CON^{+}_{DiffDfO}$, CON^{-}_{DELTA} and $CON^{-}_{DiffDfO}$

Sequence of discordant pairs: rank number derived from one parameter:
$DISC_{DELTA}$ rank number for DELTA

rank number for $DISC_{DELTA}$ = 1: highest DELTA

≡ most discordant pair

Sequence of concordant pairs: rank number derived from combination of two parameters:

all rank numbers are defined as distance from origin in a plot whereby the following parameters are assigned to:

	x axis	y axis
CON^{+}_{DELTA}	rank number for DELTA	rank number for ΣDfO
	rank number for DELTA = 1 ≡ lowest DELTA	rank number for ΣDfO =1: highest ΣDfO
	⇒ most concordant pair closest to origin	⇒ mildest affected pair closest to origin
$CON^{+}_{DiffDfO}$	rank number for DiffDfO	rank number for ΣDfO
	rank number for DiffDfO = 1 ≡ lowest DiffDfO	rank number for ΣDfO =1: highest ΣDfO
	⇒ most concordant pair closest to origin	⇒ mildest affected pair closest to origin
CON^{-}_{DELTA}	rank number for DELTA	rank number for ΣDfO
	rank number for DELTA = 1 ≡ lowest DELTA	rank number for ΣDfO =1: highest ΣDfO
	⇒ most concordant pair closest to origin	⇒ most severely affected pair closest to origin
$CON^{-}_{DiffDfO}$	rank number for DiffDfO	rank number for ΣDfO
	rank number for DiffDfO = 1 ≡ lowest DiffDfO	rank number for ΣDfO =1: lowest ΣDfO
	⇒ most concordant pair closest to origin	⇒ most severely affected pair closest to origin

DELTA and DfO: composite parameters as defined in Figure 2; DiffDfO: intra-pair difference in DfO; ΣDfO: intra-pair sum of DfO.

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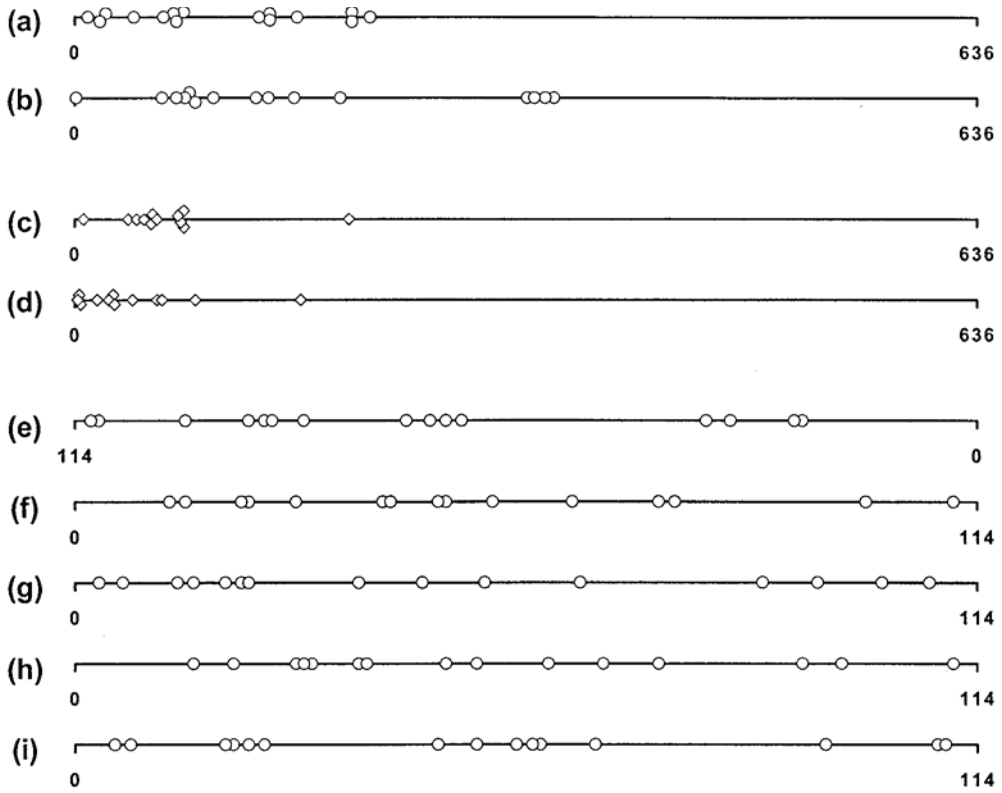

Figure 6 Intra-pair discordance (a)–(d) and rank numbers (e)–(i) for monozygous twin pairs. Intra-pair differences of rank numbers are shown for wfh% (a), (c) and FEVPerc (b), (d) for monozygous ΔF508 homozygous twins (open circles in (a), (b) and monozygous twins with other CFTR genotypes (open squares in (c), (d)). The maximal intra-pair rank number difference of 636 is displayed for a total of 318 patient pairs. Rank numbers (Table 8) for ΔF508 homozygous monozygous twins obtained from the cohort of 114 ΔF508 homozygous patient pairs are displayed in (e)–(i): (e) rank $DISC_{DELTA}$; (f) rank CON^{+}_{DELTA}; (g) rank CON^{-}_{DELTA}; (h) rank $CON^{+}_{DiffDfO}$; (i) rank $CON^{-}_{DiffDfo}$. Except for rank $DISC_{DELTA}$, no significant differences were found comparing the rank numbers between the 15 monozygous and the 99 dizygous ΔF508 homozygous patient pairs: (e) rank $DISC_{DELTA}$, P = 0.05; (f) rank CON^{+}_{DELTA}, P = 0.17; (g) rank CON^{-}_{DELTA}, P = 0.07; (h) rank $CON^{+}_{DiffDfO}$, P = 0.29; (i) rank $CON^{-}_{DiffDfO}$, P = 0.17

Table 9 Definition of patient pair categories DIS, CON^{+}, CON^{-}, ND, DC(1) and DC(2) by rank number characteristics

Category	$DISC_{DELTA}$	CON^{+}_{DELTA}	CON^{-}_{DELTA}	$CON^{+}_{DiffDfO}$	$CON^{-}_{DiffDfO}$	See Figure 9
CON^{-}	high	high	low	high	low	(a), (b), (c)
CON^{+}	high	low	high	low	high	(d), (e), (f)
ND	high	low	low	low	low	(m), (n)
DIS	low	high	high	high	high	(g), (h), (i), (j)
DC (1)	low	high	high	low	high	(l)
DC (2)	low	high	high	high	low	(k)

CON^{+}: concordant/mildly affected; CON^{-}: concordant/severely affected; ND: non-discordant (concordant/moderately affected); DIS: discordant pair; DC(1): discordant and concordant/mildly affected; DC(2): discordant and concordant/severely affected.

intra-pair concordance, did not vary significantly between monozygous and dizygous ΔF508 homozygotes (Figure 6f–i). This observation indicates that monozygous ΔF508 homozygous twins express mildly, moderately, and severely concordant affected phenotypes, and consequently rank numbers for CON^{+}_{DELTA} and the three similarly derived rank numbers did not segregate with the zygosity status of the patient pair.

Categorisation of CF patient pairs

The inter-relation of all five rank numbers $DISC_{DELTA}$, CON^{+}_{DELTA}, CON^{-}_{DELTA}, $CON^{+}_{DiffDfO}$ and $CON^{-}_{DiffDfO}$ allowed six different categories of patient pairs to be distinguished (Table 9). For a discordant patient pair (category DIS, example I in Table 7), a low rank number for $DISC_{DELTA}$, but high values for the other four rank numbers were expected. Pairs ranking low

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in $DISC_{DELTA}$ and in $CON^{+}_{DiffDfO}$ or $CON^{-}_{DiffDfO}$ were distinguishable from category DIS. These pairs were summarised respectively as discordant/concordant mild disease (DC(1); example II in Table 7) and discordant/concordant severe disease (DC(2)).

Concordant/mildy affected patient pairs (category CON^{+}, example III in Table 7) had low rank numbers for CON^{+}_{DELTA} and $CON^{+}_{DiffDfO}$, but high values for the other three rank numbers. By analogy, concordant/severely affected patient pairs (category CON^{-}, example V in Table 7) were expected to have low values for CON^{-}_{DELTA} and $CON^{-}_{DiffDfO}$, but high values for the other three rank numbers. Concordant/moderately affected pairs were summarised as non-discordant (ND, example IV in Table 7). ND pairs are expected to have similarly low rank numbers for CON^{+}_{DELTA}, CON^{-}_{DELTA}, $CON^{+}_{DiffDfO}$ and $CON^{-}_{DiffDfO}$ since, by definition, intra-pair concordance and disease severity was weighted equally for each of these rank numbers. Consequently, pairs characterised by definite intra-pair concordance but average disease severity were ranked relatively low in each of these sequences. Thus, the ND pairs were distinguished from pairs categorised as CON^{+} and CON^{-} by the low difference between their corresponding rank numbers (CON^{+}_{DELTA}–CON^{-}_{DELTA}) and/or (CON^{+}_{DELTA}–$CON^{-}_{DiffDfO}$).

To determine unambiguously the sequence of pairs within each of the categories, a pair's position in any of the sequences had to be described by the same algorithm for all CF patient pairs (Figure 7).

Categories of ⊿F508 homozygous CF twin and sibling pairs

The ranking algorithm (Figure 7) was applied to 114 ΔF508 homozygous CF twin and sibling pairs. The outcome is shown in Figures 8 and 9. As indicated in Figure 8b, 59% of the ΔF508 homozygous pairs were placed in categories DIS, CON^{+} and CON^{-} while the remaining 41% were placed in categories with intermediate phenotypes ND, DC(1) and DC(2).

To identify pairs from the three categories DIS (discordant pairs), CON^{+} (concordant/mildly diseased pairs) and CON^{-} (concordant/severely diseased pairs), we sorted the cohort of patient pairs so that subsequently ranked pairs possessed the qualities of the respective category in diminishing order: the most discordant pair (defined by rank 1 in category DIS) is followed by the second most discordant pair (defined by rank 2 in category DIS) and so forth. Likewise, pairs were ranked in categories CON^{+} and CON^{-}. This ranking is apparent from the clinical data for the patient pair cohorts shown in Figure 8c. Discordance decreased with increasing rank in the category DIS. This was true of DELTA as of intra-pair differences in wfh% and

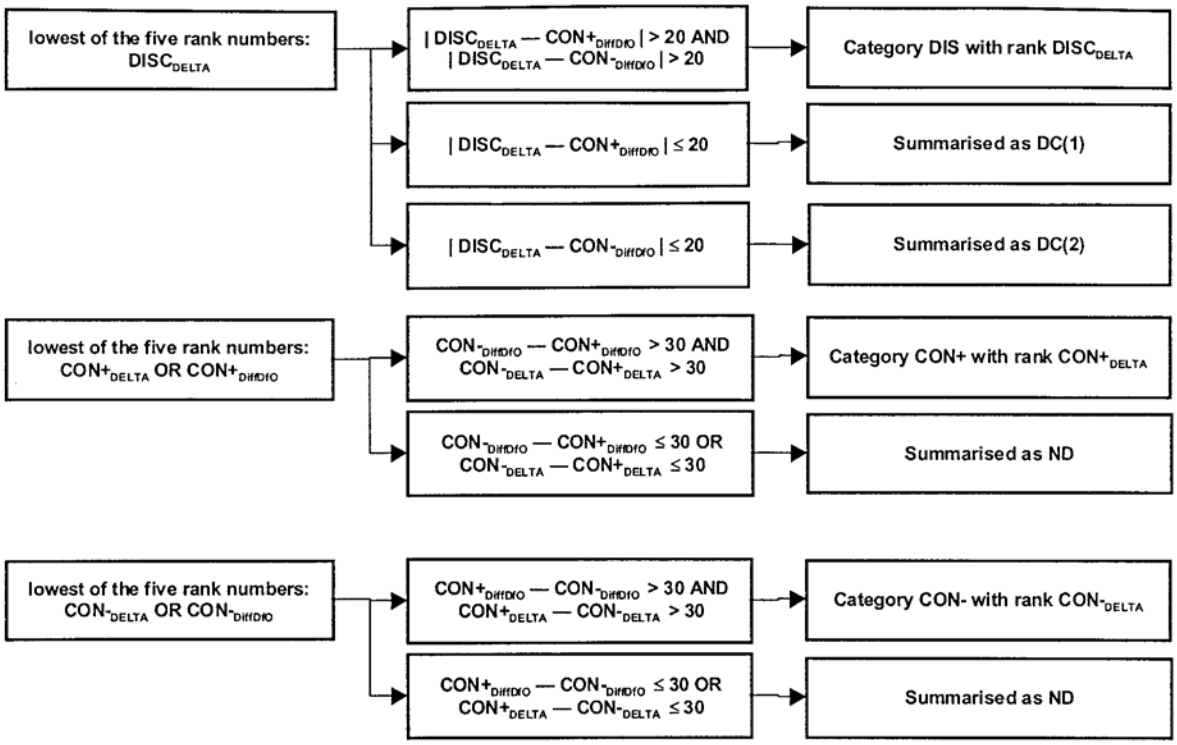

Figure 7 Flow chart for the assignment of 114 ΔF508 homozygous twin and sibling pairs to the categories DIS, CON^{+}, CON^{-}, ND, DC(1) and DC(2) based on five rank numbers derived from composite parameters (see Table 8 for definition and text for details)

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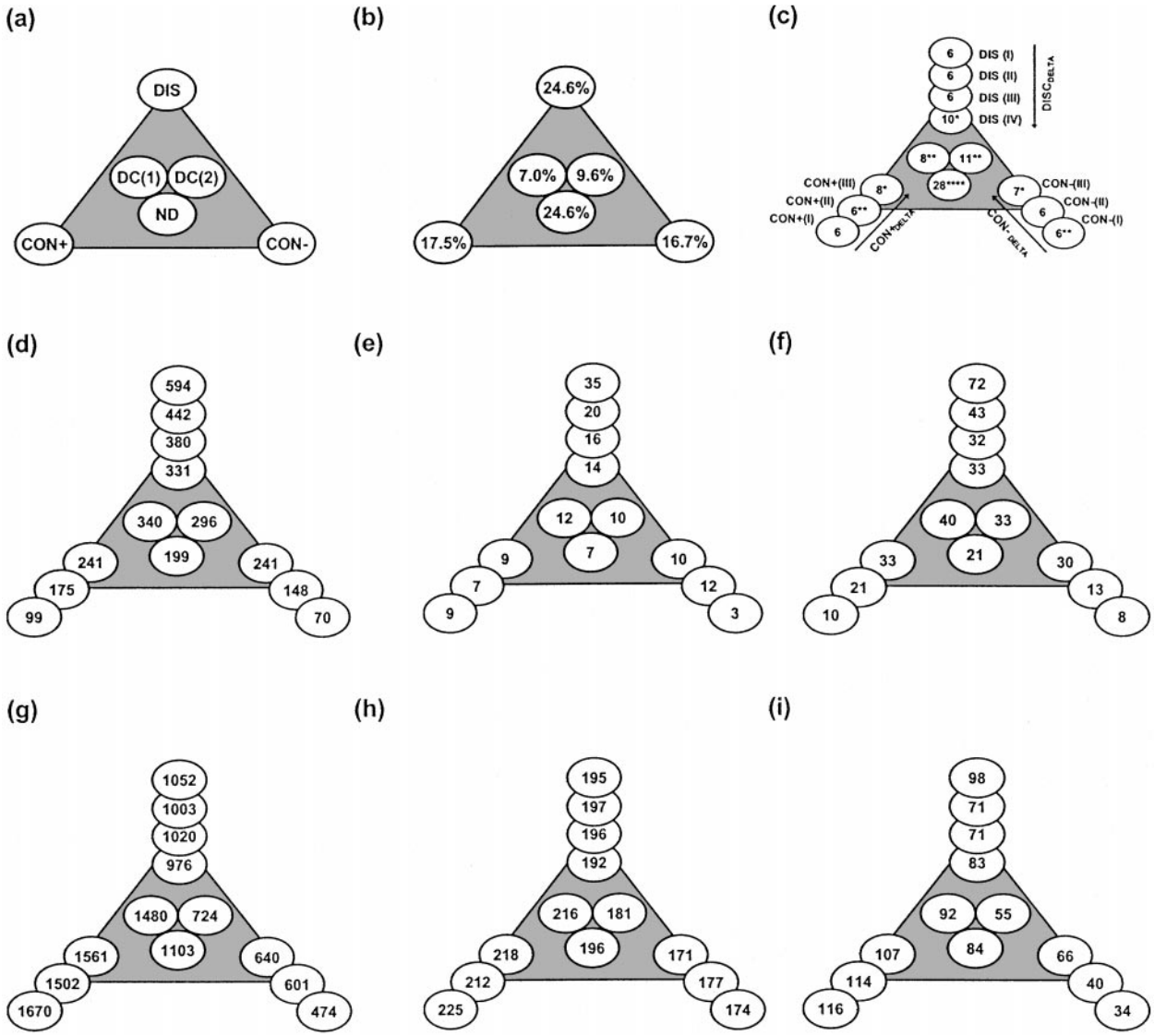

Figure 8 Disease severity and intra-pair discordance of 114 ΔF508 homozygous twin and sibling pairs assigned to the categories DIS, CON^+, CON^-, ND, DC(1) and DC(2). (a) Layout for (b)–(i) and proposed relation of the three extreme phenotypes DIS, CON^+ and CON^- to the intermediate phenotypes ND, DC(1) and DC(2). (b) Occupancy of the categories whereby 100% represents the total number of 114 ΔF508 homozygous CF twin and sibling pairs. (c) Definition of cohorts with decreasing rank numbers in the category and number of pairs per cohort. *Monozygous twins. (d)–(f) Intra-pair discordance as defined by average values within the cohorts for DELTA (d), intra-pair difference of wfh% (e) and intra-pair difference of FEVPerc (f). (g)–(i) Disease severity as defined by average values within the cohorts for the intra-pair sum of DfO (g), wfh% (h) and FEVPerc (i)

FEVPerc (Figure 8d, e and f). In the categories CON^+ and CON^-, DELTA and the intra-pair difference in FVEPerc raised with increasing rank number (Figure 8d and f). Intra-pair differences for wfh% were lower in categories CON^+ and CON^- than in category DIS. The average disease severity of pairs in category DIS were approximately half that of patient pairs ranked CON^+ or CON^- (Figure 8g, h and i).

The dissimilar, non-overlapping character of patient pairs in categories CON^+ and CON^- is evident from the dissimilar values of the average intra-pair sum in DfO, wfh% and FEVPerc (Figure 8g, h and i). Similarly, these observations are visible in Figure 9 where rank numbers for wfh% and FEVPerc for all 114 ΔF508 homozygous twin and sibling pairs are plotted against the same axes as the composite parameters DELTA and DfO (Figure 2). CON^- pairs are found in the left area of the diagram due to their low DfO (Figure 9a, b, c), whilst CON^+ pairs are located in the upper right area of the diagram indicating their high DfO (Figure 9d, e, f). Both cohorts of concordant pairs occupy distinct, non-overlapping areas within the diagram so that patients from pairs summarised as ND are located

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Figure 9 Graphical representation of rank numbers for wfh% and FEVPerc for 114 ΔF508 homozygous categorised patient pairs. Each patient pair is depicted as a set of black and white numbered data points within a diagram of the rank number FEVPerc (y axis) plotted against the rank number for wfh% (x axis) as defined in Figure 2. Both axes of all diagrams within this figure are set to the maximal rank number of 636 that can be obtained for 318 pairs. For each pair, the data point closer to the origin indicated by the black colour corresponding to the more severely affected patient. The line connecting both data points of a pair represents the composite parameter DELTA describing the intra-pair discordance. The pairs shown in figures (a) to (n) correspond to the cohorts defined in Figure 8c as is explained in the two triangular diagrams at the bottom of this figure. (a)–(c) 19 pairs categorised as CON^{-} ordered by increasing rank number for CON^{-}_{DELTA}; (d)–(f) 20 pairs categorised as CON^{+} ordered by increasing rank number for CON^{+}_{DELTA}; (g)–(j) 28 pairs categorised as DIS ordered by increasing rank number for $DISC_{DELTA}$; (k) 11 pairs summarised as DC(2); (l) 8 pairs summarised as DC(1) ordered in a sequence with decreasing DELTA; (m), (n): 28 pairs summarised as ND ordered in a sequence with increasing DELTA

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between the two extreme concordant phenotypes (Figure 9m, n).

Adjacent to the cohorts CON^+(III) (Figure 9f) and CON^-(III) (Figure 9c), more discordant pairs with high or low DfO are summarised as DC(1) (Figure 9l) and DC(2) (Figure 9k). In most of the pairs in category DIS (Figure 9g, h, i and j), the sibling with the better phenotype is characterised by higher wfh% and FEVPerc, indicated by the positive line connecting both data points of a discordant pair. In contrast, pairs summarised as DC(1) or DC(2) are concordant in wfh% but discordant in FEVPerc, or discordant in wfh% and concordant in FEVPerc, or the sibling with the better wfh% exhibits the lower FEVPerc. Thus, most data points from DC(1) and DC(2) pairs are linked by a line parallel to the y axis, or parallel to the x axis, or with a negative slope.

Discussion

The clinical phenotype of the monogenic disease CF is characterised by a broad spectrum of disease severity and variation of clinical course among patients with the same mutation genotype in the disease-causing gene, CFTR.[3,4] In recent years, modulation of CF disease by genes other than CFTR has been reported for the antiprotease alpha1-antitrypsin,[21] HLA DQB1 alleles,[22] immunoglobulin G allotypes[23] and the mannan-binding lectin.[24] Apart from these clearly identified genetic entities, the effect of residual chloride secretion on CF disease severity has been shown in intestinal tissue (modulation of basic defect)[25] and the chromosomal region 19q13 has been shown to contain a modifier for meconium ileus.[26] In summary, genetic modifiers for isolated aspects of the CF phenotype have been described, but their impact on the overall disease severity of CF remains to be evaluated.

For the multi-organ disease CF, the anthropometric parameter wfh% and the lung function parameter FEV1 are instrumental in the follow-up of CF patients for monitoring growth, development, gastrointestinal and pulmonary disease.[10] By studying affected patient pairs, ie CF twins and siblings, we have taken a classic approach to assess the effect of inherited vs environmental factors on the clinical parameters wfh% and the FEV1 derived FEVPerc.

The prominent role of the CFTR gene in CF is evident from the mode of inheritance of this autosomal recessively transmitted disease.[1] More than 800 reported CF associated CFTR mutations have been reliably classified as conferring exocrine pancreatic sufficiency or insufficiency,[27,28] but the association of CFTR mutation genotype with CF disease manifestation is less straightforward when describing nutritional status or CF pulmonary disease. The same range of disease manifestation is observed in wfh% and FEVPerc among ΔF508 homozygotes, ΔF508 compound heterozygotes and patients with non-ΔF508/non-ΔF508 genotypes (Table 5), so that the CFTR genotype–CF phenotype association is ambiguous.

To find the effect of the CFTR mutation genotype on intra-pair disease variability, we evaluated the intra-pair rank number difference (IRND) distribution among CF patient pair cohorts. When the whole cohort with various CFTR genotypes was analysed, the two members of a twin or sib pair were on average significantly more similar in both wfh% and FEVPerc than unrelated patients (Table 6 and Figure 3), demonstrating the impact of shared CFTR genotype on the CF disease phenotype. However, among ΔF508 homozygous pairs representing a cohort normalised for the genotype in the major disease-causing gene, any deviation of the observed IRND distribution from the distribution expected for unrelated couples cannot be based on the CFTR genotype. ΔF508 homozygotes differed in their IRND distribution from random couples in wfh%, but not in FEVPerc (Table 6 and Figure 3). Although more subtle effects not evident in 100 pairs would probably show up with increasing sample size, the global picture is clear. The IRNDs were apparently randomly distributed for FEVPerc, but significantly skewed to low numbers for wfh%. This can only be seen if the shared factors significantly outweigh the individual genetic and epigenetic factors.

Over-representation of shared alleles in sibs compared with unrelated subjects should account for their more similar wfh% values, because anthropometry has a strong inherited component.[29–31] However, predicted weight for height in CF is affected by eating habits and lifestyle,[32–34] type of and adherence to high-calorie diet,[32–34] administration of pancreatic enzymes and fat-soluble vitamins to treat exocrine pancreatic insufficiency and maldigestion of nutrients[35] and frequency and severity of respiratory infections.[36] All investigated patient pairs, with the exception of a few adults, shared homes, family life and CF physician, and were thus exposed to the same nutrition and medical expertise. Common medical treatment and living conditions certainly contributed to the significantly lower intra-pair variance in wfh% than inter-pair. A regime aimed at maintenance of normal weight is reflected by average values for wfh% close to 100% among CF patients[15] (see Tables 4 and 5).

As outlined above, in contrast to nutritional status, individual rather than shared factors determined lung function of sibs. Pulmonary disease in CF is characterised by a vicious cycle of infection,[37,38] inappropriate host defence,[37,38] tissue disintegration

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and remodelling[39] and irreversible loss of pulmonary function.[13,40,41] Although the airways of sib pairs were typically infected with the same bacterial strain (data not shown), differing host response seems to be more important for progression of pulmonary disease than shared environment and genetics. Generation of immune response by gene rearrangements and somatic mutation[42–44] and a high degree of polymorphism in immunorelevant loci such as the HLA[45–47] are major reasons why siblings differ more in host defence genotypes than in any other category of expressed genotypes.

The interrelation between genes determining the individual's host defence and the challenge by immunogenic, ie environmental, factors appears to have a substantial impact on the pulmonary status of CF as demonstrated by dizygous twins having a significantly lower FEVPerc compared with monozygous twins (Table 4). There is increased susceptibility to infection in CF[48] and nosocomial transmission of bacterial pathogens is a known risk[49,50] which should apply to all twin pairs irrespective of zygosity status. However, two monozygous twins are likely to possess equal host defence capabilities, whilst in dizygous twin pairs, pathogens confront susceptible individuals with a different genetic repertoire of host defences.

Taken together, these findings indicate that nutritional status in CF is modulated by few factors still detectable in the cohort of 114 ΔF508 homozygous pairs, whilst pulmonary disease in CF is modulated by numerous factors. It is therefore not surprising that the four most discordant monozygous twin pairs (Figure 6e) demonstrate intra-pair differences in FEVPerc (Figure 6b), but with wfh% intra-pair differences are inconspicuous. On the day of evaluation, these pairs were 30, 16, 9 and 9 years old. Two pairs of twins were male and two female. It remains the subject of speculation as to whether the discordance in these pairs might reflect the influence of subtle genetic differences between monozygous twins[51] such as variation in the DNA methylation pattern, the result of somatic mutations, eg at MHC loci, or differential X-inactivation in the female pairs. With equal probability, twin discordance in birth weight which has been documented among monozygous, particularly monochorionic, twin pairs[52] might give rise to differences in the twins' pulmonary status.

The comparison of intra-pair discordance in monozygous (MZ) and dizygous (DZ) twin pairs is widely accepted to separate the effect of genetic from epigenetic factors on the individual's phenotype.[53,54] The hypothesis 'a phenotypic trait is determined by inherited factors' is sustained but not proved by the observation that monozygous twin pairs are more concordant in the analysed trait than dizygous twin pairs. This applies to the parameter DELTA in the cohort of 41 CF twins with known zygosity status. The composite parameter describing intra-pair discordance based on wfh% and FEVPerc (Figure 2) was significantly lower for monozygous than for dizygous twins, indicating that monozygous CF twins are more concordant than dizygous CF twins (Table 4). However, intra-pair differences for both wfh% and FEVPerc were similar between monozygous and dizygous CF twins (Table 4). Since pulmonary function and nutritional status are clinically related[55,56] the intra-pair discordance of either parameter might be enhanced by the other. Consequently, DELTA should be more sensitive in respect of intra-pair differences than is each of the individual parameters. As a result, the concordance of monozygous twins detected by DELTA but not by wfh% and FEVPerc indicates the inherited component beside the CFTR mutation genotype that influences CF disease severity. The impact of inherited factors on CF disease is supported by the observation that DELTA is independent of intra-pair age difference in CF siblings (Figure 4): The lesser the age difference, the more have siblings shared environmental living conditions. Independence of DELTA from intra-pair age difference suggests a stronger impact of shared genetics than shared environmental factors on disease manifestation in CF. In other words, the shared time of exposure to environmental factors and the action of the environmental factors on sibs at a comparable stage of development, ie the extent of sharing a patient's history and state of development, is less important than age-independent factors.

Given the hypothesis that CF disease manifestation is substantially influenced by genes other than CFTR, methods of reverse genetics may be applied to identify the loci involved. However, the success of such an approach will be determined by the selection of appropriate candidates for such a study. For the analysis of a quantitative trait extreme phenotypes are generally considered to be more informative.[6–9] Consequently a strategy to identify these most informative patient pairs was developed. As the disease phenotype had to be described quantitatively metric data was employed to evaluate the complex multi-organ disease CF. Using wfh% and FEVPerc, two clinical parameters most sensitive to the course and prognosis of CF[10] were combined in order to describe the overall disease severity in the two major organs afflicted, ie the respiratory and gastrointestinal tracts. Furthermore the composite parameter DELTA describing the intra-pair discordance was employed in the selection procedure. As has been described in detail above, DELTA was more sensitive to the influence of genetic background on CF disease severity and therefore the employment of this parameter for patient pair selection should

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facilitate the identification of subjects informative in a genetic study.

To avoid ambiguous scores, a computer-assisted method was used to rank patient pairs in those categories of patient pairs exhibiting the phenotypes' concordant mild disease (CON^+), concordant/severe disease (CON^-) and discordant (DIS). To ensure that the selected pairs represented the extremes of a continuous spectrum of phenotypes, there had to be no overlap of clinical characteristics in the comparison of pairs from the cohorts DIS, CON^+ and CON^-. As demonstrated in Figure 8, the algorithm employed for ranking the 114 ΔF508 homozygous pairs resulted in the identification of CON^+ and CON^- patient pairs with non-overlapping wfh% and FEVPerc values. Likewise, discordance in both clinical parameters was distinct among pairs ranked DIS compared with pairs ranked CON^+ or CON^-.

In conclusion, the ΔF508 homozygous twin and sibling pairs expressed various phenotypes. Three categories of extreme phenotypes – DIS, CON^+ and CON^- and three categories with intermediate and/or uncommon phenotypes could be distinguished and were characterised as phenotypically distinct entities in respect of pulmonary function and the nutritional state of the CF patients.

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References

1 Welsh MJ, Tsui LC, Boat TF, Beaudet AL. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Basis of Inherited Disease. McGraw-Hill, New York: 1995; pp 3799–3876.
2 Kerem BS, Rommens JM, Buchanan JA, Markiewicz D, Cox RTK, Chakravarti A, Buchwald M, Tsui LC. Identification of the cystic fibrosis gene: genetic analysis. Science 1989; 245: 1073–1080.
3 Johannsen HK, Nir M, Hoiby N, Koch C, Schwartz M. Severity of cystic fibrosis in patients homozygous and heterozygous for the ΔF508 mutation. Lancet 1991; 337: 631–634.
4 Kerem E, Corey M, Kerem BS, Rommens J, Markiewicz D, Levison H, Tsui LC, Durie P. The relationship between genotype and phenotype in cystic fibrosis – analysis of the most common mutation (ΔF508). N Engl J Med 1990; 323: 1517–1522.
5 European Working Group on Cystic Fibrosis Genetics. Gradient of distribution in Europe of the major CF mutation and of its associated haplotype. Hum Genet 1990; 85: 436–445.
6 Dolan CV, Boomsma DI. Optimal selection of sib pairs from random samples for linkage analysis of a QTL using the EDAC test. Behav Genet 1998; 28: 197–206.
7 Risch N, Zhang H. Extreme discordant sib pairs for mapping quantitative trait loci in humans. Science 1995; 16: 268: 1584–1589.
8 Risch NJ, Zhang H. Mapping quantitative trait loci with extreme discordant sib pairs: sampling considerations. Am J Hum Genet 1996; 58: 836–843.
9 Eaves L, Meyer J. Locating human quantitative trait loci: guidelines for the selection of sibling pairs for genotyping. Behav Genet 1994; 24: 443–455.
10 Corey M, McLaughlin FJ, Williams M, Levinson H. A comparison of survival, growth, and pulmonary function in patients with cystic fibrosis in Boston and Toronto. J Clin Epidemiol 1998; 41: 583–591.
11 Prader A, Largo RH, Molinari L, Issler C. Physical growth of Swiss children from birth to 20 years of age. First Zurich longitudinal study of growth and development. Helv Pediatr Acta Suppl 1989; 52: 1–125.
12 Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow volume curve with growth and ageing. Am Rev Respir Dis 1983; 127: 725–734.
13 Corey M, Edwards L, Levinson H, Knowles M. Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis. J Pediatr 1997; 131: 809–814.
14 Available from URL: http://www.ERCF.org
15 Lai HC, Corey M, FitzSimmons S, Korosok MR, Farrell PM. Comparison of growth status of patients with cystic fibrosis between the United States and Canada. Am J Clin Nutr 1999; 69: 531–538.
16 Sachetti L, Calcagno G, Coto I, Tinto N, Vuttariello E, Salvatore F. Efficiency of two different nine-loci short tandem repeat systems for DNA typing purposes. Clin Chem 1999; 45: 178–183.
17 Epplen JT, Melmer G, Schmidt P, Roewer L, Hundrieser J, Epplen C, Buitkamp J. On the potential of simple repetitive DNA for fingerprinting in clinical, forensic, and evolutionary dynamic studies. Clin Invest 1992; 70: 1043–1051.
18 Weber E. Grundriss der biologischen Statistik. Gustav Fischer Verlag, Stuttgart: 1980; 190–191.
19 Weber E. Grundriss der biologischen Statistik. Gustav Fischer Verlag, Stuttgart: 1980; 184–190.
20 Weber E. Grundriss der biologischen Statistik. Gustav Fischer Verlag, Stuttgart: 1980; 204–207.

https://doi.org/10.1375/twin.3.4.277 Published online by Cambridge University Press

21 Döring G, Krogh-Johansen H, Weidinger S, Hoiby N. Allotypes of alpha-1-antitrypsin in patients with cystic fibrosis, homozygous and heterozygous for deltaF508. Pediatr Pulmonol 1994; 18: 3–7.
22 Carrington M, Krueger LJ, Holsclaw DS Jr, Iannuzzi MC, Dean M, Mann D. Cystic fibrosis-related diabetes is associated with HLA DQB1 alleles encoding Asp-57-molecules. J Clin Immunol 1994; 14: 353–358.
23 Ciofu O, Pressler T, Pandey JP, Hoiby N. The influence of allotypes on the IgG subclass response to chromosomal beta-lactamase of Pseudomonas aeruginosa in cystic fibrosis patients. Clin Exp Immunol 1997; 108: 88–94.
24 Garred P, Pressler T, Madesen HO, Fredriksen B, Svejgaard A, Høiby N, Schwartz M, Koch C. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J Clin Invest 1999; 104: 431–437.
25 Veeze HJ, Halley DJ, Bijman J, de Jongste JC, de Jonge HR, Sinaasappel M. Determinants of mild clinical symptoms in cystic fibrosis patients. Residual chloride secretion measured in rectal biopsies in relation to the genotype. J Clin Invest 1994; 93: 461–466.
26 Zielenski J, Corey M, Rozmahel R, Markiewicz D, Aznarez I, Casals T, Larriba S, Mercier B, Cutting GR, Krebsova A, Macek M Jr, Langefelder-Schwind E, Marshall BC, DeCelie-Germana J, Claustres M, Palacio A, Bal J, Nowakowska A, Ferec C, Estivill X, Durie P, Tsui LC. Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. Nat Genet 1999; 22: 128–129.
27 Santis G, Osborne L, Knight RA, Hodson ME. Independent genetic determinants of pancreatic and pulmonary status in cystic fibrosis. Lancet 1990; 336: 1081–1084.
28 Kristidis P, Bozon D, Corey M, Markiewicz D, Rommens J, Tsui LC. Genetic determinants of exocrine pancreatic function in cystic fibrosis. Am J Hum Genet 1992; 50: 1178–1184.
29 Katzmarzyk PT, Mahaney MC, Blangero J, Quek JJ, Malina RM. Potential effects of ethnicity in genetic and environmental sources of variability in the stature, mass, and body mass index of children. Hum Biol 1999; 71: 977–987.
30 Pietilainen KH, Kaprio J, Rissanen A, Winter T, Rimpela A, Viken RJ, Rose RJ. Distribution and heritability of BMI in Finnish adolescents aged 16 y and 17 y: a study of 4884 twins and 2509 singletons. Int J Obes Relat Metab Disord 1999; 23: 107–115.
31 Ginsburg E, Livshits G, Yakovenko K, Kobylianski E. Major gene control of human body weight and BMI in five ethnically different populations. Ann Hum Genet 1998; 62: 307–322.
32 Daniels LA, Davidson GP. Current issues in the nutritional management of children with cystic fibrosis. Aust Pediatr J 1989; 25: 261–266.
33 Creveling S, Light M, Gardner P, Greene L. Cystic Fibrosis, nutrition, and the health care team. J Am Diet Assoc 1997; 97: S186–S191.
34 Stark LJ, Mulvihill MM, Powers SW, Jelalian E, Keating K, Creveling S, Byrnes-Collins B, Harwood I, Passero MA, Light M, Miller DL, Hovell MF. Behavioral intervention to improve calorie intake of children with cystic fibrosis: treatment versus wait list control. J Pediatr Gastroenterol Nutr 1996; 22: 240–253.
35 Anthony H, Collins CE, Davidson G, Mews C, Robinson P, Shepherd R, Stapleton D. Pancreatic enzyme replacement therapy in cystic fibrosis: Australian guidelines. Pediatric Gastroenterological Society and the Dietians Association of Australia. J Pediatr Child Health 1999; 35: 125–129.
36 Reilly JJ, Ralston JM, Paton JY, Edwards CA, Weaver LT, Wilkinson J, Evans TJ. Energy balance during acute respiratory exacerbations in children with cystic fibrosis. Eur Respir J 1999; 13: 804–809.
37 Döring G. Cystic fibrosis respiratory infections: interactions between bacteria and host defence. Monaldi Arch Chest Dis 1997; 52: 363–366.
38 Koch C, Høiby N. Pathogenesis of cystic fibrosis. Lancet 1993; 341: 1065–1069.
39 Tomashefski JF Jr, Bruce M, Goldberg HI, Dearborn DG. Regional distribution of macroscopic lung disease in cystic fibrosis. Am Rev Respir Dis 1986; 133: 535–540.
40 Baltimore RS, Christie CD, Smith GJ. Immunohistopathologic localization of Pseudomonas aeruginosa in lungs from patients with cystic fibrosis. Implications for the pathogenesis of progressive lung deterioration. Am Rev Respir Dis 1989; 140: 1650–1661.
41 Corey M, Farewell V. Determinants of mortality from cystic fibrosis in Canada, 1970–1989. Am J Epidemiol 1996; 143: 1007–1017.
42 Storb U. Progress in understanding the mechanism and consequences of somatic hypermutation. Immunol Rev 1998; 162: 5–11.
43 Rajewski K. Clonal selection and learning in the antibody system. Nature 1996; 381: 751–758.
44 Weill JC, Reynaud CA. Rearrangement/hypermutation/gene conversion; when, where and why? Immunol Today 1996; 17: 92–97.
45 Dawkins R, Leelayuwat C, Gaudieri S, Tay G, Hui J, Cattley S, Martinez P, Kuluski J. Genomics of the major histocompatibility complex: haplotypes, duplication, retroviruses and disease. Immunol Rev 1999; 167: 275–304.
46 Hughes AL, Yeager M. Natural selection at major histocompatibility complex loci of vertebrates. Annu Rev Genet 1998; 32: 415–435.
47 Hill AV. The immunogenetics of human infectious diseases. Annu Rev Immunol 1998; 16: 593–617.
48 Tümmler B, Kiewitz C. Cystic fibrosis: an inherited susceptibility to bacterial respiratory infections. Mol Med Today 1999; 5: 351–358.
49 Tümmler B, Koopmann U, Grothues D, Weissbrodt H, Steinkamp G, von der Hardt H. Nosocomial acquisition of Pseudomonas aeruginosa by cystic fibrosis patients. J Clin Microbiol 1991; 29: 1265–1267.
50 Grothues D, Koopmann U, von der Hardt H, Tümmler B. Genome fingerprinting of Pseudomonas aeruginosa indicates colonization of cystic fibrosis siblings with closely related strains. J Clin Microbiol 1988; 26: 1973–1977.
51 Corey LA, Nance WE, Kang KW, Christian JC. Effects of type and placentation on birthweight and its variability in monozygotic and dizygotic twins. Acta Genet Med Gemellol Croma 1988; 37: 229–238.
52 Machin G. Some causes of genotypic and phenotypic discordance in monozygotic twin pairs. Am J Med Genet 1996; 61: 216–228.
53 Martin N, Boomsma D, Machin G. A twin-pronged attack on complex traits. Nat Genet 1997; 17: 387–392.
54 Phillips DIW. Twin studies in medical research: can they tell us whether diseases are genetically determined? Lancet 1993; 341: 1008–1009.
55 Steinkamp G, von der Hardt H. Improvement of nutritional status and lung function after long-term nocturnal gastrostomy feedings in cystic fibrosis. J Pediatr 1994; 124: 244–249.
56 Steinkamp G, Drommer A, von der Hardt H. Resting energy expenditure before and after treatment for Pseudomonas aeruginosa infection in patients with cystic fibrosis. Am J Clin Nutr 1993; 57: 685–689.

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