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Long term effects of birth order and age at diagnosis in cystic fibrosis; a sibling cohort study.



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3 **Long term effects of birth order and age at diagnosis in cystic fibrosis; a sibling cohort study.**
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7 M.G. Slieker, MD, PhD,^{1*} J.M.W. van den Berg, MD,² J. Kouwenberg, MD,³ F. Teding van Berkhout, MD¹,

8
9 H.G.M. Heijerman, MD, PhD,² C.K. van der Ent, MD, PhD¹

10
11 ¹Cystic Fibrosis Center Utrecht, University Medical Center Utrecht, Utrecht, the Netherlands

12
13 ²Adult Cystic Fibrosis Center, Haga Teaching Hospital, The Hague, the Netherlands

14
15 ³Juliana Children's Hospital, Haga Teaching Hospital, The Hague, the Netherlands

16
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18
19 *Correspondence to: Dr. M.G. Slieker, Cystic Fibrosis Center Utrecht, University Medical Center Utrecht,

20
21 Lundlaan 6, 3584 EA, Utrecht, the Netherlands. Phone: +31887554690. Fax: +31887554747, E-mail:

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23 m.g.slieker@umcutrecht.nl
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ABSTRACT

Background: Siblings with cystic fibrosis (CF) share many genetic and environmental factors, but may present different phenotypes. Younger sibs are mostly earlier diagnosed with CF than their older sibs, but ~~are often infected~~ might be at risk for an earlier colonization with *P. aeruginosa* (PA) ~~at an earlier age~~ than their older counterparts **due to cross-infection within families.**

Aims: To analyze the effects of birth order and age at diagnosis on lung function, PA colonization, nutritional status, and survival during the first two decades of life in siblings with CF.

Methods: A retrospective cohort study of 52 sibling pairs was performed in two Dutch CF centers. Data were analyzed both cross-sectionally and longitudinally using Kaplan-Meier curves and modified log-rank tests.

Results: Median age at diagnosis was significantly higher in the older sib compared with the younger sib (3.0 and 0.2 years, respectively, $P < 0.0001$). At the age of 5, 10 and 15 years no difference in lung function was found. However, at the age of 20 years, FEV₁ in older sibs was 19.4% (95% CI: 5.9-32.9%, $P = 0.007$) lower than in younger sibs. In the younger sibs group, FEV₁ at age 20 years was significantly better in those who had a diagnosis before the age of 6 months (difference 22.9%, 95% CI: 0.1-45.8%, $P < 0.05$). In the first 10 years of life the younger sibs tended to be earlier colonized with PA than their older counterparts. No differences in nutritional status and survival were observed.

Conclusion: In this sibling cohort study, an early diagnosis of CF was associated with better lung function **during the first after** two decades of life. Although younger siblings tended to be colonized with PA at an earlier age, they showed better lung function outcomes. This underscores the importance of early diagnosis with newborn screening and early referral to a specialized center in the prevention of long-term deleterious effects on lung function.

INTRODUCTION

There is considerable variation in the clinical course of disease in cystic fibrosis (CF), even in patients with the same CF genotype,¹ suggesting the influence of other factors such as environment, quality of care and modifier genes.²⁻⁴

Siblings with CF share the same CFTR mutations, are generally exposed to the same environment, and have similar quality of care. These factors contribute to a high concordance in CF phenotype between CF siblings.⁵

However, in countries where newborn screening (NBS) is not available (as in the Netherlands), younger sibs are generally earlier diagnosed with CF than their older sibs.^{5,6} Early asymptomatic diagnosis is associated with better lung function,⁷⁻¹⁰ nutritional status,¹¹⁻¹⁴ and survival.^{7,9,14-16} This would suggest an advantage for the younger sib.

On the other hand, since cross-infection is known to occur between patients with CF,^{17,18} it can be expected that younger siblings may acquire human adapted respiratory pathogens at an earlier age. *P. aeruginosa* (PA) infection is associated with deteriorating lung function and increased morbidity,^{19,20} which may result in a more severe CF phenotype in the younger sib.

The aim of this study was to investigate the effects of birth order and age at diagnosis on lung function, PA colonization, nutritional status, and survival in non-twin sibs with CF, aged up to 20 years.

METHODS

The study population consisted of all sibling pairs treated at the CF Centers of the University Medical Center Utrecht (Utrecht, the Netherlands) and the Haga Teaching Hospital (The Hague, the Netherlands). These centers provide care for 580 out of 1250 CF patients who are recorded in the Dutch Cystic Fibrosis Registry. This registry covers 93% of all Dutch CF-patients (www.ncfs.nl). Treatment strategies are comparable in both centers and are based on national guidelines.²¹ All patients gave informed consent for the recording of their clinical data in our database and for the use of these data for scientific purposes, including descriptive studies.

In families with more than two affected siblings, only the first two siblings were included in the study. Twins were excluded. Patients born before 1970 or without clinical data before the age of 20 years available were also excluded.

All data were retrospectively collected from patient's medical charts at each center. The diagnosis of CF was confirmed in all patients with sweat testing and/or DNA analysis for CF mutations.

The primary outcome measure was lung function (forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC)). Secondary outcome measures included age at diagnosis, nutritional status (height and weight), PA colonization and survival.

FEV₁ and FVC were measured by a pneumotachograph and converted to percentage of predicted values.^{22,23} For cross-sectional analysis, the highest FEV₁ measure within the last year of available data for the younger sib was used. This value was matched with the highest FEV₁ of the older sibling during the year in which he/she reached the same age as the younger sib. Longitudinal measures were derived from all years of available lung function data for each study subject, using the best FEV₁ measurement per year.

Z-scores for height and weight were calculated using standard growth diagrams for the Dutch population.²⁴

Matching of height and BMI measures between older and younger sibs was similar to those performed for lung function. Data on lung function and nutritional status were available from 1991 onwards.

PA colonization was considered to be present when >50% of the PA cultures in the preceding year were positive.²⁵ In the older siblings less than 4 sputum samples per year were available. In these patients the old European consensus definition for chronic PA colonization was used, i.e. at least three positive cultures over ≥ 6 months with a ≥ 1 month interval.²⁶ In patients who were colonized according to this definition, we defined date of PA colonization as date of first positive PA culture plus one year (to make this date similar to the Leeds criteria). In all patients we attempted to perform a sputum culture. When a patient was not able to produce sputum, an oropharyngeal swab was taken.

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3 Based on their functional effects, CFTR mutations were divided in 5 classes.¹ Class I, II and III mutations result
4 in absence of or non-functional CFTR, whereas classes IV and V mutations, allowing some function of CFTR,
5 usually result in less severe disease manifestations.²⁷ Patients with two class I, II or III mutations were
6 considered to have a severe CFTR genotype, whereas patients with one or two class IV or V mutations were
7 considered to have a mild genotype.¹
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13 14 15 **Statistical analysis**

16 Differences between siblings were calculated using paired Student's t-test for normally distributed continuous
17 variables, Wilcoxon signed-rank test for not-normally distributed continuous variables, and McNemar test for
18 discrete variables. In order to test if continuous variables were normally distributed, a Kolmogorov-Smirnov test
19 was performed. The level of significance was 0.05.
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24 **Power and sample size were calculated using PASS 2008 (NCSS, Kaysville, UT).** Sample size calculation
25 showed that twenty sibling pairs were needed to detect a 20% difference in lung function with an estimated SD
26 of the difference in lung function of 30%, an alpha of 0.05 and a power of 0.8.
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29 Kaplan-Meier curves and modified log rank tests were used to assess whether there were differences in survival
30 and PA colonization by birth order. Specifically, a Cox proportional hazards model (the probability model
31 implicit in the log rank test) was used, with a term denoting sibling pairs, to test for the significance of birth
32 order in the model. This model takes into account the paired nature of the siblings when calculating the standard
33 error for the birth order coefficient.
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RESULTS

In total, 52 families with at least one sibling pair with CF met the inclusion criteria. The mean age difference between the two siblings was 3.7 ± 2.2 years.

Patient characteristics are shown in Table 1. Median age at diagnosis was significantly higher in the older sib compared with the younger sib (3.0 and 0.2 years, respectively, $P < 0.0001$).

In 37 pairs (71.2%) the older sib was diagnosed first, in 11 pairs (21.2%) the younger sib was diagnosed first, and in 4 pairs (7.7%) sibs were diagnosed at the same time. Eight of the younger sibs (15.4%) were diagnosed antenatally or shortly after birth before the development of symptoms because of CF in the older sib. **Both the first and second diagnosed sibs were immediately followed in a CF center from the diagnosis. PA colonization in first and second diagnosed sibs was 12.5% and 8.3%, respectively. Sputum cultures at diagnosis in first and second diagnosed sibs were positive for PA in 12.5% and 8.3% of the patients, respectively (McNemar test $P = 0.63$).**

Older sibs tended to have respiratory symptoms at diagnosis more often (55.8% and 38.5%, respectively, $P = 0.12$) compared with younger sibs (Table 1).

In four sibling pairs no CFTR genotype was determined. Of the remaining 48 patient pairs, 39 (81.3%) had a severe CFTR genotype class.

Lung function testing was performed in 47 sibling pairs. Lung function at last clinical visit for the younger sib tended to be higher than the matched lung function for the older sib (Table 2). FEV₁ was analyzed per 5-year intervals. Unfortunately, for some sibling pairs lung function data were not available at all investigated ages because they had not reached that age or because they were first treated at another center. At the age of 5, 10, 15 and 20 years, for 15, 21, 22 and 22 patients pairs lung function data were available, respectively. FEV₁ at the age of 5, 10 and 15 years was not significantly different between the sibs. However, at the age of 20 years, FEV₁ in older sibs was 19.4% (95% CI: 5.9-32.9%, $P = 0.007$) lower than in younger sibs (Figure 1). At this age, 4 of the older sibs had a better and 18 had a poorer lung function compared to their younger sibs (Wilcoxon signed rank test $P = 0.007$).

In order to test the hypothesis that the better clinical outcome in the younger sibs is caused by earlier diagnosis of CF, we analyzed the effect of age at diagnosis on lung function in the younger sibs using an unpaired Student's t-test. Since the association between age at diagnosis and lung function could be confounded by severity of CFTR genotype,²⁸ this analysis was only performed in patients with a severe CFTR genotype. FEV₁ analyzed per 5-year intervals demonstrated a significantly higher FEV₁ at the age of 20 years in sibs diagnosed

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3 before 6 months of age compared with those diagnosed after 6 months of age (difference 22.9%, 95% CI: 0.1-
4 45.8%, $P < 0.05$). However, no significant differences were observed at the age of 5, 10 and 15 years.

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7 PA colonization at last visit was not different between the older and younger sibs (Table 2). Also, when
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9 differences in age at PA colonization between younger and older sibs were analyzed longitudinally, younger sibs
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11 were overall not earlier colonized with PA (Cox proportional hazard 1.60, 95% CI: -0.73-3.52). However, the
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13 younger sibs tended to be earlier colonized in the first 10 years of life than their older counterparts ($P = 0.08$), but
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15 this difference disappeared in the second decade (Figure 2). Height, weight and BMI did not differ between the
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17 older and the younger sibs (Table 2).

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19 Two of the older sibs and none of the younger sibs received a lung transplant before the age of 25 years. Three of
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21 the older sibs and one of the younger sibs died before the age of 25 years. ~~Survival curves for older and younger~~
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23 ~~sibs are displayed in Figure 3.~~ Survival (no death and no lung transplant) was not significantly worse in the older
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25 sibs compared with the younger sibs (Cox proportional hazard: $P = 0.21$).

DISCUSSION

This study on long-term differences in lung function, PA colonization, nutritional status, and survival between older and younger sibs with CF demonstrated that older siblings had a poorer lung function in the second decade of life compared to their younger siblings with CF. Early diagnosis was associated with better lung function outcome. PA colonization was not significantly different between younger and older sibs, but younger sibs tended to be earlier colonized in the first 10 years of life. Older and younger siblings did not appear to have a different height, weight and survival.

In this study, we hypothesized that younger siblings might be at risk of more accelerated lung disease because of exposure to cross-infection from older siblings. On the other hand, we postulated that younger sibs would be earlier diagnosed and would therefore benefit from therapy earlier in life. It was shown that younger sibs tended to be earlier colonized with PA in the first 10 years of life, but that this difference disappeared in the second decade. Despite the higher PA colonization in the first decade, this did not result in a significantly worse lung function in the first decade, probably due to an earlier diagnosis. In the second decade no difference between PA colonization was found between the sibs, and younger sibs had better lung function. This suggests that the net effect of earlier diagnosis in younger CF siblings outweighs the negative effects, including the exposure to CF pathogens from older siblings.

The beneficial effect of younger age at diagnosis on lung function was confirmed by our subgroup analysis in the younger sibs: patients diagnosed before 6 months of age had a significantly better lung function in the second decade of life. This is in accordance with the better prognosis associated with early asymptomatic diagnosis of CF through NBS.^{7-10,29} In these early diagnosed asymptomatic patients, early antibiotic therapy, nutritional supplementation, and intensive physiotherapy can delay the progression of lung disease in CF.²⁹

A better lung function in younger siblings with CF was also found in a relatively old and small study by Orenstein *et al.*³⁰ On the other hand, two recent studies could not find significant differences in FEV₁ and FVC between older and younger sibs with CF.^{5,6} However, lung function was only measured at the age of 7 and 10 years,⁵ and at the age of 8 years,⁶ respectively. The age of 10 years may be too young to identify significant differences between the two siblings, which was demonstrated by our longitudinal analysis (difference only found in second decade of life).

Similar to other studies,^{5,6} we did find a strong familial tendency for PA colonization. This was probably caused by cross-infections between the siblings. The importance of cross-infections among CF patients within families has already been confirmed by DNA fingerprinting and serology.^{18,31} In accordance with other studies,^{5,6} we

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3 demonstrated an earlier age at PA colonization in the younger siblings than in the older siblings. However, in all
4 studies the interpretation is limited by the age-of-diagnosis bias. Patients are only tested for typical CF pathogens
5 if they already have a CF diagnosis. Therefore, a positive culture at time of diagnosis prevents accurate
6 determination of true age of infection. Since the older child in a family is always diagnosed at an older age than
7 the younger sibling, regardless of who is the index case, older siblings are more likely to have positive cultures at
8 time of diagnosis, simply due to more time to acquire the pathogens. The age-of-diagnosis bias could only be
9 overcome by a study of CF siblings diagnosed through NBS, but NBS is not yet available in the Netherlands.

10 The proportion of patients chronically colonized by PA according was rather high for the age of the patients. The
11 high proportion of chronic PA colonization was probably due to the fact that early eradication protocols for PA
12 were introduced only some years ago. Until 4 years ago, there was no segregation policy in The Netherlands and
13 many children visited CF summer camps, which may have contributed to the relatively high PA rates in our
14 cohort.

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17 Since the proportion of patients chronically colonized by PA in this study was high for age, one could speculate
18 that patients who are early diagnosed and consequently early followed in CF centers are at risk of early
19 acquisition of bacterial pathogens, such as PA. However, different recent studies could not demonstrate an
20 association between early diagnosis (and early follow-up in a CF center) and early infection with PA when good
21 infection control practices are followed.^{32,33}

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23 In the younger children the definition of chronic colonization by PA were based on Leeds criteria.²⁵ The validity
24 of this definition has recently been confirmed.³⁴ The minimum sample number needed to use the Leeds criteria
25 for a given year should be four. Unfortunately, in the past less than 4 sputum cultures per year were performed in
26 our patients and, consequently, the Leeds criteria could not be used in the older siblings. This could have led to
27 under-detection of the PA colonization. The effect on the outcome of our study is probably limited, since the
28 same definition was used within siblings pairs.

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30 Similar to other sibling studies,^{5,6} we did not demonstrate any significant differences in height or BMI between
31 older and younger sibs, although one of the earlier sibling studies demonstrated a marginally significantly higher
32 height z-score in younger siblings than in older siblings.⁶ Other studies^{13,29,35} have shown dramatic differences in
33 nutritional status at diagnosis between those diagnosed through screening and those diagnosed as a result of
34 clinical signs and symptoms. However, these differences can be reduced once appropriate dietary intervention,
35 including the provision of pancreatic enzyme replacement therapy, is commenced.¹³ That we could not detect
36 any advantage of early diagnosis on nutritional status in younger sibs might be because of a significant catch-up

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3 growth achieved in the early years of life. Finally, in accordance with our study but in contrast with NBS studies,
4 a recent Belgian study could not find significant differences in anthropometric parameters between children who
5 were early and late (<2 and \geq 2 years after diagnosis) referred to a CF center.³⁶
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9 We found no significant differences in survival between older and younger sibs, but the number of patients
10 investigated may have been too small to detect these differences. Other sibling studies did not investigate
11 differences in survival between older and younger sibs.^{5,6,30} However, most of the studies on the effect of NBS
12 found that early diagnosis through NBS is associated with improved child survival.^{7,9,14-16}
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16 Although this is the largest sibling study so far on the effect of birth order on lung function in CF patients, our
17 study was limited by the population size. However, power analysis revealed that the number of sibling pairs
18 included was sufficient to detect a 20% difference in lung function between the siblings.
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22 Another limitation may have been the influence of time. Treatment regimes for sibling pairs born in the early
23 seventies were different than the ones for sibling pairs born in the nineties. These differences include improved
24 nutritional management and dietary recommendations, new airway clearance techniques and new
25 antipseudomonal antibiotics.³⁷ One could hypothesize that having a older sib could have resulted in poorer lung
26 function in younger sibs born in the seventies compared to those born in the nineties because of new
27 antipseudomonal drugs. Unfortunately, the size of our population did not allow us to analyze the influence of
28 date of birth on outcome.
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32 In conclusion, in this sibling cohort study, an early diagnosis of CF was associated with better lung function
33 ~~during the first~~ after two decades of life. Although younger siblings tended to be colonized with PA at an earlier
34 age, they showed better lung function outcomes. This underscores the importance of early diagnosis with
35 newborn screening in the prevention of long-term deleterious effects on lung function.
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References

1. Mckone EF, Goss CH, Aitken ML. CFTR genotype as a predictor of prognosis in cystic fibrosis. *Chest* 2006;130:1441-1447.
2. Schechter MS. Non-genetic influences on cystic fibrosis lung disease: the role of sociodemographic characteristics, environmental exposures, and healthcare interventions. *Semin Respir Crit Care Med* 2003;24:639-652.
3. Knowles MR. Gene modifiers of lung disease. *Curr Opin Pulm Med* 2006;12:416-421.
4. Vanscoy LL, Blackman SM, Collaco JM, Bowers A, Lai T, Naughton K, Algire M, McWilliams R, Beck S, Hoover-Fong J, Hamosh A, Cutler D, Cutting GR. Heritability of lung disease severity in cystic fibrosis. *Am J Respir Crit Care Med* 2007;175:1036-1043.
5. Picard E, Aviram M, Yahav Y, Rivlin J, Blau H, Bentur L, Avital A, Villa Y, Schwartz S, Kerem B, Kerem E. Familial concordance of phenotype and microbial variation among siblings with CF. *Pediatr Pulmonol* 2004;38:292-297.
6. Katz SL, Strug LJ, Coates AL, Corey M. Disease severity in siblings with cystic fibrosis. *Pediatr Pulmonol* 2004;37:407-412.
7. McKay KO, Waters DL, Gaskin KJ. The influence of newborn screening for cystic fibrosis on pulmonary outcomes in new South Wales. *J Pediatr* 2005;147:S47-S50.
8. Merelle ME, Schouten JP, Gerritsen J, Dankert-Roelse JE. Influence of neonatal screening and centralized treatment on long-term clinical outcome and survival of CF patients. *Eur Respir J* 2001;18:306-315.
9. Mastella G, Zanolla L, Castellani C, Altieri S, Furnari M, Giglio L, Lombardo M, Miano A, Sciuto C, Pardo F, Magazzu G. Neonatal screening for cystic fibrosis: long-term clinical balance. *Pancreatology* 2001;1:531-537.
10. Wang SS, O'Leary LA, Fitzsimmons SC, Khoury MJ. The impact of early cystic fibrosis diagnosis on pulmonary function in children. *J Pediatr* 2002;141:804-810.
11. Farrell PM, Kosorok MR, Rock MJ, Laxova A, Zeng L, Lai HC, Hoffman G, Laessig RH, Splaingard ML. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. *Pediatrics* 2001;107:1-13.
12. Sims EJ, McCormick J, Mehta G, Mehta A. Neonatal screening for cystic fibrosis is beneficial even in the context of modern treatment. *J Pediatr* 2005;147:S42-S46.

- 1
2
3 13. Farrell PM, Kosorok MR, Laxova A, Shen G, Kosciak RE, Bruns WT, Splaingard M, Mischler EH.
4
5 Nutritional benefits of neonatal screening for cystic fibrosis. Wisconsin Cystic Fibrosis Neonatal
6
7 Screening Study Group. *N Engl J Med* 1997;337:963-969.
8
- 9 14. Siret D, Bretaudeau G, Branger B, Dabadie A, Dagonne M, David V, De Braekeleer M, Moisan-Petit V,
10
11 Picherot G, Rault G, Storni V, Roussey M. Comparing the clinical evolution of cystic fibrosis screened
12
13 neonatally to that of cystic fibrosis diagnosed from clinical symptoms: a 10-year retrospective study in a
14
15 French region (Brittany). *Pediatr Pulmonol* 2003;35:342-349.
16
- 17 15. Doull IJ, Ryley HC, Weller P, Goodchild MC. Cystic fibrosis-related deaths in infancy and the effect of
18
19 newborn screening. *Pediatr Pulmonol* 2001;31:363-366.
20
- 21 16. Lai HJ, Cheng Y, Farrell PM. The survival advantage of patients with cystic fibrosis diagnosed through
22
23 neonatal screening: evidence from the United States Cystic Fibrosis Foundation registry data. *J Pediatr*
24
25 2005;147:S57-S63.
26
- 27 17. Tubbs D, Lenney W, Alcock P, Campbell CA, Gray J, Pantin C. *Pseudomonas aeruginosa* in cystic
28
29 fibrosis: cross-infection and the need for segregation. *Respir Med* 2001;95:147-152.
30
- 31 18. Renders NH, Sijmons MA, van BA, Overbeek SE, Mouton JW, Verbrugh HA. Exchange of *Pseudomonas*
32
33 *aeruginosa* strains among cystic fibrosis siblings. *Res Microbiol* 1997;148:447-454.
34
- 35 19. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other
36
37 predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr Pulmonol*
38
39 2002;34:91-100.
40
- 41 20. Nixon GM, Armstrong DS, Carzino R, Carlin JB, Olinsky A, Robertson CF, Grimwood K. Clinical
42
43 outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Pediatr* 2001;138:699-704.
44
- 45 21. van der Laag J, Sinaasappel M, Heijerman HGM, editors. *Diagnostiek en behandeling van cystic fibrosis*
46
47 [Diagnosis and treatment of cystic fibrosis]. Utrecht: Centraal Begeleidingsorgaan voor de Intercollegiale
48
49 Toetsing; 1998. 174 p.
50
- 51 22. Zapletal A, Samanek M, Paul T. Lung function in children and adolescents: methods, reference values. In:
52
53 Zapletal A, Samanek M, Paul T, editors. *Progress in respiration research*. Basel, Switzerland: Karger;
54
55 1987. p 114-218.
56
- 57 23. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced
58
59 ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community
60

- 1
2
3 for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;16:5-
4
5 40.
6
7 24. TNO Prevention and Health, Leiden University Medical Center. Growth diagrams 1997. Houten, the
8
9 Netherlands: Bohn Stafleu Van Loghum; 1998.
10
11 25. Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for
12
13 chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *J Cyst Fibros* 2003;2:29-34.
14
15 26. Doring G, Conway SP, Heijerman HG, Hodson ME, Hoiby N, Smyth A, Touw DJ. Antibiotic therapy
16
17 against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. *Eur Respir J* 2000;16:749-767.
18
19 27. Vankeerberghen A, Cuppens H, Cassiman JJ. The cystic fibrosis transmembrane conductance regulator:
20
21 an intriguing protein with pleiotropic functions. *J Cyst Fibros* 2002;1:13-29.
22
23 28. Mckone EF, Emerson SS, Edwards KL, Aitken ML. Effect of genotype on phenotype and mortality in
24
25 cystic fibrosis: a retrospective cohort study. *Lancet* 2003;361:1671-1676.
26
27 29. Waters DL, Wilcken B, Irwing L, Van Asperen P, Mellis C, Simpson JM, Brown J, Gaskin KJ. Clinical
28
29 outcomes of newborn screening for cystic fibrosis. *Arch Dis Child Fetal Neonatal Ed* 1999;80:F1-F7.
30
31 30. Orenstein DM, Boat TF, Stern RC, Tucker AS, Charnock EL, Matthews LW, Doershuk CF. The effect of
32
33 early diagnosis and treatment in cystic fibrosis: a seven-year study of 16 sibling pairs. *Am J Dis Child*
34
35 1977;131:973-975.
36
37 31. Thomassen MJ, Demko CA, Doershuk CF, Root JM. *Pseudomonas aeruginosa* isolates: comparisons of
38
39 isolates from campers and from sibling pairs with cystic fibrosis. *Pediatr Pulmonol* 1985;1:40-45.
40
41 32. Baussano I, Tardivo I, Bellezza-Fontana R, Forneris MP, Lezo A, Anfossi L, Castello M, Aleksandar V,
42
43 Bignamini E. Neonatal screening for cystic fibrosis does not affect time to first infection with
44
45 *Pseudomonas aeruginosa*. *Pediatrics* 2006;118:888-895.
46
47 33. Collins MS, Abbott MA, Wakefield DB, Lapin CD, Drapeau G, Hopfer SM, Greenstein RM, Cloutier
48
49 MM. Improved pulmonary and growth outcomes in cystic fibrosis by newborn screening. *Pediatr*
50
51 *Pulmonol* 2008;43:648-655.
52
53 34. Proesmans M, Balinska-Miskiewicz W, Dupont L, Bossuyt X, Verhaegen J, Hoiby N, De BK. Evaluating
54
55 the "Leeds criteria" for *Pseudomonas aeruginosa* infection in a cystic fibrosis centre. *Eur Respir J*
56
57 2006;27:937-943.
58
59 35. Dankert-Roelse JE, te Meerman GJ. Long term prognosis of patients with cystic fibrosis in relation to
60
early detection by neonatal screening and treatment in a cystic fibrosis centre. *Thorax* 1995;50:712-718.

- 1
2
3 36. Lebecque P, Leonard A, De BK, De BF, Malfroot A, Casimir G, Desager K, Godding V, Leal T. Early
4 referral to cystic fibrosis specialist centre impacts on respiratory outcome. *J Cyst Fibros* 2009;8:26-30.
5
6
7 37. Slieker MG, Uiterwaal CSPM, Sinaasappel M, Heijerman HGM, van der Laag J, van der Ent CK. Birth
8 prevalence and survival in cystic fibrosis - A national cohort study in the Netherlands. *Chest*
9
10
11 2005;128:2309-2315.
12
13
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15
16
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For Peer Review

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3 **Long term effects of birth order and age at diagnosis in cystic fibrosis; a sibling cohort study.**
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7 M.G. Slieker, MD, PhD,^{1*} J.M.W. van den Berg, MD,² J. Kouwenberg, MD,³ F. Teding van Berkhout, MD¹,

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9 H.G.M. Heijerman, MD, PhD,² C.K. van der Ent, MD, PhD¹

10
11 ¹Cystic Fibrosis Center Utrecht, University Medical Center Utrecht, Utrecht, the Netherlands

12
13 ²Adult Cystic Fibrosis Center, Haga Teaching Hospital, The Hague, the Netherlands

14
15 ³Juliana Children's Hospital, Haga Teaching Hospital, The Hague, the Netherlands

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19 *Correspondence to: Dr. M.G. Slieker, Cystic Fibrosis Center Utrecht, University Medical Center Utrecht,

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21 Lundlaan 6, 3584 EA, Utrecht, the Netherlands. Phone: +31887554690. Fax: +31887554747, E-mail:

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23 m.g.slieker@umcutrecht.nl
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27 Abbreviated title: Effects of age at diagnosis in siblings with CF
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31 Key words: cystic fibrosis, siblings, prognosis, lung function, Pseudomonas aeruginosa
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ABSTRACT

Background: Siblings with cystic fibrosis (CF) share many genetic and environmental factors, but may present different phenotypes. Younger sibs are mostly earlier diagnosed with CF than their older sibs, but might be at risk for an earlier colonization with *P. aeruginosa* (PA) than their older counterparts due to cross-infection within families.

Aims: To analyze the effects of birth order and age at diagnosis on lung function, PA colonization, nutritional status, and survival during the first two decades of life in siblings with CF.

Methods: A retrospective cohort study of 52 sibling pairs was performed in two Dutch CF centers. Data were analyzed both cross-sectionally and longitudinally using Kaplan-Meier curves and modified log-rank tests.

Results: Median age at diagnosis was significantly higher in the older sib compared with the younger sib (3.0 and 0.2 years, respectively, $P < 0.0001$). At the age of 5, 10 and 15 years no difference in lung function was found. However, at the age of 20 years, FEV₁ in older sibs was 19.4% (95% CI: 5.9-32.9%, $P = 0.007$) lower than in younger sibs. In the younger sibs group, FEV₁ at age 20 years was significantly better in those who had a diagnosis before the age of 6 months (difference 22.9%, 95% CI: 0.1-45.8%, $P < 0.05$). In the first 10 years of life the younger sibs tended to be earlier colonized with PA than their older counterparts. No differences in nutritional status and survival were observed.

Conclusion: In this sibling cohort study, an early diagnosis of CF was associated with better lung function after two decades of life. Although younger siblings tended to be colonized with PA at an earlier age, they showed better lung function outcomes. This underscores the importance of early diagnosis with newborn screening and early referral to a specialized center in the prevention of long-term deleterious effects on lung function.

INTRODUCTION

There is considerable variation in the clinical course of disease in cystic fibrosis (CF), even in patients with the same CF genotype,¹ suggesting the influence of other factors such as environment, quality of care and modifier genes.²⁻⁴

Siblings with CF share the same CFTR mutations, are generally exposed to the same environment, and have similar quality of care. These factors contribute to a high concordance in CF phenotype between CF siblings.⁵ However, in countries where newborn screening (NBS) is not available (as in the Netherlands), younger sibs are generally earlier diagnosed with CF than their older sibs.^{5,6} Early asymptomatic diagnosis is associated with better lung function,⁷⁻¹⁰ nutritional status,¹¹⁻¹⁴ and survival.^{7,9,14-16} This would suggest an advantage for the younger sib.

On the other hand, since cross-infection is known to occur between patients with CF,^{17,18} it can be expected that younger siblings may acquire human adapted respiratory pathogens at an earlier age. *P. aeruginosa* (PA) infection is associated with deteriorating lung function and increased morbidity,^{19,20} which may result in a more severe CF phenotype in the younger sib.

The aim of this study was to investigate the effects of birth order and age at diagnosis on lung function, PA colonization, nutritional status, and survival in non-twin sibs with CF, aged up to 20 years.

METHODS

The study population consisted of all sibling pairs treated at the CF Centers of the University Medical Center Utrecht (Utrecht, the Netherlands) and the Haga Teaching Hospital (The Hague, the Netherlands). These centers provide care for 580 out of 1250 CF patients who are recorded in the Dutch Cystic Fibrosis Registry. This registry covers 93% of all Dutch CF-patients (www.ncfs.nl). Treatment strategies are comparable in both centers and are based on national guidelines.²¹ All patients gave informed consent for the recording of their clinical data in our database and for the use of these data for scientific purposes, including descriptive studies.

In families with more than two affected siblings, only the first two siblings were included in the study. Twins were excluded. Patients born before 1970 or without clinical data before the age of 20 years available were also excluded.

All data were retrospectively collected from patient's medical charts at each center. The diagnosis of CF was confirmed in all patients with sweat testing and/or DNA analysis for CF mutations.

The primary outcome measure was lung function (forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC)). Secondary outcome measures included age at diagnosis, nutritional status (height and weight), PA colonization and survival.

FEV₁ and FVC were measured by a pneumotachograph and converted to percentage of predicted values.^{22,23} For cross-sectional analysis, the highest FEV₁ measure within the last year of available data for the younger sib was used. This value was matched with the highest FEV₁ of the older sibling during the year in which he/she reached the same age as the younger sib. Longitudinal measures were derived from all years of available lung function data for each study subject, using the best FEV₁ measurement per year.

Z-scores for height and weight were calculated using standard growth diagrams for the Dutch population.²⁴

Matching of height and BMI measures between older and younger sibs was similar to those performed for lung function. Data on lung function and nutritional status were available from 1991 onwards.

PA colonization was considered to be present when >50% of the PA cultures in the preceding year were positive.²⁵ In the older siblings less than 4 sputum samples per year were available. In these patients the old European consensus definition for chronic PA colonization was used, i.e. at least three positive cultures over ≥ 6 months with a ≥ 1 month interval.²⁶ In patients who were colonized according to this definition, we defined date of PA colonization as date of first positive PA culture plus one year (to make this date similar to the Leeds criteria). In all patients we attempted to perform a sputum culture. When a patient was not able to produce sputum, an oropharyngeal swab was taken.

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3 Based on their functional effects, CFTR mutations were divided in 5 classes.¹ Class I, II and III mutations result
4 in absence of or non-functional CFTR, whereas classes IV and V mutations, allowing some function of CFTR,
5 usually result in less severe disease manifestations.²⁷ Patients with two class I, II or III mutations were
6 considered to have a severe CFTR genotype, whereas patients with one or two class IV or V mutations were
7 considered to have a mild genotype.¹
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13 14 15 **Statistical analysis**

16 Differences between siblings were calculated using paired Student's t-test for normally distributed continuous
17 variables, Wilcoxon signed-rank test for not-normally distributed continuous variables, and McNemar test for
18 discrete variables. In order to test if continuous variables were normally distributed, a Kolmogorov-Smirnov test
19 was performed. The level of significance was 0.05.
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25 Power and sample size were calculated using PASS 2008 (NCSS, Kaysville, UT). Sample size calculation
26 showed that twenty sibling pairs were needed to detect a 20% difference in lung function with an estimated SD
27 of the difference in lung function of 30%, an alpha of 0.05 and a power of 0.8.
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30 Kaplan-Meier curves and modified log rank tests were used to assess whether there were differences in survival
31 and PA colonization by birth order. Specifically, a Cox proportional hazards model (the probability model
32 implicit in the log rank test) was used, with a term denoting sibling pairs, to test for the significance of birth
33 order in the model. This model takes into account the paired nature of the siblings when calculating the standard
34 error for the birth order coefficient.
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RESULTS

In total, 52 families with at least one sibling pair with CF met the inclusion criteria. The mean age difference between the two siblings was 3.7 ± 2.2 years.

Patient characteristics are shown in Table 1. Median age at diagnosis was significantly higher in the older sib compared with the younger sib (3.0 and 0.2 years, respectively, $P < 0.0001$).

In 37 pairs (71.2%) the older sib was diagnosed first, in 11 pairs (21.2%) the younger sib was diagnosed first, and in 4 pairs (7.7%) sibs were diagnosed at the same time. Eight of the younger sibs (15.4%) were diagnosed antenatally or shortly after birth before the development of symptoms because of CF in the older sib. Both the first and second diagnosed sibs were immediately followed in a CF center from the diagnosis. Sputum cultures at diagnosis in first and second diagnosed sibs were positive for PA in 12.5% and 8.3% of the patients, respectively (McNemar test $P = 0.63$).

Older sibs tended to have respiratory symptoms at diagnosis more often (55.8% and 38.5%, respectively, $P = 0.12$) compared with younger sibs (Table 1).

In four sibling pairs no CFTR genotype was determined. Of the remaining 48 patient pairs, 39 (81.3%) had a severe CFTR genotype class.

Lung function testing was performed in 47 sibling pairs. Lung function at last clinical visit for the younger sib tended to be higher than the matched lung function for the older sib (Table 2). FEV₁ was analyzed per 5-year intervals. Unfortunately, for some sibling pairs lung function data were not available at all investigated ages because they had not reached that age or because they were first treated at another center. At the age of 5, 10, 15 and 20 years, for 15, 21, 22 and 22 patients pairs lung function data were available, respectively. FEV₁ at the age of 5, 10 and 15 years was not significantly different between the sibs. However, at the age of 20 years, FEV₁ in older sibs was 19.4% (95% CI: 5.9-32.9%, $P = 0.007$) lower than in younger sibs (Figure 1). At this age, 4 of the older sibs had a better and 18 had a poorer lung function compared to their younger sibs (Wilcoxon signed rank test $P = 0.007$). In order to test the hypothesis that the better clinical outcome in the younger sibs is caused by earlier diagnosis of CF, we analyzed the effect of age at diagnosis on lung function in the younger sibs using an unpaired Student's t-test. Since the association between age at diagnosis and lung function could be confounded by severity of CFTR genotype,²⁸ this analysis was only performed in patients with a severe CFTR genotype. FEV₁ analyzed per 5-year intervals demonstrated a significantly higher FEV₁ at the age of 20 years in sibs diagnosed before 6 months of age compared with those diagnosed after 6 months of age (difference 22.9%, 95% CI: 0.1-45.8%, $P < 0.05$). However, no significant differences were observed at the age of 5, 10 and 15 years.

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3 PA colonization at last visit was not different between the older and younger sibs (Table 2). Also, when
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5 differences in age at PA colonization between younger and older sibs were analyzed longitudinally, younger sibs
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7 were overall not earlier colonized with PA (Cox proportional hazard 1.60, 95% CI: -0.73-3.52). However, the
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9 younger sibs tended to be earlier colonized in the first 10 years of life than their older counterparts (P=0.08), but
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11 this difference disappeared in the second decade (Figure 2). Height, weight and BMI did not differ between the
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13 older and the younger sibs (Table 2).

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15 Two of the older sibs and none of the younger sibs received a lung transplant before the age of 25 years. Three of
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17 the older sibs and one of the younger sibs died before the age of 25 years. Survival (no death and no lung
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19 transplant) was not significantly worse in the older sibs compared with the younger sibs (Cox proportional
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21 hazard: P=0.21).
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DISCUSSION

This study on long-term differences in lung function, PA colonization, nutritional status, and survival between older and younger sibs with CF demonstrated that older siblings had a poorer lung function in the second decade of life compared to their younger siblings with CF. Early diagnosis was associated with better lung function outcome. PA colonization was not significantly different between younger and older sibs, but younger sibs tended to be earlier colonized in the first 10 years of life. Older and younger siblings did not appear to have a different height, weight and survival.

In this study, we hypothesized that younger siblings might be at risk of more accelerated lung disease because of exposure to cross-infection from older siblings. On the other hand, we postulated that younger sibs would be earlier diagnosed and would therefore benefit from therapy earlier in life. It was shown that younger sibs tended to be earlier colonized with PA in the first 10 years of life, but that this difference disappeared in the second decade. Despite the higher PA colonization in the first decade, this did not result in a significantly worse lung function in the first decade, probably due to an earlier diagnosis. In the second decade no difference between PA colonization was found between the sibs, and younger sibs had better lung function. This suggests that the net effect of earlier diagnosis in younger CF siblings outweighs the negative effects, including the exposure to CF pathogens from older siblings.

The beneficial effect of younger age at diagnosis on lung function was confirmed by our subgroup analysis in the younger sibs: patients diagnosed before 6 months of age had a significantly better lung function in the second decade of life. This is in accordance with the better prognosis associated with early asymptomatic diagnosis of CF through NBS.^{7-10,29} In these early diagnosed asymptomatic patients, early antibiotic therapy, nutritional supplementation, and intensive physiotherapy can delay the progression of lung disease in CF.²⁹

A better lung function in younger siblings with CF was also found in a relatively old and small study by Orenstein *et al.*³⁰ On the other hand, two recent studies could not find significant differences in FEV₁ and FVC between older and younger sibs with CF.^{5,6} However, lung function was only measured at the age of 7 and 10 years,⁵ and at the age of 8 years,⁶ respectively. The age of 10 years may be too young to identify significant differences between the two siblings, which was demonstrated by our longitudinal analysis (difference only found in second decade of life).

Similar to other studies,^{5,6} we did find a strong familial tendency for PA colonization. This was probably caused by cross-infections between the siblings. The importance of cross-infections among CF patients within families has already been confirmed by DNA fingerprinting and serology.^{18,31} In accordance with other studies,^{5,6} we

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3 demonstrated an earlier age at PA colonization in the younger siblings than in the older siblings. However, in all
4 studies the interpretation is limited by the age-of-diagnosis bias. Patients are only tested for typical CF pathogens
5 if they already have a CF diagnosis. Therefore, a positive culture at time of diagnosis prevents accurate
6 determination of true age of infection. Since the older child in a family is always diagnosed at an older age than
7 the younger sibling, regardless of who is the index case, older siblings are more likely to have positive cultures at
8 time of diagnosis, simply due to more time to acquire the pathogens. The age-of-diagnosis bias could only be
9 overcome by a study of CF siblings diagnosed through NBS, but NBS is not yet available in the Netherlands.

10 The proportion of patients chronically colonized by PA according was rather high for the age of the patients. The
11 high proportion of chronic PA colonization was probably due to the fact that early eradication protocols for PA
12 were introduced only some years ago. Until 4 years ago, there was no segregation policy in The Netherlands and
13 many children visited CF summer camps, which may have contributed to the relatively high PA rates in our
14 cohort.

15 Since the proportion of patients chronically colonized by PA in this study was high for age, one could speculate
16 that patients who are early diagnosed and consequently early followed in CF centers are at risk of early
17 acquisition of bacterial pathogens, such as PA. However, different recent studies could not demonstrate an
18 association between early diagnosis (and early follow-up in a CF center) and early infection with PA when good
19 infection control practices are followed.^{32,33}

20 In the younger children the definition of chronic colonization by PA were based on Leeds criteria.²⁵ The validity
21 of this definition has recently been confirmed.³⁴ The minimum sample number needed to use the Leeds criteria
22 for a given year should be four. Unfortunately, in the past less than 4 sputum cultures per year were performed in
23 our patients and, consequently, the Leeds criteria could not be used in the older siblings. This could have led to
24 under-detection of the PA colonization. The effect on the outcome of our study is probably limited, since the
25 same definition was used within siblings pairs.

26 Similar to other sibling studies,^{5,6} we did not demonstrate any significant differences in height or BMI between
27 older and younger sibs, although one of the earlier sibling studies demonstrated a marginally significantly higher
28 height z-score in younger siblings than in older siblings.⁶ Other studies^{13,29,35} have shown dramatic differences in
29 nutritional status at diagnosis between those diagnosed through screening and those diagnosed as a result of
30 clinical signs and symptoms. However, these differences can be reduced once appropriate dietary intervention,
31 including the provision of pancreatic enzyme replacement therapy, is commenced.¹³ That we could not detect
32 any advantage of early diagnosis on nutritional status in younger sibs might be because of a significant catch-up
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3 growth achieved in the early years of life. Finally, in accordance with our study but in contrast with NBS studies,
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5 a recent Belgian study could not find significant differences in anthropometric parameters between children who
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7 were early and late (<2 and ≥ 2 years after diagnosis) referred to a CF center.³⁶
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9 We found no significant differences in survival between older and younger sibs, but the number of patients
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11 investigated may have been too small to detect these differences. Other sibling studies did not investigate
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13 differences in survival between older and younger sibs.^{5,6,30} However, most of the studies on the effect of NBS
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15 found that early diagnosis through NBS is associated with improved child survival.^{7,9,14-16}
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17 Although this is the largest sibling study so far on the effect of birth order on lung function in CF patients, our
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19 study was limited by the population size. However, power analysis revealed that the number of sibling pairs
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21 included was sufficient to detect a 20% difference in lung function between the siblings.
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23 Another limitation may have been the influence of time. Treatment regimes for sibling pairs born in the early
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25 seventies were different than the ones for sibling pairs born in the nineties. These differences include improved
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27 nutritional management and dietary recommendations, new airway clearance techniques and new
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29 antipseudomonal antibiotics.³⁷ One could hypothesize that having a older sib could have resulted in poorer lung
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31 function in younger sibs born in the seventies compared to those born in the nineties because of new
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33 antipseudomonal drugs. Unfortunately, the size of our population did not allow us to analyze the influence of
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35 date of birth on outcome.

36 In conclusion, in this sibling cohort study, an early diagnosis of CF was associated with better lung function after
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38 two decades of life. Although younger siblings tended to be colonized with PA at an earlier age, they showed
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40 better lung function outcomes. This underscores the importance of early diagnosis with newborn screening in the
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References

1. Mckone EF, Goss CH, Aitken ML. CFTR genotype as a predictor of prognosis in cystic fibrosis. *Chest* 2006;130:1441-1447.
2. Schechter MS. Non-genetic influences on cystic fibrosis lung disease: the role of sociodemographic characteristics, environmental exposures, and healthcare interventions. *Semin Respir Crit Care Med* 2003;24:639-652.
3. Knowles MR. Gene modifiers of lung disease. *Curr Opin Pulm Med* 2006;12:416-421.
4. Vanscoy LL, Blackman SM, Collaco JM, Bowers A, Lai T, Naughton K, Algire M, McWilliams R, Beck S, Hoover-Fong J, Hamosh A, Cutler D, Cutting GR. Heritability of lung disease severity in cystic fibrosis. *Am J Respir Crit Care Med* 2007;175:1036-1043.
5. Picard E, Aviram M, Yahav Y, Rivlin J, Blau H, Bentur L, Avital A, Villa Y, Schwartz S, Kerem B, Kerem E. Familial concordance of phenotype and microbial variation among siblings with CF. *Pediatr Pulmonol* 2004;38:292-297.
6. Katz SL, Strug LJ, Coates AL, Corey M. Disease severity in siblings with cystic fibrosis. *Pediatr Pulmonol* 2004;37:407-412.
7. McKay KO, Waters DL, Gaskin KJ. The influence of newborn screening for cystic fibrosis on pulmonary outcomes in new South Wales. *J Pediatr* 2005;147:S47-S50.
8. Merelle ME, Schouten JP, Gerritsen J, Dankert-Roelse JE. Influence of neonatal screening and centralized treatment on long-term clinical outcome and survival of CF patients. *Eur Respir J* 2001;18:306-315.
9. Mastella G, Zanolla L, Castellani C, Altieri S, Furnari M, Giglio L, Lombardo M, Miano A, Sciuto C, Pardo F, Magazzu G. Neonatal screening for cystic fibrosis: long-term clinical balance. *Pancreatology* 2001;1:531-537.
10. Wang SS, O'Leary LA, Fitzsimmons SC, Khoury MJ. The impact of early cystic fibrosis diagnosis on pulmonary function in children. *J Pediatr* 2002;141:804-810.
11. Farrell PM, Kosorok MR, Rock MJ, Laxova A, Zeng L, Lai HC, Hoffman G, Laessig RH, Splaingard ML. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. *Pediatrics* 2001;107:1-13.
12. Sims EJ, McCormick J, Mehta G, Mehta A. Neonatal screening for cystic fibrosis is beneficial even in the context of modern treatment. *J Pediatr* 2005;147:S42-S46.

- 1
2
3 13. Farrell PM, Kosorok MR, Laxova A, Shen G, Kosciak RE, Bruns WT, Splaingard M, Mischler EH.
4
5 Nutritional benefits of neonatal screening for cystic fibrosis. Wisconsin Cystic Fibrosis Neonatal
6
7 Screening Study Group. *N Engl J Med* 1997;337:963-969.
8
- 9 14. Siret D, Bretaudeau G, Branger B, Dabadie A, Dagonne M, David V, De Braekeleer M, Moisan-Petit V,
10
11 Picherot G, Rault G, Storni V, Roussey M. Comparing the clinical evolution of cystic fibrosis screened
12
13 neonatally to that of cystic fibrosis diagnosed from clinical symptoms: a 10-year retrospective study in a
14
15 French region (Brittany). *Pediatr Pulmonol* 2003;35:342-349.
16
- 17 15. Doull IJ, Ryley HC, Weller P, Goodchild MC. Cystic fibrosis-related deaths in infancy and the effect of
18
19 newborn screening. *Pediatr Pulmonol* 2001;31:363-366.
20
- 21 16. Lai HJ, Cheng Y, Farrell PM. The survival advantage of patients with cystic fibrosis diagnosed through
22
23 neonatal screening: evidence from the United States Cystic Fibrosis Foundation registry data. *J Pediatr*
24
25 2005;147:S57-S63.
26
- 27 17. Tubbs D, Lenney W, Alcock P, Campbell CA, Gray J, Pantin C. *Pseudomonas aeruginosa* in cystic
28
29 fibrosis: cross-infection and the need for segregation. *Respir Med* 2001;95:147-152.
30
- 31 18. Renders NH, Sijmons MA, van BA, Overbeek SE, Mouton JW, Verbrugh HA. Exchange of *Pseudomonas*
32
33 *aeruginosa* strains among cystic fibrosis siblings. *Res Microbiol* 1997;148:447-454.
34
- 35 19. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other
36
37 predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr Pulmonol*
38
39 2002;34:91-100.
40
- 41 20. Nixon GM, Armstrong DS, Carzino R, Carlin JB, Olinsky A, Robertson CF, Grimwood K. Clinical
42
43 outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Pediatr* 2001;138:699-704.
44
- 45 21. van der Laag J, Sinaasappel M, Heijerman HGM, editors. *Diagnostiek en behandeling van cystic fibrosis*
46
47 [Diagnosis and treatment of cystic fibrosis]. Utrecht: Centraal Begeleidingsorgaan voor de Intercollegiale
48
49 Toetsing; 1998. 174 p.
50
- 51 22. Zapletal A, Samanek M, Paul T. Lung function in children and adolescents: methods, reference values. In:
52
53 Zapletal A, Samanek M, Paul T, editors. *Progress in respiration research*. Basel, Switzerland: Karger;
54
55 1987. p 114-218.
56
- 57 23. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced
58
59 ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community
60

- 1
2
3 for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;16:5-
4
5 40.
6
7 24. TNO Prevention and Health, Leiden University Medical Center. Growth diagrams 1997. Houten, the
8
9 Netherlands: Bohn Stafleu Van Loghum; 1998.
10
11 25. Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for
12
13 chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *J Cyst Fibros* 2003;2:29-34.
14
15 26. Doring G, Conway SP, Heijerman HG, Hodson ME, Hoiby N, Smyth A, Touw DJ. Antibiotic therapy
16
17 against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. *Eur Respir J* 2000;16:749-767.
18
19 27. Vankeerberghen A, Cuppens H, Cassiman JJ. The cystic fibrosis transmembrane conductance regulator:
20
21 an intriguing protein with pleiotropic functions. *J Cyst Fibros* 2002;1:13-29.
22
23 28. Mckone EF, Emerson SS, Edwards KL, Aitken ML. Effect of genotype on phenotype and mortality in
24
25 cystic fibrosis: a retrospective cohort study. *Lancet* 2003;361:1671-1676.
26
27 29. Waters DL, Wilcken B, Irwing L, Van Asperen P, Mellis C, Simpson JM, Brown J, Gaskin KJ. Clinical
28
29 outcomes of newborn screening for cystic fibrosis. *Arch Dis Child Fetal Neonatal Ed* 1999;80:F1-F7.
30
31 30. Orenstein DM, Boat TF, Stern RC, Tucker AS, Charnock EL, Matthews LW, Doershuk CF. The effect of
32
33 early diagnosis and treatment in cystic fibrosis: a seven-year study of 16 sibling pairs. *Am J Dis Child*
34
35 1977;131:973-975.
36
37 31. Thomassen MJ, Demko CA, Doershuk CF, Root JM. *Pseudomonas aeruginosa* isolates: comparisons of
38
39 isolates from campers and from sibling pairs with cystic fibrosis. *Pediatr Pulmonol* 1985;1:40-45.
40
41 32. Baussano I, Tardivo I, Bellezza-Fontana R, Forneris MP, Lezo A, Anfossi L, Castello M, Aleksandar V,
42
43 Bignamini E. Neonatal screening for cystic fibrosis does not affect time to first infection with
44
45 *Pseudomonas aeruginosa*. *Pediatrics* 2006;118:888-895.
46
47 33. Collins MS, Abbott MA, Wakefield DB, Lapin CD, Drapeau G, Hopfer SM, Greenstein RM, Cloutier
48
49 MM. Improved pulmonary and growth outcomes in cystic fibrosis by newborn screening. *Pediatr*
50
51 *Pulmonol* 2008;43:648-655.
52
53 34. Proesmans M, Balinska-Miskiewicz W, Dupont L, Bossuyt X, Verhaegen J, Hoiby N, De BK. Evaluating
54
55 the "Leeds criteria" for *Pseudomonas aeruginosa* infection in a cystic fibrosis centre. *Eur Respir J*
56
57 2006;27:937-943.
58
59 35. Dankert-Roelse JE, te Meerman GJ. Long term prognosis of patients with cystic fibrosis in relation to
60
early detection by neonatal screening and treatment in a cystic fibrosis centre. *Thorax* 1995;50:712-718.

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2
3 36. Lebecque P, Leonard A, De BK, De BF, Malfroot A, Casimir G, Desager K, Godding V, Leal T. Early
4 referral to cystic fibrosis specialist centre impacts on respiratory outcome. *J Cyst Fibros* 2009;8:26-30.
5
6
7 37. Slieker MG, Uiterwaal CSPM, Sinaasappel M, Heijerman HGM, van der Laag J, van der Ent CK. Birth
8 prevalence and survival in cystic fibrosis - A national cohort study in the Netherlands. *Chest*
9
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11 2005;128:2309-2315.
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Table 1. Baseline demographics of the study population.

Variable	Younger sib	Older sib	P-value ^a
N	52	52	
Males (%)	21 (40.4)	20 (38.5)	1.00
Pancreatic insufficiency (%)	48 (92.3)	48 (92.3)	1.00
Age at diagnosis (yrs), median (range)	0.2 (0.0-10.7)	3.0 (0.0-13.7)	<0.0001
Presenting symptoms of CF:			
Meconium ileus (%)	11 (21.2)	8 (15.4)	0.51
Failure to thrive (%)	22 (42.3)	26 (50.0)	0.50
Respiratory symptoms (%)	20 (38.5)	29 (55.8)	0.12
Genotype:			
Homozygous Δ F508 (%)	28 (53.8)		
Heterozygous Δ F508 (%)	15 (28.8)		
Other (%)	5 (9.6)		
Not determined (%)	4 (7.7)		
Genotype classes:			
Class I-III (%) ^b	39 (75.0)		
Class IV-V (%) ^c	9 (17.3)		
Not determined (%)	4 (7.7)		

^aFrom Wilcoxon signed rank test for age at diagnosis, McNemar test for all other analyses. ^bBoth alleles belonging to class I – III. ^cAt least one allele belonging to class IV or V.

Table 2. Disease severity outcome measures at the last visit for the younger sib and at the visit for the older sib at which he/she had the same age as the younger sib. Data are presented as mean (SD), unless otherwise specified.

	Younger sib	Older sib	P-value ^a	Younger sib higher (N) ^b	Older sib higher (N)	P-value ^c
Age, years (SD)	15.2 (6.5)	15.2 (6.5)	1.00			
FEV ₁ , % predicted (SD) ^d	81.3 (22.9)	74.2 (32.4)	0.11	30	17	0.15
FVC, % predicted (SD) ^d	88.5 (17.6)	82.0 (25.8)	0.11	28	19	0.11
FEV ₁ /FVC (SD) ^d	77.2 (13.2)	73.7 (13.7)	0.08	28	19	0.10
<i>P. aeruginosa</i> colonization (%)	33 (63.5)	34 (65.4)	1.00			
Height for age, z-score (SD)	-0.76 (0.98)	-0.61 (1.13)	0.31	20	32	0.27
Weight for height, z-score (SD)	-0.23 (0.92)	-0.12 (1.17)	0.55	25	27	0.80
BMI, kg/m ² (SD)	18.6 (2.8)	18.8 (3.1)	0.66	24	28	0.96

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

^aFrom McNemar test for PA colonization, paired t-test for all other analyses. ^bColumn shows number of patients having a higher value than their older sib. ^cWilcoxon signed rank test. ^dN=47.

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7 **Figure 1.** Lung function measured at five-year intervals. At the age of 5, 10, 15 and 20 years, for 15, 21, 22 and
8
9 22 patients pairs lung function data were available, respectively. **Bold line indicates mean FEV₁.** FEV₁ at the age
10 of 20 years was significantly lower in older siblings compared with their younger counterparts (difference
11 19.4%, 95% confidence interval: 5.9–32.9, **P=0.007**).
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17 **Figure 2.** Kaplan-Meier curves of age at PA colonization by birth order, measured in 39 sibling pairs. Cox
18 proportional hazard P=0.24.
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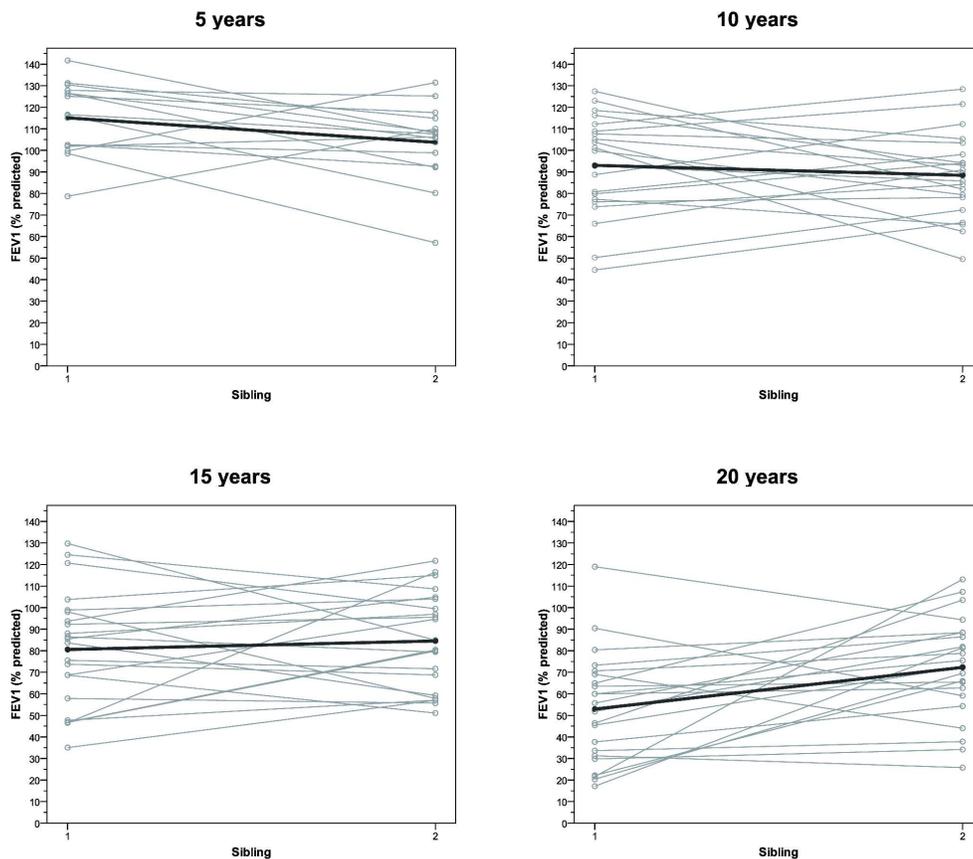


Figure 1. Lung function measured at five-year intervals. At the age of 5, 10, 15 and 20 years, for 15, 21, 22 and 22 patients pairs lung function data were available, respectively. Bold line indicates mean FEV1. FEV1 at the age of 20 years was significantly lower in older siblings compared with their younger counterparts (difference 19.4%, 95% confidence interval: 5.9–32.9, P=0.007).
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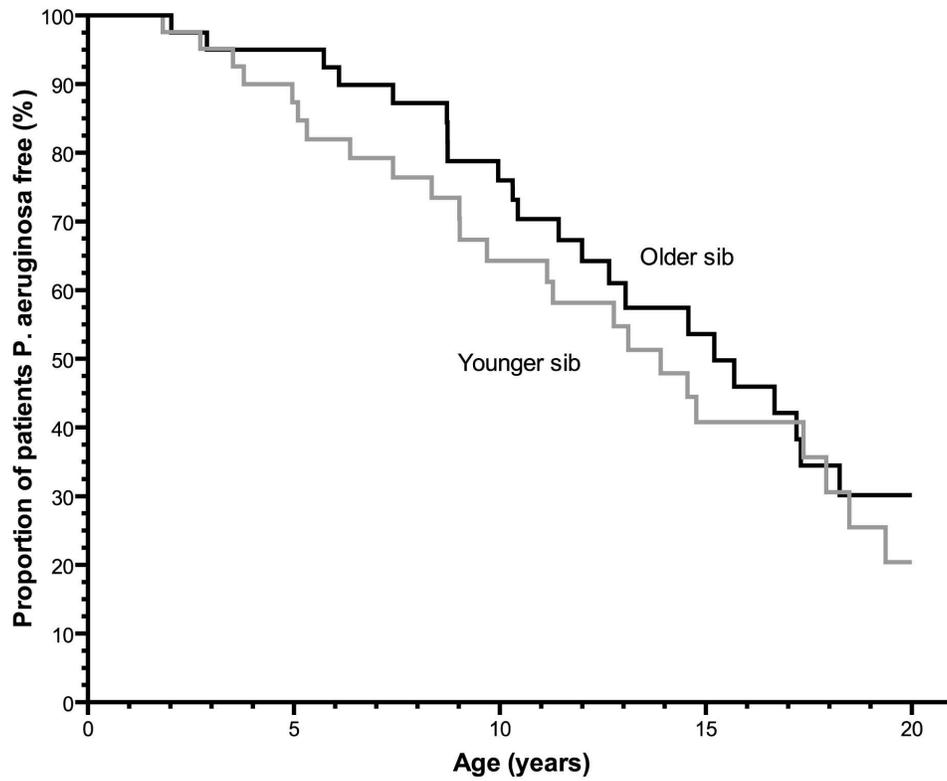


Figure 2. Kaplan-Meier curves of age at PA colonization by birth order, measured in 39 sibling pairs.
Cox proportional hazard P=0.24.
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