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Clinical expression of cystic fibrosis in a large cohort of Italian siblings

Vito Terlizzi[1*], Marco Lucarelli[2], Donatello Salvatore[3], Adriano Angioni[4], Arianna Bisogno[5], Cesare Braggion[1], Roberto Buzzetti[6], Vincenzo Carnovale[7], Rosaria Casciaro[8], Giuseppe Castaldo[9,10], Natalia Cirilli[11], Mirella Collura[12], Carla Colombo[5], Antonella Miriam Di Lullo[13], Ausilia Elce[14], Vincenzina Lucidi[15], Elisa Madarena[16], Rita Padoan[17], Serena Quattrucci[18], Valeria Raia[19], Manuela Seia[20], Lisa Termini[21] and Federica Zarrilli[22]

Abstract

Background: A clinical heterogeneity was reported in patients with Cystic Fibrosis (CF) with the same *CFTR* genotype and between siblings with CF.

Methods: We investigated all clinical aspects in a cohort of 101 pairs of siblings with CF (including 6 triplets) followed since diagnosis.

Results: Severe lung disease had a 22.2% concordance in sib-pairs, occurred early and the FEV_1% at 12 years was predictive of the severity of lung disease in the adulthood. Similarly, CF liver disease occurred early (median: 15 years) and showed a concordance of 27.8% in sib-pairs suggesting a scarce contribution of genetic factors; in fact, only 2/15 patients with liver disease in discordant sib-pairs had a deficiency of alpha-1-antitrypsin (a known modifier gene of CF liver phenotype). CF related diabetes was found in 22 pairs (in 6 in both the siblings). It occurred later (median: 32.5 years) and is strongly associated with liver disease. Colonization by *P. aeruginosa* and nasal polyposis that required surgery had a concordance > 50% in sib-pairs and were poorly correlated to other clinical parameters. The pancreatic status was highly concordant in pairs of siblings (i.e., 95.1%) but a different pancreatic status was observed in patients with the same *CFTR* mutations. This suggests a close relationship of the pancreatic status with the "whole" *CFTR* genotype, including mutations in regulatory regions that may modulate the levels of *CFTR* expression. Finally, a severe course of CF was evident in a number of patients with pancreatic sufficiency.

Conclusions: Physicians involved in care of patients with CF and in genetic counseling must be aware of the clinical heterogeneity of CF even in sib-pairs that, at the state of the art, is difficult to explain.

Keywords: CFTR, Genotype, Phenotype, Modifier genes, FEV_1, Pseudomonas aeruginosa

Background

Cystic fibrosis (CF) is the most common, severe, autosomal recessive inherited disease among Caucasians [1]. It is usually characterized by elevated sweat chloride levels (SCL), pancreatic insufficiency (PI), progressive lung disease with chronic bacterial infections of lower airways and male infertility due to obstructive azoospermia. More than 2000 variants have been identified in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene so far (www.genet.sickkids.on.ca), but only few have been functionally characterized [2]. Some of them may be grouped in six classes according to the known effect on CFTR synthesis and/or function [2, 3]. However, although life-expectance and severity of the disease depend on the class of mutations [4, 5], there is a wide clinical heterogeneity in CF patients carrying the same *CFTR* genotype and even between siblings and twins with CF [6]. Several sources contribute to such variability as mutations in non-coding regions of the *CFTR* gene [7–9], intronic variants [9–11] and complex alleles [12, 13] making the genotype-phenotype relationship more complex.

* Correspondence: terlizzivito@libero.it
[1]Dipartimento di Pediatria, Centro Regionale Toscano per la Fibrosi Cistica, Azienda Ospedaliero-Universitaria Meyer, Viale Gaetano Pieraccini 24, 50139 Florence, Italy
Full list of author information is available at the end of the article

In the last decade several studies explored the role of modifier genes of CF phenotype [14] for meconium ileus (MI) [15–17], CF-related diabetes (CFRD) [18], CF liver disease (CFLD) [19], lung disease [20, 21] and the colonization by *P. aeruginosa* [22]. However, environmental factors, such as the quality of health care, compliance to therapy [23], lifestyle, and socio-economic status [24] play a pivotal role in the outcome of CF.

The strong heterogeneity of CF impacts on the genetic counseling and on the reproductive planning of families that have CF affected children. In the present study, we evaluated a series of pairs of siblings with CF monitored since diagnosis to study the degree of clinical heterogeneity of the CF phenotype, assessing and correlating clinical aspects and complications of the disease.

Methods

Patients

The study population consisted of patients followed since diagnosis at 12 CF specialized care centers in Italy who met diagnostic criteria for CF [25] and had at least one sibling suffering from CF. According to the current Italian legislation, we obtained from all patients (or from their legal guardian) the informed consent to use anonymously clinical data for research purposes.

Methods

Sweat chloride levels were tested according to guidelines [26]. We screened for a panel of mutations and for the most common *CFTR* rearrangements [27]; thus, we carried out gene sequencing (detection rate 95%) [28, 29] in patients in which one or both mutations resulted undetected after first level analysis [30]. We analyzed 7 intragenic *CFTR* short tandem repeats [31] to verify that both members of four sibling-pairs carrying only one known mutation had the same *CFTR* genotype. Molecular analysis revealed more than 50 different *CFTR* mutations in our patients and only for a half of them functional studies had defined the molecular effect and the class; furthermore, some of these latter mutations may combine more defects and can be attributed to more classes [2]. Thus, we did not perform correlations between the *CFTR* genotype and clinical parameters. However, Additional file 1: Table S1 provides the *CFTR* genotype of each patient included in the study.

Meconium ileus was defined using the criteria described [15]. The forced expiratory volume (FEV_1), expressed as the percentage of predicted value for age, according to standardized reference equations for spirometry [32] was recorded. To avoid age-related differences between siblings of each pair, we recorded as current FEV_1 the last value of the younger sibling and that of the older sibling at the same age. In the case of patients who had died or had undergone lung transplantation we recorded the last FEV_1 and the value of the living or non-transplanted member of the sibling-pair at the same age. Given the inter-individual variability of FEV_1 and the evolution of lung damage with age [33] the patients were classified as severe or mild according with the criteria by Schluchter et al., that take into account both the FEV_1 value and age [34, 35]. The airway colonization by *P. aeruginosa* was identified by sputum or oropharyngeal swab culture. Chronic infection was defined according to the modified Leeds criteria [36].

Fecal pancreatic elastase was evaluated annually and at least 3 months before enrollment. Pancreatic sufficiency was defined on the basis of fecal pancreatic elastase-1 higher than 200 mcg/g measured in the absence of acute pancreatitis or gastrointestinal diseases. Pancreatitis was defined as acute or chronic according to the report from International study group of pediatric pancreatitis [37] excluding all known causes of pancreatitis. Liver disease was evaluated by means of clinical, biochemical or ultrasonography abnormalities recorded in two consecutive examinations within a 3-month period, in the absence of other causes of congenital or acquired chronic liver disease [38]. Patients were considered as affected by CFLD when they had liver cirrhosis, considered as the extreme phenotype to define liver disease in our study, with imaging techniques showing nodular hepatic parenchyma and signs of portal hypertension [17]. A glucose tolerance test was performed annually in all patients with CF and the diagnosis of CFRD was made according to the standard American Diabetes Association criteria [39]. Finally, the history of nasal polyposis requiring surgery was evaluated.

Statistics

The concordance for disease (symptoms or complications) within sibling-pairs was calculated as the ratio between the number of pairs concordant for the symptom/complication and the number of pairs in which at least one member had the symptom/complication [15].

Results and discussion

Study population

We studied 208 patients with CF (median age: 30 years, range 12–61 years; 106 males); of these, 40 (19.2%) were diagnosed by newborn screening (NBS) and 168 (80.8%) by symptoms or by family history. All the 208 patients were over 12 years of age and 172/208 patients (82.7%) were over 18 years. The study included 95 pairs of siblings (22 pairs of females, 27 pairs of males and 46 pairs of one female and one male) and 6 sets of 3 siblings (2 including two males and one female, 2 including two females and one male and 2 including three females).

In 80/101 pairs (79.2%) all the siblings were above 18 years of age.

Pancreatic status

As shown in Table 1, in our pairs of siblings we found a concordance for PI of 95.1% (i.e., only in three sibling-pairs the pancreatic status resulted discordant) confirming the known correlation between the pancreatic status and the *CFTR* genotype. However, pairs of siblings with the same *CFTR* mutations may have PS or PI, and it is known that mutations like the R334W, the R347P, the 2789 + 5G > A [40], or the D1152H [41] may be associated with PS or PI. This means that the high degree of concordance for the pancreatic status found in siblings depends on the "whole" *CFTR* genotype (that is quite completely shared by siblings) including either mutations in the coding regions and variants in non-coding regions like the promoter or the intronic regions [7, 8] that together define the levels of residual activity of the CFTR protein at pancreatic level and thus may modulate the pancreatic status of each patient.

Table 1 compares the clinical characteristics and the complications in sib-pairs with different pancreatic status. In sib-pairs with PI, MI, severe lung disease, CFLD and CFRD occurred more frequently (even if the small number of cases preclude a statistical comparison) according to the current literature [42, 43].

Table 1 Concordance within sib-pairs for PI and correlation between the pancreatic status and meconium ileus (MI), severity of lung disease, CF liver disease (CFLD), CF related diabetes (CFRD) and recurrent panreatitis (RP) in 101 pairs of siblings with CF. N: absence of the symptom/complication

Concordance within sib-pairs for PI (%)95.1				
		PI/PI	PI/PS	PS/PS
Number of sib-pairs		58	3	40
Meconium ileus:	MI/MI	4	0	0
	MI/N	5	0	2
	N/N	49	3	38
Lung disease:	Severe/severe	6	0	0
	Severe/mild	15	1	5
	Mild/mild	37	2	35
CF liver disease:	CFLD/CFLD	3	2	0
	CFLD/N	11	0	2
	N/N	44	1	38
CF related diabetes:	CFRD/CFRD	5	0	1
	CFRD/N	11	3	2
	N/N	42	0	37
Pancreatitis:	RP/RP	1	0	1
	RP/N	1	0	6
	N/N	56	3	33

Recurrent pancreatitis was significantly more frequent in sib-pairs with PS in agreement with the concept that recurrent pancreatitis in patients with CF is due to ductal plugging that typically occurs in patients with PS [43, 44]. On the other hand, modifier genes other than *CFTR* may enhance the risk to develop recurrent/chronic pancreatitis [45, 46] explaining some cases of patients with PI that developed recurrent pancreatitis (Additional file 1: Table S1).

Interestingly, among the patients with PS we found MI in 2 cases, severe lung disease in 6 and complications such CFLD in 5 or CFRD in 7 cases (Additional file 1: Table S1). We are confident that our patients with PS would not develop PI later either because most patients with PS have at least one mild mutation and because their age is higher than that of patients with PI (mean 34.6 years, median 33 years for patients with PS versus a mean age of 28.0 years and a median of 25 years of patients with PI). Thus, a severe clinical phenotype may occur in a percentage of patients with PS. Considering that most patients with CF and PS are nowadays diagnosed by NBS, the presence of these complicated cases suggests that patients with PS would be monitored with the same care of patients with PI.

Meconium ileus and DIOS

We found a 36.4% of concordance for MI in sib-pairs, i.e., 4 pairs were concordant for MI and 7 pairs were discordant suggesting that in addition to the *CFTR* genotype other non-genetic and genetic factors contribute to the development of MI. Among the genetic ones, in CFTR-deficient mice, that is an excellent model for human MI, three potential modifier loci for MI were found on chromosomes 1, 9 and 10, respectively [47]. Subsequent studies in humans identified at least 2 modifier loci for MI [15] and thus, the SLC4A4 gene was candidate as modifier genes for MI in patients with CF [48]. Furthermore, a role of KCNN4 as a modifier gene of MI was suggested [16, 17].

We found five patients with CF that had DIOS; such complication was revealed in both the members of two sib-pairs and in a member of a further pair (Additional file 1: Table S1). Interestingly, none of the five patients experienced MI, reinforcing the view that DIOS, once considered the adult equivalent of MI, has an independent etiology [15].

Lung disease

We found 34/208 patients (16.4%) with severe lung disease. The concordance for severe lung disease was 22.2%, i.e., in 21 pairs the lung status was discordant (i.e., severe versus mild) between siblings (Fig. 1) while in 6 pairs both the siblings had a severe lung disease suggesting that environmental factors and genes inherited

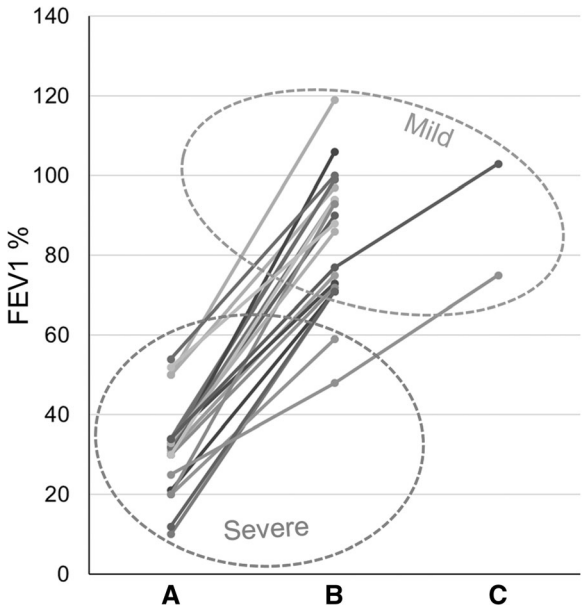

Fig. 1 FEV_1% of predicted for age in 21 pairs of siblings with cystic fibrosis discordant for the severity of lung disease. The classification as mild or severe was performed according to the criteria by Schluchter et al. [30] that take into account both the FEV_1 value and age

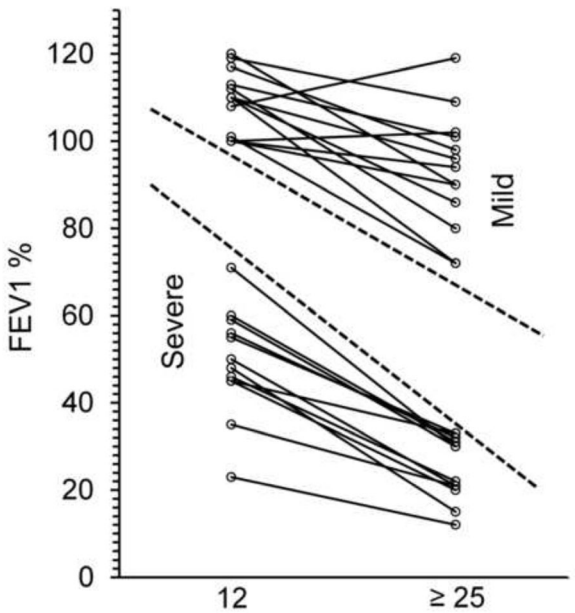

Fig. 2 FEV_1% of predicted for age in 25 patients with cystic fibrosis. For each patient we report the value measured at the age of 12 years and the current value at the age of 25 years or later. The classification of mild (13 cases) or severe (12 cases) was performed according to criteria by Schluchter et al. [30] that take into account both the FEV_1 value and age

independently by *CFTR* contribute to the pathogenesis of severe lung disease. This agree with the results of Vanscoy et al. on 231 pairs of siblings [49], and of Collaco et al. [21], that concluded that genetic and environmental factors contribute equally to lung function in patients with CF. Finally, an excellent study on 6365 patients with CF revealed five loci that modulate the severity of lung disease in patients with CF [20]. The severe lung disease correlated with PI (e.g., all the 6 pairs of siblings with severe lung disease had PI, see Additional file 1: Table S1) and with *P. aeruginosa* colonization (see below).

It is interesting to observe the longitudinal data of 25 patients from our study for which the values of FEV_1 at the age of 12 years and at the age of 25 years or later were available (Fig. 2). Even if the number of cases is small, the classification of severe ($n = 12$) or mild ($n = 13$) lung disease performed at 12 years invariably coincides with that obtained in the adulthood in the same patient, suggesting that the FEV_1% in young patients with CF is predictive of the lung function in the adult age.

On the other hand, it is known that structural lung damage is an early event in patients with CF [50]. This was revealed by high-resolution chest tomography [51] and by the presence of severe pulmonary inflammation and structural lung disease in still asymptomatic patients diagnosed by NBS [52].

Colonization by Pseudomonas aeruginosa

We found colonization by *P. aeruginosa* in 92/208 (44.2%) patients with CF. Among our 101 pairs of siblings, we found colonization by *P. aeruginosa* in all the members of 34 sibling-pairs, among which the three siblings of two triplets and only one member of 20 sibling-pairs (and in two of three members of a triplet), with a high concordance for colonization (i.e., 61.8%). This result agrees with previous studies on 50 [52] and 11 [19] pairs of siblings. Interestingly, among our siblings discordant for colonization, in 11/21 cases both the siblings lived in the same house, and the sibling colonized by *P. aeruginosa* was colonized since at least 3 years. These data indicate a limited role of the environment in the colonization and confirms a contribution of *CFTR* genotype and genes inherited independently by *CFTR* that may predispose to colonization by *P. aeruginosa,* as reviewed by Cutting [3]. Nevertheless, we cannot rule out an early intervention (e.g. antibiotics) to prevent nosocomial infection in non-colonized siblings.

In patients with colonization there is a significantly higher occurrence of severe lung disease (data not shown). However, colonization is likely a consequence of the severe pulmonary damage more than a causal contribution. Indeed, the mean age of colonization in our patients was 18.5 years (median 22.6), while the occurrence of the structural lung damage is an early

event in patients with CF (as discussed in the previous paragraph).

CF liver disease

We found CFLD in 24/208 (11.5%) patients with CF. It is difficult to compare this figure with previous data because different parameters may be used to define CFLD (i.e., altered aminotransferase levels, focal biliary cirrhosis, US alterations, portal hypertension). As described in materials and methods, we used very stringent parameters to define CFLD and our results agree with the evidence that about 10% of patients with CF develop a severe liver damage and about 5% require liver transplantation. Our data confirm that CFLD is an early event since it was diagnosed at a mean age of 14.7 years (median 15 years old, range 7–27 years) in agreement with the view that CFLD peaks in adolescence [42].

CFLD was found in both the members of 5 sibling-pairs and in one member of 13 sibling-pairs (among which two triplets) and in two members of a further triplet. No previous data are available on the comparison of CFLD liver disease in siblings and in twins with CF, but the concordance for CFLD of 27.8% obtained in our sibling-pairs indicate a scarce contribution of genes in the pathogenesis of CFLD reinforcing the role of environmental, mostly still unknown, risk factors [38]. In fact, the largest two-stage control study (about 2000 patients with CF) on modifier genes of CFLD revealed mutations in the SERPIN1 gene encoding for alpha-1-antitrypsin only in about 2% of patients with CFLD [19]. In agreement, among the 15 sibling-pairs discordant for CFLD, only in 2 cases the sibling suffering from CFLD had levels of serum alpha-1-antitrypsin under the lower reference limit, compatible with the deficiency of the protein, while the siblings free from CFLD had normal levels of the protein.

Cystic fibrosis related diabetes (CFRD)

We found CFRD in all the members of 6/101 sibling-pairs (among which one triplet) and in one member of 16/101 sibling-pairs. The concordance for CFRD was 27.3% and this figure compares with that of 18.0% reported in a population of 588 sibling-pairs [53]. These data, added to the concordance for CFRD of 73.0% obtained in 68 pairs of monozygotic twins with CF [53] that share 100% of DNA, support the view that genetic modifiers play a marginal role in the development of diabetes in patients with CF. This is also confirmed by the observation that the occurrence of CFRD is not an early complication in patients with CF [39]. In fact, the diagnosis of CFRD in our patients was performed at the mean age of 32.2 years and in more than a half of patients > 30 years. Furthermore, we excluded also the gender as a risk factor for CFRD: we found such complication in 13/106 males and in 15/102 females (p not significant). Most studies on larger populations reported the female gender as a risk factor for CFRD [42], but a more recent study on 588 sibling-pairs did not find a statistically significant role of gender as risk factor for CFRD [53].

As shown in Table 1, PI was significantly more frequent in sib-pairs with CFRD, confirming PI as a risk factor for CFRD since it causes a progressive pancreatic fibrosis that gradually damages the insulae [54]. However, observing that 7/28 patients with CFRD had PS (Additional file 1: Table S1), we suggest that also CF patients with PS must be included in the annual screening for CFRD based on the glucose tolerance test [42].

In addition, CFLD contributes to the risk for CFRD [55] since we found a higher occurrence of CFLD among sib-pairs with CFRD than in those free from this complication. Finally, our data are in agreement with the view that severe lung disease is a risk factor for CFRD in turn [56, 57]. In fact, in 19 sib-pairs in which one or both patients had CFRD, in 12 cases one or both the siblings had a severe lung disease, a condition observed only in 15/79 pairs in which both the siblings were free from CFRD. This correlation is more evident in 16 sibling-pairs discordant for CFRD, in which the sibling with CFRD, at the same age, had the worst FEV_1% ($p = 0.004$, Wilcoxon signed rank test) in 14/16 cases (Fig. 3).

Nasal polyposis

We found nasal polyposis requiring surgery in both the siblings of 13 pairs and only in one sibling from 12 sibling-pairs (concordance for disease: 52.0%). There are no previous data on sibling pairs to which compare our results. The poor correlation of nasal polyposis to other clinical manifestations such as the pancreatic status and the severity of lung disease (data not shown) imply that modifier genes play a role in determining nasal polyposis. In fact, a recent study firstly described the potential role of interferon-related developmental regulator 1 (a known modifier gene for CF pulmonary disease severity) as a modifier gene of nasal polyposis in patients with CF [58].

Conclusions

Our study confirms the clinical heterogeneity of CF also in a percentage of pairs of siblings with CF. Physicians involved in genetic counseling must be aware of a so wide and mostly unpredictable variability. Stochastic, environmental and genetic factors also independent by *CFTR* contribute to such variability, even if with a different weight on each variable (i.e., nasal polyposis and *P. aeruginosa* colonization may be more influenced by genetic factors while CFRD and CFLD or the severe lung disease may be influenced either by genetic and by

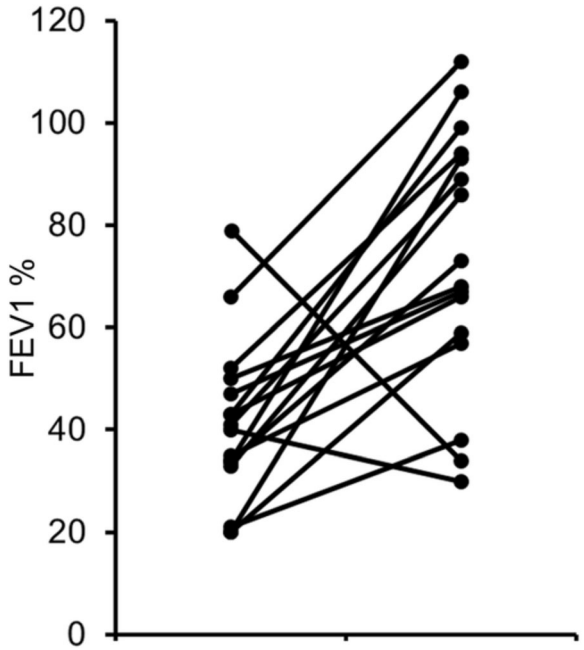

Fig. 3 Current FEV_1% of predicted for age in 15 pairs of siblings discordant for cystic fibrosis related diabetes (CFRD). **a** sibling with CFRD; **b** sibling free from CFRD. We reported as current FEV_1 the last value of the younger sibling and that of the older sibling at the same age

environmental factors). However other variables like medical care (that significantly improved in the last years), strongly influenced the clinical expression of each patient of our cohort that includes CF patients in the wide range between 12 to 61 years. Some working hypotheses emerged from our study: i) the classification of the lung damage as severe or mild based on the FEV_1% assessed at the age of 12 years coincides with that obtained in the adulthood; ii) CFRD is influenced by the severity of liver disease; iii) a severe course of the disease (including the occurrence of complications) may occur in a percentage of patients with CF and PS suggesting that also such cases, usually revealed by NBS and once classified as mild CF, should be monitored as the patients with PI.

Additional file

Additional file 1: Table S1. Clinical and genetic data of the 208 patients with CF included in the study. (XLSX 44 kb)

Abbreviations

CF: Cystic fibrosis; CFLD: CF liver disease.; CFRD: CF-related diabetes; CFTR: Cystic fibrosis transmembrane conductance regulator; MI: Meconium ileus; PI: Pancreatic insufficiency; PS: Pancreatic sufficiency; SCL: Sweat chloride levels

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Availability of data and materials

All data are available.

Authors' contributions

V.T. and G.C conceived and designed the study, analyzed and interpreted the data and wrote the manuscript; M.L. and D.S. contributed to data interpretation and manuscript preparation, collected the data and revised the manuscript; RB analyzed statistically data; C.C., AM. DL.,V.L, V.C., C.B., N.C., R.P., R.C., L.T., E.M., M.C., S.Q., M.S., A.B., A.E., A.M.D.L., F.Z., A.A., V.R. collected and analyzed the data and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

We obtained from all patients (or from their legal guardian) the written consent to participate. In according to National Guidelines, observational studies do not require the formal approval by the Ethics Committee. In any case, we asked to the local ethics committee (University of Naples Federico II) that replied confirming what above and sent us the reference of the guidelines: Ministry of Health, Italian Medicines Agency, Guidelines for the Classification and Conduct of Observational Drugs Studies (2008).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1]Dipartimento di Pediatria, Centro Regionale Toscano per la Fibrosi Cistica, Azienda Ospedaliero-Universitaria Meyer, Viale Gaetano Pieraccini 24, 50139 Florence, Italy. [2]Dipartimento di Biotecnologie Cellulari ed Ematologia, Istituto Pasteur Fondazione Cenci Bolognetti, Sapienza Università e Policlinico Umberto I, Rome, Italy. [3]Centro Regionale Fibrosi Cistica, Centro Pediatrico Bambino Gesù Basilicata, AOR San Carlo, Potenza, Italy. [4]Laboratorio di Genetica Medica, Ospedale Pediatrico Bambino Gesù, Rome, Italy. [5]Centro Regionale Fibrosi Cistica, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy. [6]Freelance Epidemiologist, Bergamo, Italy. [7]Centro Regionale Fibrosi Cistica Adulti, Dipartimento di Scienze Mediche Traslazionali, Università di Napoli Federico II, Naples, Italy. [8]Centro Regionale Fibrosi Cistica, U.O.C. Pneumologia, IRCCS G. Gaslini, Genua, Italy. [9]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy. [10]CEINGE-Biotecnologie avanzate, Naples, Italy. [11]Centro Regionale Fibrosi Cistica, Dipartimento Materno-Infantile, Ospedali Riuniti Ancona, Ancona, Italy. [12]CRR Fibrosi Cistica, Ospedale dei Bambini, ARNAS Civico, Palermo, Italy. [13]Dipartimento di Neuroscienze, Scienze Riproduttive ed Odontostomatologica, Università di Napoli Federico II, Naples, Italy. [14]Università Telematica Pegaso, Naples, Italy. [15]Unità Regionale di Fibrosi Cistica, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy. [16]Centro Regionale Fibrosi Cistica, Ospedale Giovanni Paolo II, Lamezia, Italy. [17]Centro Regionale di supporto Fibrosi Cistica, Dipartimento di Pediatria, Università di Brescia, AO Spedali Civili, Brescia, Italy. [18]Centro Fibrosi Cistica Regione Lazio, Dipartimento di Pediatria e Neuropsichiatria Infantile, Sapienza Università-Policlinico Umberto I, Rome, Italy. [19]Centro Regionale Fibrosi Cistica, Sezione Pediatrica, Dipartimento di Scienze Mediche Traslazionali, Università di Napoli Federico II, Naples, Italy. [20]Laboratorio di Genetica

Medica, Fondazione IRCCS Policlinico Ca' Granda Ospedale, Milan, Italy. [21]Ospedale dei Bambini G. Di Cristina, Centro Regionale Fibrosi Cistica, Palermo, Italy. [22]Dipartimento di Bioscienze e Territorio, Università del Molise, Isernia, Italy.

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Additional file 1: Table S1: Clinical and genetic data of the 208 patients with CF included in the study

	PAIR NUMBER	DIAGNOSED BY NEONATAL SCREENING (NBS)	MECONIUM ILEUS (MI)	DIOS (D)	SWEAT CHLORIDE AT DIAGNOSIS (mEq/L)	CFTR GENOTYPE	PANCREATIC STATUS (I: INSUFFICIENCY; S: SUFFICIENCY)	RECURRENT PANCREATITIS, REC	I FEV1%	INTERMEDIATE FEV1%	LAST FEV1% AVAILABLE SEVERE LUNG DISEASE	P. AERUGINOSA COLONIZATION	MRSA COLONIZATION	S. AUREUS COLONIZATION	S. MALTOPHILIA COLONIZATION	B. CEPACIA COLONIZATION	CF LIVER DISEASE	CF RELATED DIABETES	NASAL POLYPOSIS REQUIRING SURGERY (NP)
1	1 A				112	F508del/E831X	I		100		99								
2	1 B				120	F508del/E831X	I		100		101								
3	2 A				104	I1234V/I1234V	S		26	30	10	CHR							
4	2 B				96	I1234V/I1234V	S		70	64	71								
5	3 A	NBS			107	G85E/[R117L,L997F]	S		110		96								NP
6	3 B				80	G85E/[R117L,L997F]	S		100		90			CHR					NP
7	4 A				110	R334W/G85E	S		81		70			CHR				CFRD	
8	4 B				100	R334W/G85E	S		81		60	CHR		CHR				CFRD	NP
9	5 A	NBS			88	F508del/2789+5G>A	S				111			CHR					
10	5 B				90	F508del/2789+5G>A	S				91			CHR					
15	8 A				73	Q220X/A1006E	S	REC	95		75	CHR							
16	8 B				63	Q220X/A1006E	S		120	90	82	CHR	CHR						NP
17	9 A	NBS			88	N1303K/G1244E	I		110		103				CHR				NP
18	9 B				133	N1303K/G1244E	I		100		98			CHR	CHR				
19	10 A	NBS			54	F508del/P5L	S		98		91			CHR					
20	10 B	NBS			68	F508del/P5L	S		115		112			CHR					
21	11 A				58	F508del/P5L	S		120		111			CHR					
22	11 B				84	F508del/P5L	S				107			CHR					
23	12 A				84	R347P/2184insA	S		78		70			CHR					
24	12 B				88	R347P/2184insA	S				68			CHR					
25	13 A				89	F508del/U	I				85			CHR					
26	13 B				80	F508del/U	I				88			CHR					
27	14 A	NBS			>60	F508del/G542X	I				59			CHR					
28	14 B	NBS			>60	F508del/G542X	I				65			CHR					
29	15 A	NBS			93	F508del/N1303K	I				83			CHR					
30	15 B	NBS			99	F508del/N1303K	I				83			CHR					
31	16 A	NBS			80	2789+5G>A/1342-2A>C	S				93								
32	16 B	NBS			85	2789+5G>A/1342-2A>C	S				91								
33	17 A	NBS			116	F508del/W1282X	I		85		80			CHR					NP

	PAIR NUMBER	DIAGNOSED BY NEONATAL SCREENING (NBS)	MECONIUM ILEUS (MI)	DIOS (D)	SWEAT CHLORIDE AT DIAGNOSIS (mEq/L)	CFTR GENOTYPE	PANCREATIC STATUS (I: INSUFFICIENCY; S: SUFFICIENCY)	RECURRENT PANCREATITIS, REC	I FEV1%	INTERMEDIATE FEV1%	LAST FEV1% AVAILABLE SEVERE LUNG DISEASE	P. AERUGINOSA COLONIZATION	MRSA COLONIZATION	S. AUREUS COLONIZATION	S. MALTOPHILIA COLONIZATION	B. CEPACIA COLONIZATION	CF LIVER DISEASE	CF RELATED DIABETES	NASAL POLYPOSIS REQUIRING SURGERY (NP)
34	17 B		MI		120	F508del/W1282X	I				71			CHR					
35	18 A				79	E1104X/E1104X	I				97	CHR							NP
36	18 B				83	E1104X/E1104X	I				50	CHR							
37	19 A				100	F508del/F508del	I		83		39	CHR		CHR				CFRD	
38	19 B				93	F508del/F508del	I		114		37	CHR		CHR				CFRD	
41	21 A				92	R374P/R374P	S		110		86			CHR					
42	21 B				60	R374P/R374P	S				84								
43	22 A				110	F508del/2789+5G>A	S		91		79				CHR				
44	22 B	NBS			125	F508del/2789+5G>A	S				84			CHR					
45	23 A				77	F508del/711+3A>G	S				66			CHR					
46	23 B				101	F508del/711+3A>G	S		79		86								
47	24 A				109	F508del/S1455X	I				120								
48	24 B				102	F508del/S1455X	I				96								
49	25 A	NBS			101	F508del/G542X	I				80								
50	25 B				113	F508del/G542X	I				68			CHR					
51	26 A		MI		111	F508del/E585X	S				92	CHR							
52	26 B	NBS			108	F508del/E585X	S				80	CHR							
53	27 A	NBS	MI		110	F508del/G542X	I												
54	27 B	NBS			164	F508del/G542X	I												
63	32 A				95	L1077P/[E504X-1898+73T->G]	S				91			CHR					NP
64	32 B	NBS			127	L1077P/[E504X-1898+73T->G]	S				78	CHR							
65	33 A				76	F508del/E831X	S		119		109								
66	33 B				89	F508del/E831X	S		117		98								
67	34 A				80	DELe2/C1002-1110_1113DEL	S		60	34	31								
68	34 B				63	DELe2/C1002-1110_1113DEL	S		101	80	75								
71	36 A	NBS			100	F508del/F508del	I				62								
72	36 B				125	F508del/F508del	I				67								
73	37 A	NBS			103	2789+5G->A/DELe2	S				93								
74	37 B				96	2789+5G->A/DELe2	S				105								
75	38 A	NBS			113	F508del/DUPe19	I				109								

	PAIR NUMBER	DIAGNOSED BY NEONATAL SCREENING (NBS)	MECONIUM ILEUS (MI)	DIOS (D)	SWEAT CHLORIDE AT DIAGNOSIS (mEq/L)	CFTR GENOTYPE	PANCREATIC STATUS (I: INSUFFICIENCY; S: SUFFICIENCY)	RECURRENT PANCREATITIS, REC	I FEV1%	INTERMEDIATE FEV1%	LAST FEV1% AVAILABLE SEVERE LUNG DISEASE	P. AERUGINOSA COLONIZATION	MRSA COLONIZATION	S. AUREUS COLONIZATION	S. MALTOPHILIA COLONIZATION	B. CEPACIA COLONIZATION	CF LIVER DISEASE	CF RELATED DIABETES	NASAL POLYPOSIS REQUIRING SURGERY (NP)
76	38 B	NBS			105	F508del/DUPe19	I				106								
77	39 A	NBS			93	F508del/R334W	I				104			CHR			NO		
78	39 B	NBS			93	F508del/R334W	I				95						CIRRHOSIS		NP
79	40 A		MI		101	F508del/N1303K	I		69		50								
80	40 B	NBS			115	F508del/N1303K	I		108	104	119	CHR		CHR					
81	41 A				94	F508del/3878delG	S				57	CHR					NO		
82	41 B				99	F508del/3878delG	S				68	CHR					CIRRHOSIS		
83	42 A				134	F508del/N1303K	I				39					CHR		CFRD	
84	42 B				120	F508del/N1303K	I				62					CHR		CFRD	
87	44 A				124	F508del/F508del	I				91	CHR					CIRRHOSIS		
88	44 B				103	F508del/F508del	I				76	CHR					NO		
93	47 A				95	F508del/G1244E	I				64	CHR							
94	47 B				90	F508del/G1244E	I				54	CHR							
95	48 A				109	[R74W,V201M,D1270N]/N1303K	S				119								
96	48 B				65	[R74W,V201M,D1270N]/N1303K	S				95								
99	50 A				95	F508del/F508del	I				54	CHR		CHR			CIRRHOSIS		
100	50 B				91	F508del/F508del	I				100	CHR		CHR			NO		
101	51 A				84	G542X/4016insT	I				113			CHR			CIRRHOSIS		
102	51 B				84	G542X/4016insT	I				95			CHR			NO		
103	52 A				114	F508del/F508del	I				80				CHR				
104	52 B				90	F508del/F508del	I				93								NP
105	53 A				100	F508del/F508del	I		78		71	CHR					BILIARY CIRRHOSIS	CFRD	
106	53 B				100	F508del/F508del	I		43	21	21	CHR					NO	CFRD	
107	54 A		MI		89	F508del/F508del	I		96		84	CHR							
108	54 B		MI		120	F508del/F508del	I		110	102	72	CHR							
109	55 A				69	F508del/3849+10kbC	S				46	CHR							
110	55 B				70	F508del/3849+10kbC	S				68	CHR							
111	56 A	NBS			84	F508del/G542X	I							CHR					
112	56 B				105	F508del/G542X	I							CHR					
113	57 A				103	3272-26A>G/E585X	S		48		32	CHR		CHR	CHR				

	PAIR NUMBER	DIAGNOSED BY NEONATAL SCREENING (NBS)	MECONIUM ILEUS (MI)	DIOS (D)	SWEAT CHLORIDE AT DIAGNOSIS (mEq/L)	CFTR GENOTYPE	PANCREATIC STATUS (I: INSUFFICIENCY; S: SUFFICIENCY)	RECURRENT PANCREATITIS, REC	I FEV1%	INTERMEDIATE FEV1%	LAST FEV1% AVAILABLE SEVERE LUNG DISEASE	P. AERUGINOSA COLONIZATION	MRSA COLONIZATION	S. AUREUS COLONIZATION	S. MALTOPHILIA COLONIZATION	B. CEPACIA COLONIZATION	CF LIVER DISEASE	CF RELATED DIABETES	NASAL POLYPOSIS REQUIRING SURGERY (NP)
114	57 B				110	3272-26A>G/E585X	S		94		90	CHR		CHR					
115	58 A				113	G542X/711+3A>G	S			44	39			CHR					
116	58 B				86	G542X/711+3A>G	S		113	101	114								
117	59 A				102	G542X/U	I				100			CHR					
118	59 B				81	G542X/U	I				106			CHR					
119	60 A				119	F508del/F508del	I		56		32	CHR				CHR			
120	60 B				94	F508del/F508del	I		100		94	CHR				CHR			
121	61 A				60	F508del/U	S				39			CHR					
122	61 B				67	F508del/U	S				51			CHR					
123	62 A				115	F508del/G542X	I		46		22	CHR		CHR				CFRD	
124	62 B				79	F508del/G542X	I		48		15	CHR		CHR				CFRD	
127	64 A				114	F508del/U	S		69		63	CHR							
128	64 B				80	F508del/U	S		94		65			CHR					
129	65 A				100	F508del/876-10del8	I				63	CHR				CHR			
130	65 B				110	F508del/876-10del8	I				69	CHR				CHR			
131	66 A				61	W1282X/Q1291R	S		80		79	CHR		CHR					
132	66 B				85	W1282X/Q1291R	S		88		96								
133	67 A				124	F508del/F508del	I				80	CHR	CHR	CHR					
134	67 B	NBS			75	F508del/F508del	I				72	CHR		CHR					
135	68 A				115	F508del/R553X	I				25	CHR					CIRRHOSIS	CFRD	
136	68 B				121	F508del/R553X	I		35		21	CHR		CHR			CIRRHOSIS	CFRD	
137	69 A	NBS			67	F508del/DELe22-24	I				84	CHR	CHR	CHR	CHR		CIRRHOSIS		NP
138	69 B				82	F508del/DELe22-24	I				76		CHR	CHR			NO		NP
139	70 A				87	F508del/D110H	S		99		87								NP
140	70 B				77	F508del/D110H	S	REC			100								NP
145	73 A				64	R553X/2789+5G>A	S	REC	118		71								
146	73 B	NBS			63	R553X/2789+5G>A	S		101		83			CHR					
147	74 A				84	F508del/W1282X	I				95			CHR					
148	74 B	NBS			84	F508del/W1282X	I				92			CHR					
149	75 A				75	F508del/I1234V	I		81		78			CHR					

	PAIR NUMBER	DIAGNOSED BY NEONATAL SCREENING (NBS)	MECONIUM ILEUS (MI)	DIOS (D)	SWEAT CHLORIDE AT DIAGNOSIS (mEq/L)	CFTR GENOTYPE	PANCREATIC STATUS (I: INSUFFICIENCY; S: SUFFICIENCY)	RECURRENT PANCREATITIS, REC	I FEV1%	INTERMEDIATE FEV1%	LAST FEV1% AVAILABLE SEVERE LUNG DISEASE	P. AERUGINOSA COLONIZATION	MRSA COLONIZATION	S. AUREUS COLONIZATION	S. MALTOPHILIA COLONIZATION	B. CEPACIA COLONIZATION	CF LIVER DISEASE	CF RELATED DIABETES	NASAL POLYPOSIS REQUIRING SURGERY (NP)
150	75 B				98	F508del/I1234V	I				83	CHR		CHR					
151	76 A				81	G542X/S549R(T>G)	I			95	72	CHR		CHR					NP
152	76 B				98	G542X/S549R(T>G)	I		59	21	30	CHR		CHR					NP
153	77 A				65	F508del/Q1476X	S		100		109								NP
154	77 B				84	F508del/Q1476X	S				104								NP
155	78 A				84	N1303K/2789+5G>A	S		116		107	CHR		CHR					NP
156	78 B				128	N1303K/2789+5G>A	S		103		89	CHR		CHR					NP
157	79 A				78	F508del/S945L	S		83		94	CHR		CHR					NP
158	79 B				110	F508del/S945L	S	REC	60		30	CHR	CHR	CHR					NP
159	80 A				146	F508del/S549N	I				46	CHR							NP
160	80 B				154	F508del/S549N	I				86	CHR		CHR					NP
163	82 A		MI		97	R1066C/L1077P	I		99		90						CIRRHOSIS		
164	82 B		MI		75	R1066C/L1077P	I		78		83			CHR			CIRRHOSIS		
167	84 A				81	F508del/N1303K	I		129		88	CHR							
168	84 B				91	F508del/N1303K	I		97		52	CHR		CHR					
169	85 A				71	R334W/[R117L;L997F]	S				80	CHR		CHR					NP
170	85 B				75	R334W/[R117L;L997F]	S		107		120								NP
171	86 A				86	F508del/F508del	I		54	69	71	CHR							
172	86 B				104	F508del/F508del	I		23	12	12	CHR							
173	87 A				82	2789+5G>A/H1375P	S		116		113								
174	87 B				91	2789+5G>A/H1375P	S		67		108	CHR		CHR					
175	88 A				80	2789+5G>A/L1077P	S				96			CHR					
176	88 B				88	2789+5G>A/L1077P	S	REC	86		108								
179	90 A	NBS			109	L1065P/T338I	S				90								
180	90B	NBS			100	L1065P/T338I	S				103			CHR					
181	91 A	NBS		D	97	F508del/dele22_24	I				60			CHR					NP
182	91 B	NBS		D	138	F508del/dele22_24	I				49			CHR					
183	92 A			D	115	F508del/2789+5G>A	I				117	CHR							
184	92 B			D	95	F508del/2789+5G>A	I	REC			112			CHR					
185	93 A			D	116	F508del/G178R	I				45	CHR							

	PAIR NUMBER	DIAGNOSED BY NEONATAL SCREENING (NBS)	MECONIUM ILEUS (MI)	DIOS (D)	SWEAT CHLORIDE AT DIAGNOSIS (mEq/L)	CFTR GENOTYPE	PANCREATIC STATUS (I: INSUFFICIENCY; S: SUFFICIENCY)	RECURRENT PANCREATITIS, REC	I FEV1%	INTERMEDIATE FEV1%	LAST FEV1% AVAILABLE SEVERE LUNG DISEASE	P. AERUGINOSA COLONIZATION	MRSA COLONIZATION	S. AUREUS COLONIZATION	S. MALTOPHILIA COLONIZATION	B. CEPACIA COLONIZATION	CF LIVER DISEASE	CF RELATED DIABETES	NASAL POLYPOSIS REQUIRING SURGERY (NP)
186	93 B			D	114	F508del/G178R	I				47	CHR	CHR						
187	94 A				110	F508del/2789+5G>A	S	REC			93			CHR					NP
188	94 B				102	F508del/2789+5G>A	S				101			CHR					
191	96 A	NBS			62	F508del/D579G	S				103		CHR						
192	96 B				110	F508del/D579G	S				77								
193	96 C				84	F508del/D579G	S				34	CHR							
194	97 A		MI		107	F508del/DELe14B_17B	I				124						NO		NP
195	97 B				107	F508del/DELe14B_17B	I				93	CHR					NO		NP
196	97 C		MI		131	F508del/DELe14B_17B	I				101	CHR					CIRRHOSIS		
197	98 A				102	F508del/G1244E	I				48	CHR							
198	98 B				103	F508del/G1244E	I				25	CHR							
199	98 C				92	F508del/G1244E	I				75	CHR							
200	99 A				97	F508del/D614G	I				81	CHR							
201	99 B				79	F508del/D614G	I				56	CHR							
202	99 C				65	F508del/D614G	I				83	CHR				CHR			
203	100 A				106	N1303K/G542X	I				117			CHR			NO		
204	100 B				80	N1303K/G542X	I				74						BILIARY CIRRHOSIS		
205	100 C				98	N1303K/G542X	I				88			CHR			NO		
206	101 A				115	2789+5G>A/2789+5G>A	S		78		39	CHR					CIRRHOSIS		
207	101 B				85	2789+5G>A/2789+5G>A	S		92		44						CIRRHOSIS		
208	101 C				85	2789+5G>A/2789+5G>A	S		96		93			CHR			NO		
189	95 A				114	2789+5G>A/2789+5G>A	S			64	52	CHR						CFRD	
190	95 B				112	2789+5G>A/2789+5G>A	S			98	94								
177	89 A				105	F508del/F508del	I				40	CHR		CHR				CFRD	
178	89 B				110	F508del/F508del	I		71		30	CHR		CHR					
165	83 A				117	DELe22_24/DELe22_24	I		64	69	33	CHR		CHR				CFRD	
166	83 B				125	DELe22_24/DELe22_24	I		90	69	86			CHR					
161	81 A				67	F508del/S549R(A>C)	I			102	93			CHR					
162	81 B				108	F508del/S549R(A>C)	I			33	20			CHR				CFRD	
141	71 A				67	F508del/G1349D	I		112	91	59	CHR		CHR					NP

	PAIR NUMBER	DIAGNOSED BY NEONATAL SCREENING (NBS)	MECONIUM ILEUS (MI)	DIOS (D)	SWEAT CHLORIDE AT DIAGNOSIS (mEq/L)	CFTR GENOTYPE	PANCREATIC STATUS (I: INSUFFICIENCY; S: SUFFICIENCY)	RECURRENT PANCREATITIS, REC	I FEV1%	INTERMEDIATE FEV1%	LAST FEV1% AVAILABLE SEVERE LUNG DISEASE	P. AERUGINOSA COLONIZATION	MRSA COLONIZATION	S. AUREUS COLONIZATION	S. MALTOPHILIA COLONIZATION	B. CEPACIA COLONIZATION	CF LIVER DISEASE	CF RELATED DIABETES	NASAL POLYPOSIS REQUIRING SURGERY (NP)
142	71 B				73	F508del/G1349D	I		45	33	20	CHR		CHR				CFRD	NP
143	72 A				79	R334W/N1303K	S	REC	64		47	CHR		CHR				CFRD	NP
144	72 B				103	R334W/N1303K	S	REC	79		67	CHR		CHR					NP
125	63 A				100	DELe 22_24/DELe 22_24	I			61	41	CHR						CFRD	
126	63 B				125	DELe 22_24/DELe 22_24	I			89	89			CHR					
97	49 A		MI		92	F508del/G85E	I			93	106		CHR	CHR					
98	49 B		MI		83	F508del/G85E	I		34		30	CHR	CHR					CFRD	
89	45 A				89	F508del/R553X	S				50	CHR					CIRRHOSIS	CFRD	
90	45 B				100	F508del/R553X	I				68	CHR					CIRRHOSIS		
91	46 A				125	F508del/F508del	S		32	35	34	CHR					CIRRHOSIS	CFRD	
92	46 B				86	F508del/F508del	I		51		73	CHR					CIRRHOSIS		
85	43 A				81	F508del/N1303K	S				43	CHR						CFRD	
86	43 B				75	F508del/N1303K	I				66	CHR							
69	35 A				98	F508de/F508del	I				38	CHR					CIRRHOSIS, PORTAL HYP		NP
70	35 B	NBS			105	F508del/F508del	I		50		21	CHR					NO	CFRD	
55	28 A				93	F508del/F508del	I		55	40	33		CHR	CHR			CIRRHOSIS	CFRD	
56	28 B	NBS			98	F508del/F508del	I			83	99						CIRRHOSIS		
39	20 A				113	F508del/F508del	I		109		57	CHR							
40	20 B				100	F508del/F508del	I		99		35	CHR						CFRD	
11	6 A				93	F508del/G542X	I		100	102	112	CHR					NO		NP
12	6 B				74	F508del/G542X	I			97	86						CIRRHOSIS, PORTAL HYP	CFRD	NP
13	7 A		MI		98	F508del/621+1G->T	I		54	48	34					CHR	CIRRHOSIS, PORTAL HYP		
14	7 B		MI		86	F508del/621+1G->T	I		100	94	79	CHR		CHR		CHR	NO	CFRD	