Differences in Outcomes between Early and Late Diagnosis of Cystic Fibrosis in the Newborn Screening Era

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Objectives To evaluate children with cystic fibrosis (CF) who had a late diagnosis of CF (LD-CF) despite newborn screening (NBS) and compare their clinical outcomes with children diagnosed after a positive NBS (NBS-CF).

Study design A retrospective review of patients with LD-CF in New South Wales, Australia, from 1988 to 2010 was performed. LD-CF was defined as NBS-negative (negative immunoreactive trypsinogen or no *F508del*) or NBS-positive but discharged following sweat chloride < 60 mmol/L. Cases of LD-CF were each matched 1:2 with patients with NBS-CF for age, sex, hospital, and exocrine pancreatic status.

Results A total of 45 LD-CF cases were identified (39 NBS-negative and 6 NBS-positive) with 90 NBS-CF matched controls. Median age (IQR) of diagnosis for LD-CF and NBS-CF was 1.35 (0.4-2.8) and 0.12 (0.03-0.2) years, respectively ($P < .0001$). Estimated incidence of LD-CF was 1 in 45 000 live births. Compared with NBS-CF, LD-CF had more respiratory manifestations at time of diagnosis (66% vs 4%; $P < .0001$), a higher rate of hospital admission per year for respiratory illness (0.49 vs 0.2; $P = .0004$), worse lung function (forced expiratory volume in 1 second percentage of predicted, 0.88 vs 0.97; $P = .007$), and higher rates of chronic colonization with *Pseudomonas aeruginosa* (47% vs 24%; $P = .01$). The LD-CF cohort also appeared to be shorter than NBS-CF controls (mean height *z*-score −0.65 vs −0.03; $P = .02$).

Conclusions LD-CF, despite NBS, seems to be associated with worse health before diagnosis and worse later growth and respiratory outcomes, thus providing further support for NBS programs for CF. *(J Pediatr 2017;181:137-45).*

Cystic fibrosis (CF) is a life-shortening recessive disorder, caused by mutations in the *CF transmembrane conductance regulator* (*CFTR*) gene, which affects approximately 1 in 3000 newborns in Caucasian populations.[1-7] CF is now commonly diagnosed via newborn screening (NBS) in many countries,[8] with the state of New South Wales (NSW) in Australia implementing screening in July 1981. Screening in NSW initially began with a 2-tier immunoreactive trypsinogen (IRT) protocol, with dried blood spots collected on days 3-5 (subsequently changed to days 2-4) of life and again at 4-6 weeks if increased.[9] In 1993 NSW changed to an IRT-DNA system: IRT level > 99th percentile and *F508del* constituting a positive test. Testing during this period was for the *F508del* mutation only (which was at the time present as either one or 2 copies in 94% of NSW patients with CF[10]). Dependent on whether *F508del* was found on 1 or both alleles, referral to a Cystic Fibrosis Clinic for treatment, sweat testing, and possibly further genotyping was recommended.

In 2009 a Cochrane review of NBS for CF highlighted that the Wisconsin NBS trial was the only one meeting randomized, controlled trial criteria.[11] This review concluded that severe malnutrition was less common among screened babies[12] and the NBS provided potential for better respiratory outcomes[13]; however, the later

BMI	Body mass index
CF	Cystic fibrosis
CFSPID	CF screen positive inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
FEV1%	Forced expiratory volume in 1 second percentage of predicted
IRT	Immunoreactive trypsinogen
LD-CF	Late diagnosis of cystic fibrosis
LD-NBS-neg	Late diagnosis newborn screen negative
LD-NBS-pos	Late diagnosis newborn screen positive
MI	Meconium ileus
NBS	Newborn screening
NBS-CF	Newborn screen diagnosed CF
NSW	New South Wales
PI	Pancreatic insufficient
PS	Pancreatic sufficient
SC	Sweat chloride

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findings were confounded by a high infection rate of *Pseudomonas aeruginosa*. The prolonged debate over the benefit of NBS has been largely due to the lack of limited randomized, controlled trial data. Although good quality evidence was available, limitations often included the retrospective nature and selection criteria of studies.[14-21] Beyond those diagnosed by NBS, the outcomes of those who are missed by NBS are unknown. The first attempt to look for missing cases was undertaken by Massie et al in 2000.[22] In Australian populations, the IRT-DNA protocol has performed with a sensitivity of 94% and a specificity of 99.9%,[10] which is comparable with other US and European centers.[8] Accounting for differences in IRT cutoffs and the number of mutations in DNA panels worldwide, the sensitivity of NBS varies between 86.6% and 97.5%.[8] To date, there are no publications specifically analyzing those patients with a false-negative NBS result.

Interest in the subset of patients with an inconclusive diagnosis of CF after NBS has emerged, with the terms "CFTR-related metabolic syndrome"[23] and "CF screen positive inconclusive diagnosis" (CFSPID)[24,25] proposed by US and European groups, respectively. In a prospective study, approximately 11% of infants with an initial inconclusive diagnosis will subsequently receive a diagnosis of CF, thus, arguing the need for follow-up of this population.

Due to the opportunity for early intervention and preventative care of patients with CF, data about those with a missed diagnosis are important. These data includes incidence, characteristics (including genotype and phenotype), and long-term outcomes. Given the long history of NBS in NSW, we are provided with a unique opportunity to evaluate this subset of patients. Our study aimed to provide observations from the important group of patients with CF who had a late diagnosis of CF (LD-CF) despite NBS, and determined whether their clinical outcomes differ from children diagnosed with CF after a positive NBS (newborn screen diagnosed CF [NBS-CF]) during the same time period.

Methods

A retrospective review of patients with LD-CF from April 1988 (commencement of the Australian CF Data Registry) to December 2010 within NSW was performed. All cases with LD-CF were identified through the Australian CF Data Registry, CF clinics (Sydney Children's Hospital Randwick, Children's Hospital Westmead, and John Hunter Children's Hospital), and the NSW NBS Programme. This study was approved by the human research ethics boards of all participating institutions (LNR/11/SCHN/310).

Patients were included if they met the diagnostic criteria for CF and were defined as LD-CF (as per definitions below). Patients were excluded if they (1) did not meet the diagnostic criteria for CF, (2) did not receive NBS, (3) were born before April 1988, from outside NSW or there was insufficient data for any other reason, or (4) were not diagnosed late.

As a comparator, each subject with LD-CF was matched 1:2 with patients with NBS-CF for age, sex, exocrine pancreatic status (determined by either the coefficient of fat absorption from 72-hour fecal fat collection and/or fecal elastase), and CF clinic (ie, same clinic) using the Australian CF Data Registry. If multiple matches were identified, the control with the smallest age difference to the subject was selected. If pancreatic function status was unknown for the subject, they were matched with pancreatic insufficient (PI) controls. Demographic, clinical (including calculated *z*-scores for growth variables), and laboratory data were collected from the aforementioned databases.

During the study period (April 1988-December 2010), the NBS protocol in NSW included a dried blood spot IRT level for all newborns. From April 1988 to March 1993, those patients who had an elevated IRT (top 0.7% and/or >100 μg/L whole blood) had a repeat IRT performed and if the second was also elevated (>75 μg/L whole blood), they would subsequently undergo sweat testing. Mutation screening for *F508del* began in April 1993 and those with an elevated IRT (top 1% and/or >75 μg/L whole blood) would undergo *F508del* mutation analysis. If *F508del* was identified, either 1 or 2 copies, sweat testing, and, at the discretion of treating clinician, an extended genetic mutation panel was performed.

CF as defined by the US CF Foundation Consensus Report[20] consists of ≥1 characteristic phenotypic feature(s) of disease plus sweat chloride (SC) ≥ 60 mmol/L, and/or identification of CF disease-causing mutations on both alleles. Alternatively in the absence of symptoms, a diagnosis of CF in a sibling is sufficient as phenotypic criteria. Mutations were classified as disease-causing using the CFTR2 project.[26,27]

LD-CF was defined as an individual who fulfilled the criteria for CF and was either (1) NBS negative (LD-NBS-neg) with a negative IRT or genotype (ie, no *F508del* identified) or (2) NBS positive (LD-NBS-pos) but discharged after a SC < 60 mmol/L.

Chronic *Pseudomonas aeruginosa* infection was defined by 3 consecutive respiratory cultures within a 12-month period and/or isolation of mucoid *P aeruginosa*.[28] Chronic *Staphylococcus aureus* infection was defined by 3 consecutive positive respiratory cultures within a 12-month period.

Statistical Analyses

Case-control matching analysis of age (on December 31, 2010) was performed using Pearson correlation to assess effectiveness of matching. Cohort analysis was performed with comparisons between LD-CF (including LD-NBS-neg and LD-NBS-pos subsets) and NBS-CF cohorts made by utilizing Student *t* test or Mann-Whitney *U* test for continuous variables (presented as mean [SD] and median [IQR] for normally and not normally distributed data, respectively) and by Fisher exact test for categorical variables. Linear mixed models analysis was utilized to assess clinical measurements performed throughout the study period and is presented as mean (SD). $P < .05$ was considered statistically significant. All statistical calculations and graphs were performed in SPSS 22.0 (SPSS Inc, Chicago, Illinois).

Results

A total of 45 LD-CF cases were identified and included along with 90 matched NBS-CF controls. Of the initial 68 LD-CF cases identified, 4 did not meet the diagnostic criteria for CF, 1 did not receive NBS, 1 was born before April 1988, 1 was born outside NSW, 1 moved from NSW, 4 were lost to follow-up, 1 was a duplicate patient, 3 patients with CF had meconium ileus (MI), and 7 patients with NBS-CF in whom there was a documented reason for delay in performing a sweat test and/or the diagnosis of CF was not late.

The demographic data for LD-CF and patients with NBS-CF are summarized in **Table I**. The estimated incidence of CF in NSW during the study period was 1 in 2200 live births, whereas the incidence of LD-CF was 1 in 45 000 live births. Thus, approximately 1 in every 21 patients with CF were LD-CF. The LD-CF and patients with NBS-CF were matched for sex, hospital location, exocrine pancreatic sufficiency status, and significantly matched for age (Pearson correlation, $P < .0001$). Median age (IQR) of diagnosis for LD-CF and NBS-CF was 1.35 years (0.36-2.81) and 0.12 years (0.03-0.16) respectively ($P < .0001$).

Of the LD-CF cases, 39 were LD-NBS-neg (22 negative IRT and 17 negative genotype for *F508del*) and 6 were LD-NBS-pos (2 with a SC < 30 mmol/L and 4 with a SC 30-60 mmol/L) (**Figure 1**; available at www.jpeds.com).

Genotypic and Phenotypic Data

The genotypic and phenotypic data for each patient with LD-CF and NBS-CF–matched control is listed in **Table II**. Almost one-half of the patients with LD-CF (49%) had a negative IRT < 99th percentile. Of the patients with LD-CF, 31 (69%) had *CFTR* mutations identified on both alleles, 8 (18%) had only 1 mutation identified, and 6 (13%) had no mutations identified on either allele. Two disease-causing mutations (ie, not including those with varying clinical consequence or unknown significance) were identified in 23 (51%) and 72 (80%) of patients with LD-CF and NBS-CF, respectively ($P = .001$). SC levels were not significantly different between patients with LD-CF and patients with NBS-CF with the mean (SD) initial SC values being 83.2 (4.4) and 89.1 (2.1) respectively ($P = .2$). Pancreatic function status was known in 37 of the patients with LD-CF (82%), and of those, 27 (73%) were PI and 10 (27%) were pancreatic sufficient (PS). The rate of PI was significantly higher in the LD-NBS-neg cohort compared with the LD-NBS-pos cohort (81% vs 20% respectively; $P = .01$).

Table I. Demographic characteristics

Characteristics	LD-CF (n = 45)	NBS-CF (n = 90)	Matching
Age (years)			
At diagnosis*	1.35 (0.36-2.81)	0.12 (0.03-0.16)	$P < .0001$
At 31/12/2010	9.73 (0.84)	10.02 (0.59)	$P < .0001$†
Sex			
Male	24	48	100%
Female	21	42	100%
Hospital			
CHW	21	42	100%
JHCH	7	14	100%
SCH	17	34	100%
Pancreatic function‡			
PI	27	54	100%
PS	10	20	100%
Unknown§	8	16 (PI)	-

CHW, Children's Hospital at Westmead; *JHCH*, John Hunter Children's Hospital; *SCH*, Sydney Children's Hospital Randwick.
Data presented as median (IQR) or mean (SD).
*Age at diagnosis significantly different *$P < .0001$.
†Age at December 31, 2010 (for matching analysis), significantly effective using Pearson correlation.
‡Pancreatic function determined by the coefficient of fat absorption from 72-hour decal fat collection and/or fecal elastase-1.
§Cases with unknown pancreatic function status matched with PI controls.

Clinical Data

At the time of diagnosis, 38 patients with LD-CF (84%) and 90 patients with NBS-CF (100%) had manifestations of CF recorded. None of the LD-CF cohort had MI; MI was recorded in 12% of the NBS-CF cohort ($P = .02$). The LD-CF cohort presented with significantly more respiratory (66% vs 4%; $P < .0001$), gastrointestinal (24% vs 6%; $P = .005$), and failure-to-thrive manifestations (29% vs 3%; $P < .0001$) when compared with NBS-CF controls at the time of diagnosis. Subset analysis revealed both LD-NBS-neg (54% vs 4%; $P < .0001$) and LD-NBS-pos (67% vs 8%; $P = .02$) cohorts to have significantly more respiratory manifestations than matched patients with NBS-CF.

Hospitalization, clinical, and laboratory data were recorded during follow-up at CF clinics, with data over a median of 6.5 (3.0-9.0) and 8.0 (3.0-11.0) years for the LD-CF (n = 38) and NBS-CF (n = 88) cohorts respectively ($P = .3$). The median number of hospital admissions per year for CF-related respiratory illness was significantly higher for patients with LD-CF compared with patients with NBS-CF (0.49 [0.2-1.1] vs 0.2 [0-0.5] respectively; $P = .0004$); it was also significantly higher for the LD-NBS-neg subset compared with patients with NBS-CF (0.44 [0.2-1.0] vs 0.2 [0-0.5] respectively; $P = .001$). The total number of hospital days for CF-related respiratory illness was also significantly higher for patients with LD-CF compared with patients with NBS-CF (25.0 [10.5-69.3] vs 5.0 [0-23.0], respectively; $P = .001$) and patients who were LD-NBS-neg compared with patients with NBS-CF (25.0 [10.0-83.0] vs 7.0 [0-29.0] respectively; $P = .004$). There were fewer hospital admissions per year and no difference between the LD-CF and NBS-CF cohorts in regards to CF-related gastrointestinal illness (0 [0-0.03] and 0 [0-0], respectively; $P = .5$) and other CF-related illness requiring admission (0 [0-0.2] and 0 [0-0.1], respectively; $P = .2$).

All growth variables and lung function testing results were analyzed over the duration of follow-up using linear mixed models (**Figure 2**). The mean (SD) height *z*-score was significantly lower for patients with LD-CF compared with NBS-CF (–0.65 [0.22] vs –0.03 [0.15]; $P = .02$). No difference between the LD-CF and NBS-CF cohorts in regard to mean (SD) *z*-scores for weight (–0.48 [0.21] vs 0.01 [0.14], respectively; $P = .06$) or body mass index (BMI) (–0.11 [0.17] vs –0.03

Table II. Genotype and phenotype of patients with LD-CF with matched NBS-CF controls (1:2)

	PF	Initial (peak) SC	NBS-CF 1	SC	NBS-CF 2	SC
NBS Negative (LD-NBS-neg)						
IRT Negative						
F508del/F508del	PI	112	**F508del/F508del**	-	**F508del/F508del**	-
F508del/F508del*	PI	102	**F508del/F508del**	81	**F508del/G542X**	110
F508del/F508del*	PI	82	**F508del/F508del**	100	**F508del/F508del**	106
F508del/F508del*	PI	82	**F508del/F508del**	80	**F508del**/Unknown	82
F508del/F508del	PS	112	**F508del/F508del**	-	**F508del**/Unknown	70
F508del/G551D	PI	96	**F508del/Q493X**	-	**F508del**/2789 + 2insA	67
F508del/1717-1G > A	PI	109	**F508del/F508del**	105	**F508del/F508del**	60
F508del/G542X	PI	80	**F580del/F508del**	92	**F508del/F508del**	-
F508del/R1162X	-	104 (108)	**F508del/F508del**	-	**F508del**/*c.2252G > T*	74
F508del/*R117H*	PS	78	**F508del**/Unknown	105	**F508del**/Unknown	70
3659delC/621 + 1G > T	PI	93	**F508del/F508del**	75	**F508del**/Unknown	105
Q493X/2789 + 2insA	-	78	**F508del/F508del**	-	**F508del/F508del**	92
R117H*/*R117H	PS	27 (68)	**F508del/L206W**	45	**F508del/F508del**	92
F508del/Unknown*	PI	124	**F508del/F508del**	97	**F508del/F508del**	90
F508del/Unknown	PI	98	**F508del**/Unknown	85	**F508del/F508del**	128
F508del/Unknown*	PI	96	**F508del/F508del**	94	**F508del/F508del**	-
F508del/Unknown	PS	29 (65)	**F508del**/Unknown	63	**F508del/*R117H***	86
***R117H*/Unknown**	PS	34 (62)	**F508del**/Unknown	78	**F508del/1717-1G > A**	97
***R117H*/Unknown**	PS	49 (67)	**F508del**/Unknown	96	**F508del/F508del**	-
Unknown/Unknown*	PI	104	**F508del/F508del**	99	**F508del/F508del**	121
Unknown/Unknown	-	78	**F508del/3659delc**	84	**F508del**/Unknown	73
Unknown/Unknown	-	40 (64)	Unknown/Unknown	95	**F508del/F508del**	110
***F508del* Negative**						
G551D/V520F	PI	105	**F508del/G551D**	72	**F508del/F508del**	86
G551D/1525-1G > A	PI	120	**F508del/G551D**	85	**F508del/F508del**	74
G551D/N1303K	PI	98	**F508del/N1303K**	132	**F508del/F508del**	81
E822X/E822X	PI	102	**F508del/F508del**	92	**F508del/F508del**	88
2183AA > G/	PI	88	**F508del/F508del**	115	**F508del/F508del**	-
2183AA > G						
621 + 1G > T/M1V	PI	80 (98)	**F508del**/Unknown	92	**3659delC/621 + 1G > T**	93
R1066C/1154insTC	PI	102	**F508del/G542X**	100	**F508del/W1282X**	105
R1066C/1154insTC	-	102	**F508del/394delTT**	-	**F508del/F508del**	97
S4X/S4X	PI	107	**F508del/F508del**	90	**F508del/F508del**	-
c.3386-2A > G/*c.3469-65C > A*	PI	90	**F508del/F508del**	98	**F508del/W846X**	90
G551D/Unknown	PI	121	**F508del/1717-1G > A**	103	**F508del/F508del**	86
E822X/Unknown	PI	114	**F508del/F508del**	96	**F508del/G551D**	85
621 + 1G > T/Unknown	-	93	**F508del/E60X**	-	**F508del/F508del**	-
c.3890_3891insT/Unknown	-	106	**F508del/F508del**	78	**F508del/G542X**	100
Unknown/Unknown	PI	107	**F508del/F508del**	99	**F508del/Q493X**	84
Unknown/Unknown	PI	80	**F508del/621 + 1G > T**	45	**F508del/F508del**	-
Unknown/Unknown	PI	75	**F508del/621 + 1G > T**	92	**F508del/R1162X**	79
NBS Positive (LD-NBS-pos)						
Initial SC < 30 mmol/L						
F508del/S945L	PS	29 (100)	**F508del/G551D**	85	**F508del**/Unknown	65
F508del/3849 + 10kbC > T	PS	13 (44)	**F508del/*R117H***	68	**F508del/F508del**	-
Initial SC 30-59 mmol/L						
F508del/P67L†	PI	52	**F508del/F508del**	95	**F508del/F508del**	-
F508del/S945L†	PS	44 (46)	**G542X/S945L**	51	**F508del**/*c.234delC*	140
F508del/*5T*†	PS	36 (67)	**G542X/S945L**	74	**F508del/G551D**	105
F508del/*5T*†	-	42 (72)	**F508del/F508del**	-	**F508del/394delTT**	-

PF, pancreatic function.
CF-causing mutations as defined by CFTR2[26] are highlighted in **bold** font, those with varying clinical consequence are in ***bold-italics***, and those with uncertain significance are underlined. *Italicized* mutations were identified in the CFTR1[27] database only. All cases of LD-CF were matched for pancreatic exocrine status and those with unknown status were matched with PI controls.
*Patients born during IRT/IRT algorithm period.
†Patients initially classified as CFSPID.
Unavailable data indicated with a '-'.

[0.12], respectively; $P = .7$) were evident. The LD-NBS-neg cohort had significantly lower mean height (–0.79 [0.22] vs –0.08 [0.14]; $P = .008$) and weight (–0.60 [0.21] vs –0.06 [0.14]; $P = .03$) *z*-scores than NBS-CF, whereas the LD-NBS-pos cohort had a significantly lower mean BMI *z*-score than NBS-CF controls (0.76 [0.12] vs 1.36 [0.11]; $P = .003$). Regarding lung function testing, 149 measurements were recorded from 25 of the patients with LD-CF and 308 measurements were recorded from 57 of the patients with NBS-CF. Given the earliest lung function testing is performed at is 6 years of age, 15 of the 45 patients with LD-CF born between 2004 and 2010 did not have testing performed. Mean (SD) lung function variables were significantly worse for LD-CF compared with NBS-CF, including forced vital capacity (2.1 L [0.10] vs 2.5 L [0.06], respectively; $P = .003$), forced expiratory volume in 1 second (1.7 L [0.08] vs 2.0 L [0.06] respectively; $P = .003$), and forced expiratory

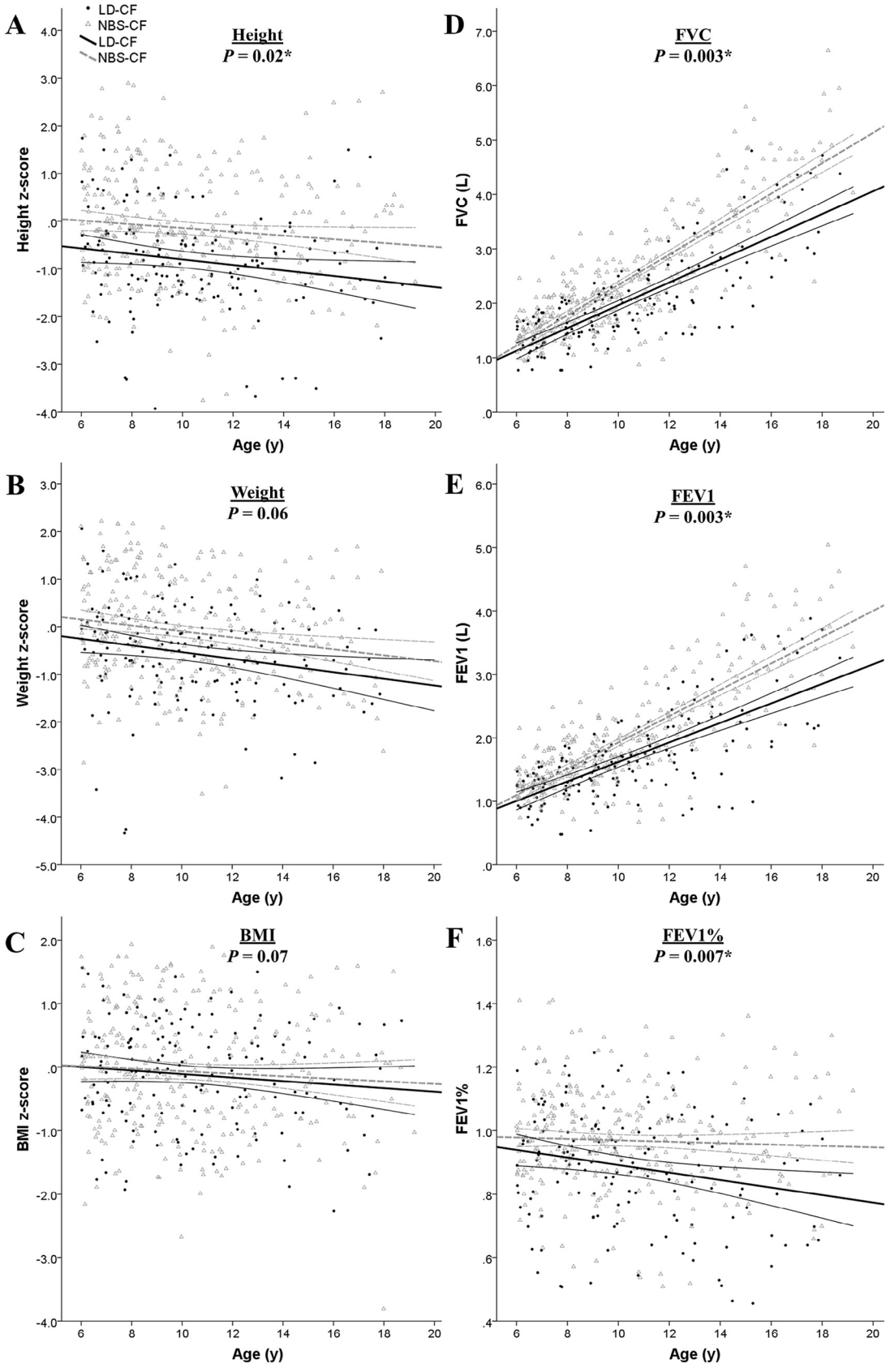

Figure 2. All recorded growth and lung function variables for all patients with LD-CF (*circles*) and all patients with NBS-CF (*triangles*). Lines represent mean (*center lines*; LD-CF is *solid black* and NBS-CF is *dashed grey*) with standard deviation bars (*outside lines*). *FEV1*, forced expiratory volume in 1 second; *FVC*, forced vital capacity.

volume in 1 second percentage of predicted (FEV1%) (0.88 [0.03] vs 0.97 [0.02], respectively; $P = .007$). Subset analysis revealed worse FEV1% for both the LD-NBS-neg (0.88 [0.03] vs 0.98 [0.02]; $P = .01$) and LD-NBS-pos (0.86 [0.02] vs 0.93 [0.01]; $P = .002$) cohorts compared with NBS-CF–matched controls.

Microbiology data from sputum and/or bronchoscopy cultures were recorded for all patients with LD-CF and patients with NBS-CF (**Table III**). A significantly higher rate of isolation of *P aeruginosa* over the duration of follow-up was identified in the LD-CF cohort compared with the NBS-CF cohort (82% vs 69%, respectively; $P = .03$); however, there was no difference in the age of first isolation (5.1 years [2.8-8.3] vs 4.4 years [1.5-6.9], respectively; $P = .3$). A significantly higher proportion of patients with LD-CF had chronic colonization with *P aeruginosa* (47% vs 24%; $P = .01$) and isolation of mucoid *P aeruginosa* (42% vs 20%; $P = .008$). The isolation rate of *P aeruginosa* was not different for the LD-NBS-neg cohort compared with NBS-CF cohort (79% vs 68%, respectively; $P = .3$); however, it was significantly higher for the 6 patients who were LD-NBS-pos (100% vs 33%; $P = .01$) when compared with NBS-CF matched controls. Mucoid *P aeruginosa* isolation rates were significantly higher for the LD-NBS-neg cohort (44% vs 22%; $P = .02$) but not for the LD-NBS-pos cohort (33% vs 8%; $P = .2$) when compared with NBS-CF–matched controls. High isolation rates of *S aureus* were also identified in both the LD-CF and NBS-CF cohorts (73% vs 76% respectively; $P = .8$); however, there was no difference in age of first isolation (4.0 years [2.4-5.4] vs 2.2 years [0.7-5.7], respectively; $P = .1$) or chronic colonization (27% vs 34%; $P = .4$).

Discussion

In this study, we report the differences in outcomes of children with a LD-CF in the NSW NBS era. Little is known about patients with LD-CF and they represent an important subset of patients with CF. Children with LD-CF, who mostly received a false negative NBS result, had worse respiratory outcomes including poorer lung function, higher rates of chronic *P aeruginosa* colonization, and increased frequency of hospitalization for CF-related respiratory illness at follow-up when compared with matched controls.

From 1988 to 2010, the estimated incidence of CF in NSW was 1 in 2200 live births, which is comparable with data from Victoria, Australia.[6] This study calculated the incidence of LD-CF, which occurred in 1 in 45 000 live births, or alternatively 1 in every 21 patients with CF. It should be noted that the sensitivity and specificity of different CF NBS programs varies throughout the world[8] and screening algorithms have been revised over time to improve detection.[29,30] The findings of this study should not be taken to mean that sensitivity should be improved above all else, especially at the expense of specificity. This is particularly relevant in light of the issues related to the identification of infants with a positive NBS result but an inconclusive diagnosis of CF (CFSPID or CFTR-related metabolic syndrome).[23,31] Of the 39 patients missed by NBS in our study, 22 (56%) had IRT levels that were not increased and 17 (44%) were not carriers for *F508del*. Of the 22 patients missed by IRT testing, 5 (23%) were homozygous for *F508del* and 12 (55%) were PI (**Table II**). The reason for patients with 2 severe mutations (without MI) not having an

Table III. Microbiology data

	LD-CF		NBS-CF		
Variables	**n**	**Result**	**n**	**Result**	***P***
P aeruginosa					
Isolation	45	37 (82%)	90	57 (63%)	.03
Age first isolation (y)	37	5.1 (2.8-8.3)	57	4.4 (1.5-6.9)	.3
Chronic colonization*	45	21 (47%)	90	22 (24%)	.01
Age first chronic colonization (y)	21	6.3 (0.9)	22	7.2 (0.8)	.7
3 × cultures†	45	8 (18%)	90	13 (14%)	.6
Age first 3 × cultures* (y)	8	7.3 (1.1)	13	7.9 (1.1)	.7
P aeruginosa (mucoid)					
Isolation	45	19 (42%)	90	18 (20%)	.008
Age first isolation (y)	19	6.4 (0.7)	18	7.5 (0.8)	.3
P aeruginosa (non-mucoid)					
Isolation	45	19 (42%)	90	39 (43%)	.99
Age first isolation (y)	19	4.7 (0.5)	39	5.7 (0.6)	.3
P aeruginosa (other)					
Isolation	45	13 (29%)	90	27 (30%)	.99
Age first isolation (y)	13	4.5 (0.9)	27	5.0 (0.8)	.9
S aureus					
Isolation	45	33 (73%)	90	68 (76%)	.8
Age first isolation (y)	33	4.0 (2.4-5.4)	68	2.2 (0.7-5.7)	.1
Chronic colonization*	45	12 (27%)	90	31 (34%)	.4
Age first 3 × cultures* (y)	12	7.7 (1.0)	31	8.3 (0.8)	.7
MRSA	45	1 (2%)	90	12 (13%)	.06

Data presented as number (percentage), mean (SD), or median (IQR).
*Chronic colonization defined by 3 consecutive cultures within a 12-month period or isolation of mucoid *Pseudomonas* spp.
†3 × cultures, 3 consecutive cultures.

elevated IRT remains unclear. Six of the patients with LD-CF were born during the IRT/IRT screening protocol and 1 of these would have been detected by the subsequent IRT/DNA protocol.

Late diagnosis patients with CF had significantly worse health at time of diagnosis, including more respiratory ($P < .0001$), gastrointestinal ($P = .005$), and failure-to-thrive manifestations ($P < .0001$), which is not surprising because they were likely the cause(s) for clinical attention. Subset analysis also revealed that patients who are LD-NBS-neg and patients who are LD-NBS-pos have significantly more respiratory manifestations than NBS-CF matched controls ($P < .0001$ and $P = .02$, respectively). In the era of CFTR-targeted therapies,[32,33] early diagnosis and rescue therapy before irreversible organ damage occurs (ie, pancreatic atrophy in utero[34] and within months postnatally[35] leading to organ failure and becoming PI) becomes increasingly important.

To date, several studies support the notion that NBS protocols reduce the therapeutic burden, hospitalization rates, and morbidity associated with CF[36-41]; however, sparing the Wisconsin CF Study Group, high-quality, randomized, controlled trials are lacking.[11] Our study provides further support for NBS with LD-CF having a significantly higher median number of hospital admissions per year for CF-related respiratory illness compared with NBS-CF controls (0.49 vs 0.20, respectively; $P = .0004$).

Waters et al[15] in 1999 found that in the 3 years before and after the implementation of NBS in NSW, NBS was associated with greater height *z*-scores at 5 years of age and improved FEV1% at 5 and 10 years of age. The most convincing evidence regarding the nutritional and growth benefits of NBS come from the Wisconsin CF randomized longitudinal studies.[12,42]

Several other studies comparing historical cohorts with NBS have also shown nutritional and/or respiratory benefits.[16,18,43,44] Interestingly, in our study, the mixed models analysis for height *z*-scores of patients with LD-CF was significantly lower than NBS-CF controls (−0.65 [0.22] vs −0.03 [0.15], respectively; $P = .02$). Although not significant, the LD-CF also had a trend toward lower weight *z*-scores and BMI compared with NBS-CF. The LD-NBS-neg cohort, however, had both lower height ($P = .008$) and weight ($P = .03$) *z*-scores compared with matched NBS-CF controls. Although the numbers were small (n = 6), patients who were LD-NBS-pos had significantly lower BMI scores over the duration of follow-up compared with NBS-CF controls ($P = .003$).

One of the strengths of this study is the use of the mixed models analysis of lung function, which takes into account all recorded measures for each individual patient over their entire duration of follow-up. The LD-CF cohort had a significantly worse mean FEV1% compared with NBS-CF controls (0.88 vs 0.98, respectively; $P = .01$). This result was also evident in both the LD-NBS-neg and LD-NBS-pos subset cohorts (when compared with their respective NBS-CF control cohorts). Although the association between NBS and improved long-term lung function has been limited,[18,42] this finding has previously been reported in our NSW cohort.[18,21] The worse lung function in the LD-CF cohort is probably associated with the significantly higher rate of mucoid *P aeruginosa* isolation compared with NBS-CF controls (42% vs 20%; $P = .008$). Although a proportion of the LD-NBS-neg cohort have a severe phenotype (ie, probably PI at birth and thus lower IRT) this alone does not account for their worse lung function, because they also have higher isolation rates of mucoid *P aeruginosa* compared with matched NBS-CF controls (44% vs 22%, respectively; $P = .02$). The remaining 6 patients who were LD-NBS-pos who are predominantly PS, also had a higher rate of mucoid *P aeruginosa* (33% vs 8%), but this failed to attain significance ($P = .2$), which may be owing to the sample size. Isolation of *P aeruginosa* and in particular chronic or mucoid *P aeruginosa* infection has long been associated with worse lung function and imaging scores in patients with CF.[45-47] A recent multivariable model from the Wisconsin group identified several modifiable extrinsic risk factors for the progression of lung disease in children with CF, which included colonization with mucoid *P aeruginosa*.[48] Early identification and eradication is likely to have long-lasting impacts in children with CF,[49,50] as are preventative measures.[51,52]

The intriguing group of patients who are screened positive and have sweat tests with an initially normal (<30 mmol/L) or intermediate SC (30-59 mmol/L) result are highlighted in this study. Of the 4 patients with CF initially identified as CFSPID (**Table II**), 2 carried 2 disease-causing mutations (*F508del/P67L* and F508del/S945L) with 1 being PI (*F508del/P67L*) at most recent review (a small but important finding that differs from previous reports[53]). Interestingly, the 2 patients with CFSPID with a *5T* mutation (which is thought to be of variable clinical significance[26]) progressed to having a positive SC result (≥60 mmol/L). **Table II** also includes 2 patients with an initially normal SC (<30 mmol/L) who were heterozygotes for *F508del* and a PS mutation, *S945L* and *3849 + 10kbC > T*.[26] A concerning finding for the patients who were LD-NBS-pos was that they all had *P aeruginosa* isolated, and this parallels previous reports.[53-56]

This study is limited by the small sample size; however, it is also reassuring that the number of patients with LD-CF is small and the NBS program performs well. The optimal NBS program for CF is yet to be determined[57] and, as adjuncts to the NSW program, both positive family history and MI protocols have been developed; however, the impact of these measures are yet to be evaluated. Further limitations include the retrospective nature and the number of patients <6 years of age by 2010 who were too young to undergo lung function testing. Not all patients had extensive genotyping or gene sequencing performed; thus, not all *CFTR* mutations were identified. The documented phenomenon of patients progressing from PS to PI[58] also provides a limitation. Patients who are yet to receive a LD-CF and those who have done so after transitioning to adult care (in Australia, data are sent voluntarily to the national CF registry) may also be missed by this analysis. The ever-expanding list of disease-causing mutations provides further limitations. NBS and this study are not designed to capture those patients who also lie on the spectrum of CFTR dysfunction including those with CFTR-related disorders such as congenital bilateral absence of the vas

deferens or idiopathic recurrent/chronic pancreatitis,[59,60] both of which are more likely to present in adult life. In addition, the heterogeneity of the LD-CF cohort made matching with NBS-CF controls challenging. Despite these challenges, the SC levels were not different between the LD-CF and NBS-CF cohorts (83.2 vs 89.1, respectively; $P = .2$). This finding, along with the matching of pancreatic function status, helps to control for the differences in initial presentation and the potential effect of improvement over time. Furthermore, matching for CF clinic aimed to reduce any variance in clinical practice. The strength of this study lies in the 2 decades of NBS experience. Although problematic to perform, long-term prospective studies may provide more insight into those patients who are missed by NBS and receive a LD-CF.

In conclusion, this study serves to highlight the outcomes of those patients with CF who have a missed or late diagnosis despite NBS. Patients who receive a late diagnosis seem to be different genotypically and phenotypically from patients with NBS-CF, as are the differences for those who are LD-NBS-neg and LD-NBS-pos. A late diagnosis seems to be associated with worse health at diagnosis and worse outcomes with regard to growth and respiratory outcomes (FEV1% and *P aeruginosa* colonization). Coupled with emerging CFTR-targeted therapies, these findings provide further support for NBS programs for CF. ■

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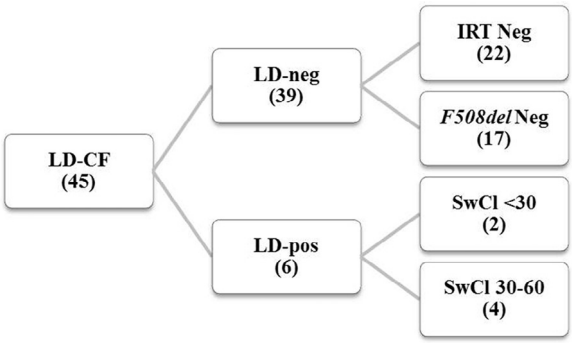

Figure 1. Distribution of LD-CF cases. *SwCl*, sweat chloride.