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Case series

Cystic fibrosis newborn screening enables diagnosis of elder siblings of recalled infants – additional benefit

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Abstract: The different clinical manifestations of cystic fibrosis, with variable intensity and timing, often delay the diagnosis of this genetic autosomal recessive disorder. Many countries have introduced newborn screening for cystic fibrosis to facilitate diagnosis prior to the development of the disease. The advantages and harms of such screening programmes are regularly reassessed. In the five families presented in this article the elder siblings of screened infants were diagnosed thanks to CF NBS. This is an example of a benefit for children not even directly covered by the screening programme, adding another CF NBS advantage to the balance.

Key words: cystic fibrosis, newborn screening, siblings, diagnosis

Introduction

The diversity of the clinical symptoms of cystic fibrosis and the manifestation in different periods of life with variable intensity, often lead to a delay in CF clinical diagnosis. The main goals of newborn screening programmes are the early diagnosis of inborn diseases, before the symptoms appear, to enable early introduction of therapy [1].

In Poland CF NBS started in January 1999. Until 2003 it was a pilot regional programme based on IRT/F508del/IRT protocol [2]. In September 2006 a move to National CF NBS was initiated, extending gradually to cover the whole of Poland by July 2009 [3].

Screening for CF is based on a four tiers protocol IRT/DNA/IRT/sweat test (covering the 16 most common mutations in the Polish population, and thanks to gene sequencing allowing the diagnosis of over 500 mutations). Intermediate tier consists of IRT resampling in infants with the highest IRT values (99th percentile cut-off). The final diagnosis is based on *CFTR* gene mutation analysis and sweat test (conventional pilocarpine-iontophoresis and conductometric Nanoduct) combined with clinical examination.

Infants with positive neonatal CF screening results (increased second IRT at 4 weeks and/or one or two mutations) are called to the CF Centre at our Institute for verification. The same day 2 sweat tests are performed – a classical pilocarpine iontophoresis and a conductometric Nanoduct. Values of Cl^- in sweat >40 mmol/L and NaCl >60 mmol/L are considered diagnostic for CF.

There are ongoing discussions about the rationale for CF NBS. We would like to add another positive argument towards the balance in favour of NBS. We present families where elder siblings were diagnosed thanks to CF NBS programmes covering newborns who were CF or *CFTR* gene carriers.

Case reports

In Table 1 the data of the 5 families described are presented. Four of them (numbers: 1-4) were diagnosed in the pilot CF NBS. An infant from family number 5 and the youngest child from family number 4 were diagnosed in the current CF NBS programme [3].

The diagnosis of pancreatic function was based on clinical symptoms and faecal elastase assessment.

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Abbreviations: CF – cystic fibrosis; CFTR – Cystic Fibrosis Transmembrane Conductance Regulator; IRT – immunoreactive trypsin; NBS – newborn screening; CXR – chest x-ray;

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Table 1. Characteristics of infants identified by CF NBS and their CF siblings (sweat tests values, genotypes). Preliminary DNA analysis – from the blood spot at the filter paper taken on the 3rd day of life.

Family no	Newborns from CF NBS					Siblings			
	preliminary DNA analysis	chloride concentration (Cl mmol/l)	conducto-metric test (NaCl mmol/l)	further DNA analysis	diagnosis	age of sibling at diagnosis (years)	chloride concentration (mmol/l)	DNA analysis	diagnosis
1	F508del	I-36,9; II-63,0	62	F508del	Carrier	5	I-77,8; II-65,6	F508del/ 1717-1	CF
2	F508del	I-89,7; II-70,2	115	F508del/ R347P	CF	5	I-75,9; II-80,1	F508del/ R347P	CF
3	no mutation identified	I -73,1	98	R553X/ 3272-26A>G	CF	10	I-43,1; II-55,8; III-77	R553X/ 3272-26A>G	CF
4	F508del	I-25,8; II-18,8; III-30,7 IV(at the age of 6y)- 68,0	46	F508del/ 3849 +10kbC>T	CF	10	I -31,6; II-38,6,	F508del/ 3849 +10kbC>T	CF
	F508del/ 3849 +10kbC>T	I-35,9; II-38,1	67	F508del/ 3849 +10kbC>T	CF	13	I-49,2; II-52,5; III-37,1	F508del/ 3849 +10kbC>T	CF
5	F508del	I-78	111	F508del/ 3659delC	CF	4	I- 109; II-93	F508del/ 3659delC	CF

Family 1

A baby girl was called to our CF centre due to remaining increased IRT still in the second sample and one mutation F508del identified. The girl at the time of consultation had no clinical symptoms and borderline sweat tests. Further DNA analysis and a second sweat test were performed at the age of 6 months.

The mother brought also her 5 year old son, who was being followed by a gastroenterologist due to rectal prolaps and steatorrhea. Periodically he was treated with pancreatic enzymes. From the second year of life he had recurrent respiratory infections. He was short and underweight with visible finger clubbing. He had chest hyperresonance in physical examination and fibrotic changes CXR. His sweat tests were high. His genotype was F508del/1717-1. For the girl the 1717-1 mutation was not present. This fact was decisive for her diagnosis as an F508del carrier.

Family 2, 3, 5

Infants from these families, recalled to our centre due to positive screening tests, had CF diagnosis confirmed with sweat tests and *CFTR* mutations. Their siblings were invited for sweat tests. Those with elevated values were subsequently DNA tested. All 3 of them had typical respiratory symptoms. Two of them (from families number 2 and 5) also had symptoms of pancreatic insufficiency (malnutrition, diarrhea) confirmed with faecal elastase.

Family 4

A baby girl from multiple children family was picked up in a pilot screening programme due to increased IRT and one F508del mutation. She was in good clinical status. Both sweat tests were within normal limits. In her 5th month of age the sweat tests were repeated, again the values were normal. The parents did not agree to keep DNA for further procedures and did not come for future consultations. Four years later the girl's 10 years old sister was referred to us for consultation because of nasal polyps and chronic sinusitis. She had normal somatic development and was pancreatic sufficient. Sweat tests were within norms, as were spirometric values. Further DNA analysis was performed and two *CFTR* mutations: F508del/3849+ 10kb>T were identified in both sisters. After that diagnosis, the mother brought to our centre the eldest brother (13 years old). He had a chronic cough and frequent respiratory tract infections. His somatic development was normal, but there were abnormalities observed in his respiratory system. Inflammatory changes were heard in his lungs in auscultation, the thickening of bronchial walls was seen in his CXR and sinal polyps were identified by CT, his lung function was below norms: FEV1 80%, FVC 75%. Sweat tests values were equivocal. DNA analysis revealed the same genotype as by his sisters. The seventh child of the family did not undergo prenatal diagnosis due to the advanced pregnancy at the

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time of the other siblings' diagnosis. She was covered by an extended DNA analysis within the current CF NBS. The identical genotype as by her elder siblings was discovered. Her sweat tests were borderline. The child's somatic development and pancreatic function were normal. The remaining 3 children were consulted in our centre. Their sweat tests values were within normal limits and no abnormalities were found during physical examination. Because in CF patients with 3849+10kbC>T mutations the sweat test readings may not be elevated, DNA analysis was performed and revealed that two of them were 3849+10kbC>T carriers. One child was free from *CFTR* mutations.

Discussion

In all the described families elder siblings of infants with positive screening test results owe their diagnosis to the screening programme, which did not cover them directly. All of them were worrying their families presenting the symptoms typical of CF which had been reported to health professionals.

The symptoms are typical but not specific for CF, and can be misdiagnosed as allergies, food intolerance and common respiratory tract infections, despite regular CF educational campaigns and postgraduate CF training.

Cases with borderline sweat tests and pancreatic sufficiency are the most problematic. Polish guidelines recommend a value of 40 mmol/l (pilocarpine iontophoresis) [3], international guidelines suggest to lower the infant sweat test value limit to 30 mmol/l [4-6].

However, in family number 4, children with CF had sweat test values even below 30mmol/. In their situation a larger panel of mutations incorporated in the standard CF NBS procedure (including 3849+10kb) was diagnostic.

Based on our experience with described families we conclude as follows:

- In the case of a carrier or CF diagnosed within CF NBS programme, family history concerning siblings should be reviewed.
- Sweat testing of the siblings of diagnosed CF persons is recommended by international and Polish standards of CF care [3,4,6,7]. In the case of carrier or CF diagnosis in CF NBS, genetic counselling of the parents is very important [1,3].
- Appropriate screening strategy for the given population enables diagnosis even in the case of equivocal sweat tests. The panel of chosen mutations should include CF causing mutations common in the given population associated with normal or equivocal sweat electrolyte values (in particular, 3849+10kb>T) [1].
- The cases presented in this article can be a memorable illustration why the opportunity to diagnose older siblings after CF screening of an infant must not be missed.

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