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ARTICLE

Mild *CFTR* mutations and genetic predisposition to lactase persistence in cystic fibrosis

Edyta Mądry[1], Ewa Fidler[2], Agnieszka Sobczyńska-Tomaszewska[3], Aleksandra Lisowska[2], Patrycja Krzyżanowska[2], Andrzej Pogorzelski[4], Łukasz Minarowski[5], Beata Oralewska[6], Ewa Mojs[7], Ewa Sapiejka[8], Ryszard Marciniak[9], Dorota Sands[10], Anna Korzon-Burakowska[11], Jarosław Kwiecień[12] and Jarosław Walkowiak*[,2,13]

Taking into account the reported incidence of hypolactasia in cystic fibrosis (CF) and the possible impact of milk products on nutritional status we aimed to assess the genetic predisposition to adult-type hypolactasia (ATH) and its incidence in CF. Single nucleotide polymorphism upstream of the lactase gene (*LCT*) was assessed in 289 CF patients. In subject with −13910C/C genotype (C/C) predisposing to ATH, hydrogen-methane breath test (BT) with lactose loading was conducted and clinical symptoms typical for lactose malabsorption were assessed. The percentage of CF patients with C/C was similar to that observed in healthy subjects (HS) (31.5 *vs* 32.5%). Eleven out of 52 (24.5%) CF C/C patients had abnormal BT results. The recalculated frequency of lactose malabsorption was similar for the entire CF and HS populations (6.9 *vs* 7.2%). Similarly as in the control group, few CF patients have identified and linked to lactose consumption clinical symptoms. The frequency of *LCT* polymorphic variants in CF patients having and not having severe mutations of *CFTR* gene showed significant differences. The C allele was more frequent in homozygotes of the severe mutations than in patients carrying at least one mild/unknown mutation ($P<0.0028$) and in patients with at least one mild mutation ($P<0.0377$). In conclusion, CF patients carrying mild *CFTR* mutations seem to have lower genetic predisposition to ATH. Lactose malabsorption due to ATH in CF is not more frequent than in the general population. Symptomatic assessment of lactose malabsorption in CF is not reliable.
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INTRODUCTION

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in the Caucasian population. Nutrition and nutritional status, beside pulmonary dysfunction, are of major – survival-limiting – clinical importance in CF patients.[1] The increased life expectancy of CF subjects, associated with improved health care and new therapeutic modalities stimulates the search for modifying factors that may have an impact on further improvement of survival and clinical status, for example, nutritional status.[2] Most CF patients require a high-energy diet, and those with exocrine pancreatic insufficiency should also receive pancreatic enzyme replacement therapy.[3,4] The deficiency of pancreatic elastase-1 (not included in pancreatic supplements), the enzyme necessary for digestion of elastin fibers (component of meat), points to dairy products as being potentially an important source of nutrients for CF patients.

Lactase-lactose-phlorizin hydrolase (LCT (MIM 603202)) is necessary for the digestion of lactose, the major carbohydrate found in milk.[5] In case of decreased LCT activity (hypolactasia), milk consumption can lead to bloating, flatulence, cramps, nausea and diarrhea[6] and a consequent exclusion of these products from the diet.[7] Adult-type hypolactasia (ATH) is the genetically determined most common cause of milk intolerance in children, adolescents and adults and the most common enzyme deficiency in humans.[8] The prevalence of ATH is highly variable, ranging from 4% in Ireland to almost 100% in some Asian populations.[9] ATH is inherited as an autosomal recessive trait leading to downregulation of lactase activity in the intestinal mucosa.[8] The homozygous form of allelic variant C at position 13910 (C/C_{13910}) upstream of the *LCT* gene (NM_005915.4:c.1917+326C>T; rs4988235) is tightly associated with ATH, whereas homo- or heterozygous genotype of T allele (T/T_{-13910} or C/T_{-13910}) results in lactase persistence.[10] The strong correlation has been shown between C/C_{-13910}, T/T_{-13910} and C/T_{-13910} genotypes and the final adult level of the lactase activity in intestinal biopsies.[10,11] However, the process of *LCT* gene silencing and its determinants remain uncertain in humans.

From the late 60s of the twenth century the increased incidence of hypolactasia in CF population was reported. The conclusions were

[1]Department of Physiology, Poznań University of Medical Sciences, Poznań, Poland; [2]Department of Pediatric Gastroenterology & Metabolism, 1st Chair of Pediatrics, Poznań University of Medical Sciences, Poznań, Poland; [3]Health Care Center GENOMED, Warsaw, Poland; [4]Department of Bronchology & Cystic Fibrosis, National Institute for Tuberculosis & Lung Diseases, Pediatric Branch, Rabka, Poland; [5]Department of Lung Diseases and Tuberculosis, Medical University of Bialystok, Bialystok, Poland; [6]Department of Pediatric Gastroenterology, Hepatology & Immunology, Child Memorial Health Institute, Warszawa, Poland; [7]Clinical Psychology Department, Poznań University of Medical Sciences, Poznań, Poland; [8]Out-Patient Clinic for CF Patients, Gdańsk, Poland; [9]Department of General, Gastroenterological, and Endocrinological Surgery, University of Medical Sciences, Poznań, Poland; [10]Institute of Mother and Child, Pediatric Department, Cystic Fibrosis Centre, Warsaw, Poland; [11]Department of Hypertension and Diabetology, Medical University of Gdańsk, Gdańsk, Poland; [12]Department of Pediatrics, The School of Medicine and Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Poland; [13]Department of Dietetics, Chair of Human Nutrition & Hygiene, Poznań University of Life Sciences, Poznań, Poland
*Correspondence: Professor J Walkowiak, Department of Pediatric Gastroenterology & Metabolism, 1st Chair of Pediatrics, University of Medical Sciences, Szpitalna 27/33, Poznań 60-572, Poland. Tel: +0048 61 8480310; Fax: +0048 61 8483362; E-mail: jarwalk@ump.edu.pl
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drawn on the basis of significant lowering of lactase activity in the small intestinal biopsies,[12–14] however, the type of hypolactasia was not considered. Recently, Modiano *et al* speculated on the possible correlation between lactase persistence and *CFTR* (cystic fibrosis transmembrane conductance regulator) gene mutation.[15] Taking into account that milk products may have the pivotal dietary role for most of CF patients, and many authors reporting increased incidence of hypolactasia in CF population, we aimed to assess the genetic predisposition to ATH and its incidence in CF patients, comparing it against data from the general population.

SUBJECTS AND METHODS

The survey was carried out in the years 2006–2009. A total of 289 CF patients of Polish origin (147 females and 142 males) aged from 6 to 42 years (median age 18) consented and entered the study. Their BMI (*Z*-score) ranged from −2.2 to 3.7 (median −0.4). The exclusion criteria comprised age below 6 years (before the onset of clinical manifestation of ATH). Diagnosis of CF was based on history, clinical manifestation and increased sweat chloride concentrations and confirmed by the *CFTR* gene mutations identification. Lung function was differentiated, FEV1 ranged from 30.3 to 140.1% (median 78.8%). Subsequent frequency of gastrointestinal manifestations was found: pancreatic insufficiency – 82.0%, CF-related diabetes – 9.3%, liver cirrhosis – 3.5%, history of meconium ileus – 8.3%. Abnormalities of liver function tests were detected in 25.2% of CF patients studied.

In all subjects, peripheral blood was drawn and total genomic DNA was extracted from fresh or frozen blood. The kit MutaGEL Lactase (Immundiagnostik AG, Bensheim, Germany) was used for the indication of 13910 C>T in the *LCT* gene polymorphism (NM_005915.4:c.1917+326C>T; rs4988235) by direct allele-specific detection of genotype as described earlier.[16]

In 52 subjects with C/C genotype hydrogen-methane breath test (BT) with lactose loading was conducted to determine the current state of lactase activity. BT was performed after overnight fast. Patients were instructed not to eat or drink for at least 12 h before the test and to avoid slowly digesting foods such as beans and similar vegetables, brans or high-fiber cereals the day before the test. Subjects were not allowed to smoke, sleep or exercise vigorously for at last 1 h before or at any time during the test. Every patient received lactose dissolved in water orally in a dose of 25 g. Breath samples were collected at baseline (fasting) and at 30, 60, 90, 120, 150 and 180 min after lactose ingestion. The samples were analyzed with QuinTron MicroLyser DP Plus (Quintron, Milwaukee, WI, USA). A positive BT was defined as breath H_2 level increase of at least 20 ppm over the lowest preceding value within the test period or breath CH_4 level increase of at least 12 ppm over the baseline within the test period or combined increase of at least 15 ppm within the test period. Abnormal BT results were considered to be equivalent to lactose malabsorption.

Clinical symptoms in all subjects who underwent BT were assessed using the questionnaire adopted for the project. The subsequent issues were addressed: (1) drinking milk or ingesting other dairy products (at all, irregular or regular), (2) symptoms after ingestion of dairy products (abdominal pain, flatulence, bloating, cramps, diarrhea, others). The presence of the symptoms listed above was considered to be indicative of lactose intolerance.

The results of molecular and clinical analysis of randomly selected 200 young adults (100 females and 100 males) of Polish origin, aged 18–20 years (median 19 years), performed previously[16] according to the same protocol, served for the purpose of comparison of ATH predisposition and its clinical manifestation. BMI ranged from 18.6 to 24.9 kg/m^2 (median 21.7) Both in CF patients and healthy subjects (HSs) the presence of celiac disease was excluded according to accepted standards. [17,18]

The differences in prevalence of allelic variants of *LCT* gene and the frequency of lactose maldigestion and malabsorption were assessed using χ^2-test. The level of significance was set at $P<0.05$. The protocol of the investigation was approved by the Bioethical Committee of the Poznań University of Medical Sciences, Poland.

RESULTS

The distribution of allelic variants of *LCT* (rs4988235) gene polymorphism in the studied group has been presented in Table 1. Genetically determined lactase persistence (genotype C/T and T/T) was expected in 67.5% of CF patients. The percentage of CF subjects with genotype predisposing to ATH was similar to that observed in HS (32.5 *vs* 31.5%). The frequency of *LCT* gene polymorphic variants in CF patients having and not having F508del mutation of *CFTR* gene was not different (Table 1). However, the frequency of *LCT* gene polymorphic variants in CF patients having and not having severe mutations of *CFTR* gene showed significant differences (Tables 2 and 3). C allele was more frequent ($P< 0.0028$) in homozygotes of the severe mutations than in the other CF patients and rarer ($P< 0.0316$) in CF patients not having two severe mutations than in HS (Table 2). C allele was also more frequent ($P<0.0377$) in homozygotes of the severe mutations than in patients carrying at least one mild mutation (Table 3).

Eleven out of 52 (24.5%) CF patients with C/C genotype were proved to be lactose malabsorbers on the basis of BT (Table 4). Recalculating the data for the entire studied CF population implies the incidence of lactose malabsorption in 6.9% of the subjects. The observed frequency was very similar to that documented in HSs (7.7%). Similarly as in the control group, few CF patients (2 out of 11–18.2%) with abnormal BT results, have identified and linked to lactose consumption clinical symptoms (Table 4).

DISCUSSION

The prevalence of C/C_{-13910} genotype predisposing to ATH, lactose malabsorption as assessed with the use of highly reliable hydrogen-methane BT and the frequency of clinical symptoms in CF patients diagnosed as lactose malabsorbers, were not different than that in randomly selected HSs. According to literature search, it is the first study directly comparing hypolactasia and its genetic predisposition, due to −13910 C>T of the *LCT* gene polymorphism, in the CF and general populations. Our data show the lack of increased genetic

Table 1 The frequency of *LCT* (NM_005915.4:c.1917+326C>T; rs4988235) polymorphic variants and alleles in CF patients and HSs

	−13910 LCT genotype n (%)			*−13910 LCT allele n (%)*	
Studied groups	*C/C*	*C/T*	*T/T*	*C*	*T*
CF					
All	94 (32.5)	120 (41.5)	75 (26.0)	308 (53.3)	270 (46.7)
F508del homozygotes	41 (35.1)	48 (41.0)	28 (23.9)	130 (55.6)	104 (44.4)
F508del heterozygotes	27 (27.8)	44 (45.4)	26 (26.8)	98 (50.5)	96 (49.5)
Other genotypes	26 (34.7)	28 (37.3)	21 (28.0)	80 (53.3)	70 (46.7)
HS	63 (31.5)	90 (45.0)	47 (23.5)	216 (54.0)	184 (46.0)

Abbreviations: CF, cystic fibrosis; HS, healthy subject.

Table 2 The frequency of *LCT* polymorphic variants (NM_005915.4:c.1917+326C>T; rs4988235) and alleles in CF patients and HSs

	−13910 LCT genotype n (%)			*−13910 LCT allele n (%)*	
Studied groups (CF according to mutations)	*C/C*	*C/T*	*T/T*	*C*	*T*
CF					
Severe/severe	66 (36.7)	76 (42.2)	38 (21.1)	208 (57.8)*,**,†	152 (42.2)
Others					
Total	27 (24.8)	44 (40.3)	38 (34.9)	98 (45.0)*,‡	120 (55.0)
Severe/other[a]	19 (25.7)	30 (40.5)	25 (33.8)	68 (45.9)**	80 (54.1)
Other/other[a]	8 (22.9)	14 (40.0)	13 (37.1)	30 (42.9)†	40 (57.1)
HS	63 (31.5)	90 (45.0)	47 (23.5)	216 (54.0)‡	184 (46.0)

Abbreviations: CF, cystic fibrosis; HS, healthy subject.
*$P<0.0028$; **$P<0.015$; †$P<0.0216$; ‡$P<0.0316$.
[a]Other=mild or unknown

Table 3 The frequency of *LCT* polymorphic variants (NM_005915.4:c.1917+326C>T; rs4988235) and alleles in CF patients with defined genotype

	−13910 LCT genotype n (%)			*−13910 LCT allele n (%)*	
Studied groups (according to mutations)	*C/C*	*C/T*	*T/T*	*C*	*T*
CF					
Severe/severe	66 (36.7)	76 (42.2)	38 (21.1)	208 (57.8)*	152 (42.2)
Others					
Total	5 (20.0)	11 (44.0)	9 (36.0)	21 (42.0)*	29 (58.0)
Severe/mild[a]	5 (23.8)	9 (42.9)	7 (33.3)	19 (45.2)	23 (54.8)
Mild/mild[a]	0 (0)	2 (50.0)	2 (50.0)	2 (25.0)	6 (75.0)

Abbreviation: CF, cystic fibrosis.
*$P<0.0377$.
[a]Other=mild or unknown.

Table 4 The frequency of LM as assessed using breath test and LI based upon clinical symptoms in CF patients and HSs with *LCT* C/C$_{-13910}$ (rs4988235) genotype predisposing to adult-type hypolactasia

Studied group	*LM n/N (%)*	*LI n/N (%)*
CF	11/52 (21.2)	2/11 (18.2)
HS	13/53 (24.5)	3/13 (23.1)

Abbreviations: CF, cystic fibrosis; HS, healthy subject; LI, lactose intolerance; LM, lactose malabsorption; n, number of cases; N, number of assessed subjects.

predisposition (the frequency of C/C$_{-13910}$ genotype) to ATH in the CF population (32.5%) as compared with HSs (31.5%). However, the link between mild *CFTR* gene mutations and lactase persistence was stated.

In a subset of 52 CF patients homozygotic for C allele the genotype-phenotype correlation was evaluated using BT with lactose loading. The percentage of subjects diagnosed as being lactose malabsorbers was similar in CF (21.2%) and HS (24.5%) populations (Table 2). Recalculating obtained data into the entire studied populations, the incidence of lactose malabsorption does not differ between CF patients and general populations (6.9 *vs* 7.7%). In the studies relating C/C$_{-13910}$ genotype to lactose malabsorption, all participating subjects were symptomatic.[10,11] The studied groups were not cross-sectional and all participants were preselected. In the present study we randomly selected a cohort of CF patients and assessed their lactose digestion and absorption with the use of hydrogen-methane BT. It seems that in many patients with genetic predisposition to ATH lactose malabsorption may develop in an older age. Median age of the CF patients and HSs enrolled in the present study was similar, the majority were adolescents and young adults. Therefore, the obtained results are expected to be comparable. Very frequent false negative results (9 out of 11 patients) of symptomatic assessment of lactose malabsorption point to the need of the use of objective measures.

Lactase activity lowering is a process that takes place progressively over time; in most mammals lactose activity is high at birth and during breast-feeding, then declines at weaning to low adult levels.[19] This physiological dependence makes the prevalence of lactose malabsorption in C/C$_{-13910}$ genotype subjects to increase significantly with age.[20] Thus the C/C$_{-13910}$ genotype indicates the predisposition to ATH rather than the current clinical state. It can be used as a first stage screening test for the selection of the group with genetic predisposition to ATH. It is of particular importance in CF population, where every factor that can affect the nutritional status may have an impact on the life expectancy. We suggest to carry out molecular analysis in all CF patients in order to select the C/C genotype group and periodically repeat the BT in predisposed subjects. BT is a cheap, simple and reliable method for monitoring the current state of lactase activity, allowing for early detection of its deficiency and possible early introduction of dietary intervention. The frequent incidence of abdominal discomfort in CF patients, which may be identical to the symptoms of ATH, seems to make the above suggested procedure deeply justified and beneficial for patients – not only for those with ATH-predisposed genotype (C/C) but also for non-ATH-predisposed group with the genotypes C/T, T/T. In this group BT may allow for the exclusion of secondary hypolactasia as the potential reason of abdominal discomfort and may be helpful in differential diagnosis.

Increased incidence of hypolactasia in the CF population has been suggested by many authors. The lowered lactase activity in structurally normal intestinal biopsies of CF patients prompted the hypothesis of

the primary nature of this finding. [12–14] However, the age of the studied subjects was predominantly low (mostly below 3 years), which precludes ATH and indicates the secondary or other than ATH primary ground of the observed lactase deficiency. The data obtained in the present study clearly documented that the frequency of lactose malabsorption and the genetic predisposition to ATH do not differ in the CF and HS populations. Our results remain consistent with Levindon's report. The prevalence of carbohydrate malabsorption in CF children with abnormal stool pattern was assessed using BT. As compared with children with non-CF-related stool abnormalities, CF subjects had no increase in lactose malabsorption. However, selected subgroups of both CF patients and HSs were assessed.[21]

Van Biervliet *et al* assessed disaccharide activities and intestinal alkaline phosphatase (IAP) in small intestinal biopsy specimens of 61 newly diagnosed CF patients aged from 1 month to 14 years. Interestingly, in a significant number of patients (n=19) Shmerling type II and III mucosa was stated. In order to eliminate the influence of morphological damage as a confounding factor the authors analyzed separately 42 patients with normal stereomicroscopic and histological features of small intestine. IAP and LCT activity were significantly lower in CF patients than in non-CF age-matched subjects with normal intestinal morphology who underwent intestinal biopsy due to the failure to thrive or non-specific gastrointestinal complaints. The observed decrease was also present in subjects younger than 2 years. Other disaccharidases activities, although lower, were not different from those in non-CF control subjects. Authors speculated that observed changes were related to basic defect of CFTR, influencing processing of IAP and LCT. The majority of CF patients studied were infants and toddlers, 32 out of 41 subjects were younger than 2 years. It rather precludes ATH as a cause of lactase decline. The decrease of LCT activity was only partial, potentially not leading to lactose malabsorption. Moreover, majority of subjects were pancreatic insufficient and did not receive pancreatic enzyme replacement therapy before the study.[14] In the present study we enrolled the older CF patients in whom the decline of LCT activity due to ATH could be present. Proper treatment precluded the potential influence of maldigestion due to pancreatic insufficiency. We did not routinely biopsied all subjects, what needs to be pointed as the limitation of the study, but at the same time seems to be understandable given the invasiveness of the procedure. However, celiac disease was excluded in all of the subjects. As documented earlier, in treated CF patients villous atrophy is not a frequent finding.[17] Therefore, we can assume that observed lactose malabsorption was related to ATH. The potential influence of the *CFTR* on LCT activity with exclusion of the influence of maldigestion demands further studies.

Interestingly, Modiano *et al*[15] speculated recently on a possible correlation between lactase persistence and CF. The two 'Europe-restricted' features, the extremely high frequency of lactose tolerance (T allele of *LCT*) and the high frequency of CF alleles, are considered as profoundly different adaptation to the severe diarrhea attacks following lactose ingestion. Assuming that CF mutation carriers are more resistant to the diarrhea-causing factors, which had impact on survival, it should lead to a selective advantage sufficient to maintain the CF alleles at a very high frequency for a lethal allele. The authors suggested that there is no stringent indication that Europeans were preferentially exposed to cholera as it was proposed previously.[22,23] Modiano hypothesized that the role of major adaptogen had been played by the diary-milk diet adopted by Europeans when they were still lactose intolerant. The 'emergency' adaptive response of an immediate expansion of the already available CF alleles (the age of F508del is estimated at around 52 000 years) resulted in a very high frequency of *CFTR* gene mutation carriers. With the appearance of *LCT* gene polymorphism a progressive decrease to the present values of *CFTR* gene allele frequency took place. Highly significant positive correlation ($P<0.001$) between the frequency of T_{-13910} allelic variant of *LCT* gene and the proportion of F508del among all CF alleles has been documented with well-known north-south cline. According to Modiano the simplest explanation is that diary-milk diet was established in a single region (5000–10 000 years ago) remaining restricted to this area for a period of time sufficient to allow the T_{-13910} and the F508del alleles to attain high values. Then both adaptive genes were exported with diary-milk culture to the rest of Europe.[15] Even if this hypothesis is correct, the frequency of T_{13910} allele of *LCT* gene in CF patients having and not having F508del mutation should not differ at present, which has been proved in the present study. However, more frequent occurrence of genetic predisposition to lactase persistence in CF patients carrying mild mutations was stated. We speculate that normal lactose digestion and absorption could promote the consumption of dairy products and thus contribute to the improvement of nutritional status in that subgroup of CF patients. Therefore, they were more frequently able to survive into adulthood than other lactose malabsorbers carrying the same genotype, passing 'T-trait'.

In conclusion, CF does not seem to be a risk factor for developing adult-type lactose intolerance. Moreover, patients carrying mild *CFTR* gene mutations have less frequently genetic predisposition to its occurrence. It is also worth to mention the usefulness of molecular analysis in assessing the genetic predisposition to ATH and BT in order to confirm/exclude the presence of lactose malabsorption, which is being quite often missed upon clinical symptoms.

CONCLUSION

CF patients carrying mild *CFTR* mutations seem to have lower genetic predisposition to ATH. Lactose malabsorption due to ATH in CF is not more frequent than in general population. Symptomatic assessment of lactose malabsorption in CF is not reliable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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