

## Age of menarche in girls with cystic fibrosis

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**Abstract:** Malnutrition, delayed growth and puberty are commonly observed in children suffering from cystic fibrosis. The aim of this study was to assess the age of menarche in girls with CF using status quo analysis. The relationship between types of CFTR mutations and onset of the first menstruation was also evaluated. Material was based on somatic data gathered from medical history records of 47 girls with cystic fibrosis, aged 11-18 years. All girls were patients of the Mother and Child Institute in Warsaw (Poland). Results: The age of menarche in the girls in the study group was 14.65±1.21 years. In comparison with the healthy child population, girls with cystic fibrosis experienced menarche with 2 years' delay. Menstruating girls were found to be statistically older and taller than their non-menstruating consorts. Regarding body mass and BMI, a marked tendency towards higher parameter values was noted in the menstruating group, although the differences did not reach statistical significance. A significant relationship between onset of menarche and type of CFTR mutation was found. Girls with cystic fibrosis enter puberty later than their peers, in spite of intensive medical care. The issue of growth and puberty in children with CF requires further detailed investigation under clinical and auxological aspects.

**Key words:** cystic fibrosis, delayed puberty, menarche, CFTR gene mutation, pulmonary function

### Introduction

Cystic fibrosis is one of the most common genetic metabolic disorders, following the pattern of autosomal recessive inheritance and leading to premature death. The incidence of cystic fibrosis in Poland is 1:2500 live births [1]. A mutation in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, acting as a cellular chloride channel pore and ion transport regulator, is the cause of the disease [2]. Over 1000 different mutations in the CFTR gene have been described, of which F508del accounts for almost 70% of all cases [3].

Malnutrition, delayed growth and delayed puberty are commonly observed in children suffering from cystic fibrosis [4-7]. However, the results of some studies demonstrate that intensive medical care, administered in specialized cystic fibrosis centers, significantly improves growth rate and nutritional status, sometimes to levels comparable to those observed in the healthy population [8,9]. Considerable delays in onset as well as disturbances of puberty are however still noted in children with CF [10,11].

Longitudinal observation of growth and maturation processes in children with CF reveals a delayed and strongly reduced pubertal growth spurt [10,12-14]. Most marked growth delays were observed in patients with F508del/F508del mutation types [12]. It should be stressed that, in CF patients, decreased levels of hormones crucial to the growth process have been detected – i.e. insulin-like growth factors IGF-I, corresponding binding proteins IGFBP3 and thyroid hormones [15-18]. Slower development of II- and III-sex characteristics has also been observed in children with CF [19].

Menarcheal age in sick children is generally delayed by 2 years in comparison with the healthy child population and usually occurs after the age of 14 [4,19,20]. Malnutrition is the main cause for delayed puberty in CF, but late menarche was also observed in girls who manifested normal nutritional status in spite of the disease [5,10]. The age at onset of menarche depends on the type of mutation, and is most delayed in patients with the F508del/ F508del genotype [10].

Puberty observations in CF adolescents have however proven equivocal with respect to clinical symptoms and disease severity. While some studies have demonstrated a significant relationship between the age of menarche and respiratory system condition –

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girls with worse respiratory parameters (low FEV<sub>1</sub> values) were found to reach puberty later – other research has shown no relationship between menarche and severity of the disease [7,10].

Most studies concerning menarcheal age in female children with CF were conducted on small study populations and the occurrence of the first period was determined exclusively by means of retrospective analysis. The aim of this study was to assess the age of menarche in 47 girls with cystic fibrosis using status quo analysis, more appropriate and accurate in the case of a developmental population. The relationships between types of CFTR mutations and age at the first menstruation as well as somatic parameter values and respiratory system condition, were also evaluated.

## Materials and methods

Material was based on somatic data gathered from the medical history records of 47 girls with cystic fibrosis, aged 11-18 years (mean age 13.8±2.2 y.o.). All girls were patients of the Institute of Mother and Child in Warsaw (Poland). In all patients, the diagnosis was confirmed by positive sweat tests. The mean age at the moment of diagnosis was 4.5 years (minimum 1 month, maximum 15 years). All study subjects were Caucasian. Data for the study were gathered in 2007 during standard developmental profile assessment procedures. All girls as well as their parents/guardians gave informed consent to the trial.

Anthropometric measurements of height and body mass were performed. Height measurements were taken using a stadiometer with a 1mm accuracy, while body mass was assessed with a 0.1 kg accuracy. All measurements were performed in accordance with the methods described by Martin and Knusmann [21]. Body Mass Index was accordingly calculated [BMI=body mass (kg)/body height (m<sup>2</sup>)]. Anthropometric parameters were expressed in terms of standard deviations away from age-specific and sex-specific reference means for the population of Poland [22].

Respiratory system deficiency was evaluated basing on spirometry results. Recorded data included Forced Vital Capacity (FVC) and Forced Expiratory Volume in one second (FEV<sub>1</sub>). All spirometric parameters were measured using a Lungtest 1000 spirometer in accordance with the procedures recommended by the Polish Respiratory Society [23]. All results were recorded as percentages of the predicted values, standardized for age, height and gender [24].

Molecular DNA was analyzed in all patients in order to determine the type of CFTR gene mutation. The studies were carried out at the Medical Genetics Laboratory of the Institute of Mother and Child at the time the patients were admitted.

All patients were treated in accordance with current CF treatment standards [25]. The therapeutic approach to the alimentary tract included diet modification together with vitamin and enzyme supplementation. Other therapy included: physiotherapy, nebulised treatment and antibiotics in the case of bronchopulmonary complications, also in case of *Pseudomonas aeruginosa* chronic colonization.

The age of menarche was determined using the status quo (probit) method, asking girls about presence or lack of menstruation. Thus, each class included girls who menstruate as well as those still pre-menarche. The age of menarche was also determined for the girls in the study group using probit analysis in accordance with the method described by Finney [26]. Using the probit method, the following menarcheal age distribution param-

**Table 1.** Number of menstruating study subjects with cystic fibrosis in subsequent age categories.

Age categories	Number of study subjects	Number of menstruating girls	
		N	%
11.0	5	0	0.0
11.5	4	1	25.0
12.0	4	0	0.0
12.5	7	1	14.0
13.0	1	0	0.0
13.5	4	0	0.0
14.0	2	1	50.0
14.5	3	3	100.0
15.0	3	1	33.3
15.5	2	1	50.0
16.0	2	2	100.0
16.5	3	3	100.0
17.0	3	3	100.0
17.5	2	2	100.0
18.0	2	2	100.0

eters were calculated: means probit-based value and standard deviation.

**Statistical analysis.** A comparison of the mean somatic and respiratory parameter values was carried out using the Student's t-test. Normality was analyzed using the Shapiro-Wilk test. The correlation between CFTR mutation type and the onset of the first menstruation was assessed with a  $\chi^2$  test. In order to determine the way menarche onset and type of CFTR mutation affect somatic and spirometric parameters, a two-way analysis of variance was performed. The R<sup>2</sup> coefficient of determination indicated the percentage of variance of independent variables. Statistical significance was considered when p<0.05. The analyses were carried out using STATISTICA 8.0.

## Results

From among the 47 girls aged 11 through 18, 20 study subjects menstruated (42.5%). The mean age of menarche calculated using probit analysis amounted to 14.65±1.21 years (Table 1).

Mean values of somatic and respiratory parameters were compared in terms of menarche occurrence.

Menstruating girls were found to be statistically older and taller than their non-menstruating consorts. Regarding body mass and BMI, a marked tendency towards higher parameter values was noted in the menstruating group, although the differences were not statistically significant.

**Table 2.** Mean height, body mass, BMI and respiratory parameter values, expressed in terms of standard deviations away from the means of reference values for the population of Poland, in menstruating and non-menstruating girl with cystic fibrosis.

Parameter	menarche				Student's t-test
	yes (n=20)		no (n=27)		
	$\bar{X} \pm SD$	min-max	$\bar{X} \pm SD$	min-max	
Age in years	15.67±1.78	11.26-17.86	12.53±1.28	10.70-15.59	7.01***
Body height	-0.02±1.33	-2.79-2.96	-1.04±1.20	-4.21-1.78	2.73**
Body weight	-0.59±1.34	-3.14-1.98	-1.14±1.19	-2.90-1.87	1.47
Body Mass Index	-0.65±1.10	-2.42-2.37	-0.94±1.04	-2.92-2.53	0.82
FVC%	86.62±19.23	47-123	71.72±19.62	43-109	2.33*
FEV <sub>1</sub> %	85.75±20.72	37-119	68.27±25.21	31-108	2.27*

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Statistically higher FVC% and FEV<sub>1</sub>% respiratory parameters were found in menstruating study subjects (Table 2).

In all girls, molecular DNA tests were performed in order to determine the type of CFTR mutation. Three study groups were formed depending on the DNA test results. The first group (N=16; 34%) included study subjects with the F508del/F508del genotype, a severe mutation; the second group (N=16, 34%) those with the F508del/Mt genotype, a less severe mutation (where Mt signifies a mutation other than F508del, i.e. R334W). The third group (n=15; 32%) included study subjects with a Mt/Mt mutation type, i.e.

3849+10kbC → T/3849+10kbC → T,  
3849+10kbC → T/3659delC.

A significant correlation between the onset of menarche and the type of CFTR mutation was found ( $\chi^2=6.41$ , df=2, p=0.04) (Table 3). More non-menstruating girls were observed in the F508del/F508del mutation group.

Table 4 presents the cumulative, independent influence of menarcheal onset and type of CFTR mutation over somatic and spirometric parameters in study subjects. The occurrence of the first period statistically

**Table 3.** Number of menstruating and non-menstruating girls with CF depending on CFTR mutation type.

CFTR mutation type	Menstruating	Non-menstruating
F508del/F508del	3 (19%)	13 (81%)
F508del/Mt	10 (62%)	6 (38%)
Mt/Mt	7 (47%)	8 (53%)

differentiated body height values, while no such influence was observed in terms of body mass, BMI or respiratory parameters.

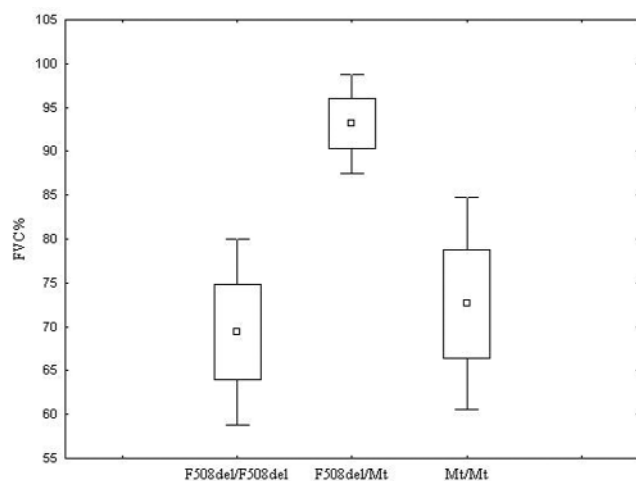
The type of CFTR gene mutation also statistically differentiated respiratory parameter values: Forced Vital Capacity (FVC%) and Forced Expiratory Volume in one second (FEV<sub>1</sub>%) in the studied girls. The lowest

FVC% and FEV<sub>1</sub>% values were determined in F508del/F508 patients, while the highest were in F508del/Mt subjects (Fig. 1, 2).

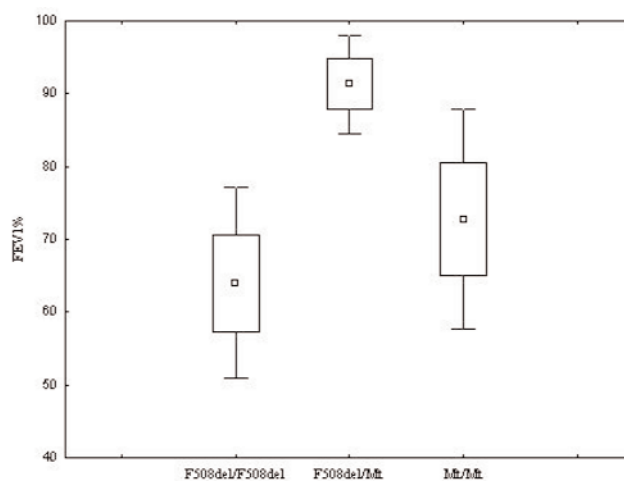
A statistically significant correlation was observed between onset of menarche and type of CFTR mutation in terms of body height and weight. (Table 4). Greatest height and weight deficiencies were observed

**Table 4.** Results of two-way variance analysis of standardized anthropometric and respiratory parameters grouped according to menarche occurrence and type of CFTR mutation.

Factor	Body height		Body weight		BMI		FVC%		FEV <sub>1</sub> %	
	F	p	F	p	F	p	F	p	F	p
Menarche	10.80	<b>0.002</b>	3.45	0.070	0.87	0.357	1.54	0.223	1.47	0.235
CFTR mutation type	2.08	0.138	2.27	0.116	1.44	0.247	4.09	<b>0.026</b>	3.40	<b>0.046</b>
Interactions:										
Menarche x CFTR mutation type	6.01	<b>0.005</b>	5.08	<b>0.011</b>	2.97	0.067	0.23	0.799	0.73	0.489
R <sup>2</sup>	28,7%		19,1%		8,3%		20,2%		17,5%	



**Fig. 1.** FVC% values for different types of CFTR gene mutation (means, standard deviations and 95% confidence intervals).



**Fig. 1.** FEV<sub>1</sub>% values for different types of CFTR gene mutation (means, standard deviations and 95% confidence intervals).

in non-menstruating F508del homozygotes (data not shown).

## Discussion

Mean menarcheal age currently noted in the Polish population varies from 12.7 y.o. in girls from Warsaw to 13.4 y.o. in girls from the rural areas of the country [27,28].

Thus, in comparison with the healthy child population, girls with cystic fibrosis treated at the Institute of Mother and Child experienced menarche with 2 years' delay. In this group, the age of menarche, at  $14.65 \pm 1.27$  years old, confirms reports of delayed puberty in patients with cystic fibrosis by other authors, in spite of intensive medical care [19,29-31]. One of the works did note menarche onset in sick girls within the healthy population age range, the authors however failed to establish the mean menarcheal age in study subjects [32].

Delayed puberty in CF patients has a complex etiology. Malnutrition and related adipose tissue depletion is the main factor answering for delayed onset and disturbances in puberty [5]. Neinstein and al. [20] furthermore demonstrated by means of step regression analysis that body mass is the main predictor for menarcheal age in girls with CF. According to the Frisch-Revelle hypothesis, a critical body mass of 47.8 kg is indispensable for the onset of menstruation [33]. In those studies, as well as in the current analysis, body mass in menstruating CF girls showed a marked tendency towards reaching higher values as compared to non-menstruating study subjects.

On the other hand, study subjects with very low body mass were also present in the menstruating group, while some non-menstruating girls had a body mass exceeding the critical values, a fact which proves

that reaching the determined body mass is not the sole criterion for menarche onset. It should be stressed that a wide range of variance and high standard deviations of the analyzed somatic parameters were noted in both groups of girls, which indicates a relatively high level of phenotypic differentiation in study subjects. It is however common knowledge that body mass in CF children has a tendency to oscillate depending on abdominal and respiratory symptom intensification [3].

In studies by Johannesson *et al.* [10], menarche in girls with CF was delayed by almost 2 years in comparison with the healthy girl population (14.9 and 13.0 years respectively), although the groups did not differ in terms of nutritional status. There are suspicions that the mutated CFTR gene may cause disturbances in the secretion of gonadotropin-releasing hormone (GnRH) and thus delay puberty in spite of proper nutritional status [10,34,35]. CFTR gene expression has been observed in uterine endometrium and oviducts, but remains unconfirmed in ovaries [36].

The Swedish study mentioned also reported the longest delays in menarche in girls with the F508del/F508del mutation type [10]. The researchers sought interpretation of the results in differences in resting energy expenditure in dependence of CFTR mutation type. In CF patients, resting energy expenditure is generally over 10% higher than in healthy subjects due to breathing effort, which further disturbs energy balance and hinders normal physical development in children [8,37,38]. The greatest energy expenditure was determined in F508del homozygotes, which may have contributed to even more severe delays and disturbances in puberty in this patient group as compared with other CFTR mutations [37].

In girls with cystic fibrosis, hormonal imbalances tend to occur significantly more often than in the

healthy population, resulting in irregular menstrual cycles and leading to the development of Polycystic Ovary Syndrome (PCOS) [20,32,39]. In the hormonal profile of 18 menstruating girls with cystic fibrosis aged from 12 through 23, increased levels of LH, FSH/LH and prolactin (PRL), as well as decreased level of SHBG (sex hormone-binding globulin) were observed [32]. In the same study, polycystic ovary syndrome was diagnosed in almost half of the patients, without however typical symptoms in the form of hirsutism and acne on the account of low adipose tissue quantity.

Advances in pharmacological treatment and nutritional approach (including amongst other things high calorie diet implementation and chronic pancreatic enzyme supplementation) considerably contribute to the improvement of nutritional status in CF, sometimes leading to the development of overweight or obese children suffering from this condition [40]. It is widely known that obesity in the healthy child population is connected with accelerated growth and puberty processes. As of yet, no reports have been published on growth and puberty in children with cystic fibrosis combined with excess body mass, which may be conducive to puberty normalization. The issue of growth and puberty in children with cystic fibrosis thus requires further detailed investigation under clinical and auxological aspects.

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