Long-term complications in Estonian galactosemia patients with a less strict lactose-free diet and metabolic control

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A B S T R A C T

The main aim of our study was to retrospectively evaluate long-term complications and measure urinary galactose and galactitol excretion in classical galactosemia patients in Estonia who have been treated with a less restricted lactose-free diet and metabolic control. Our study group consisted of five classical galactosemia patients aged 7–14 years and diagnosed since 1996 in Estonia. Their diet eliminates lactose present in dairy foods, but we did not restrict the consumption of mature cheeses, fruits and vegetables. All patients had normal growth, except for one patient who was overweight at the last evaluation. In three patients mental and speech development was normal. One patient, number 1, who was diagnosed latest (at 6 weeks of age), had moderate mental retardation, verbal dyspraxia, extrapyramidal signs and bilateral cataracts. In both patients with developmental problems, a brain MRI showed bilateral subcortical changes in the cerebral white matter. Of four females, only patient 4 (p.Q188R homozygote) has premature ovarian insufficiency. Urinary galactose and galactitol content were retrospectively measured using high-performance liquid chromatography and refractive-index detection from urinary samples that were preserved during the years 1996–2009. Galactose ranged from 60 to 600 mmol/mol creatinine (normal=4–6), and galactitol ranged from 70 to 1200 mmol/mol creatinine (normal=2–4), which was 10–100 and 17–300 times higher than the respective reference ranges for galactose and galactitol. We conclude that a less strict lactose-free diet and metabolic control performed in Estonian classical galactosemia patients does not change long-term outcome compared to previously published studies.

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1. Introduction

Classical galactosemia (OMIM 230400) is an autosomal recessive disorder of galactose metabolism caused by a deficiency of the enzyme galactose-1-phosphate uridylytransferase (GALT; EC 2.7.12) [1]. The incidence of this disorder in European populations varies greatly, ranging from 1:23,000 and 1:44,000 [2]. The incidence of classical galactosemia in Estonia was recently evaluated to be one in 19,700, which is relatively high compared to incidence in Europe as a whole [3]. The gene that encodes GALT is located on chromosome 9p13, and almost 200 mutations have been identified so far (ARUP Laboratories GALT Mutation Database) [4–6]. The most common mutation in classical galactosemia is p.Q188R, which is the most frequent mutation in all Caucasian populations, with the highest frequency (65%) in Western Europe [2,7]. If untreated, GALT deficiency usually presents with jaundice, hepatomegaly, hepatic insufficiency, renal tubular disease, cataracts, cerebral edema or sepsis in the newborn period, after intake of lactose-containing breast milk or infant formula, and can be potentially lethal [1]. In many countries galactosemia is part of the newborn screening program. Diagnostic strategies include measurement of galactose-1-phosphate (Gal-1-P) in red blood cells (RBC), quantification of galactose and galactitol, and in the second tier, mutation analysis [2].

Even with early and adequate therapy with galactose restriction, the long-term outcome in older children and adults with classic galactosemia can include cataracts, speech defects, poor growth, abnormalities of the brain white matter, poor cognitive function, neurological deficits and premature ovarian failure [1,2]. There has been considerable debate

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concerning the ideal stringency of diet after the first year of life, as endogenous galactose production is an order of magnitude higher than that ingested from foods other than milk [8–10].

Herein we report the long-term complications and results of urinary galactose/galactitol excretion in five classical galactosemia patients in Estonia who have been on a less restricted diet since the suspicion and confirmation of the diagnosis.

2. Patients and methods

2.1. Patients

Our study group consists of five classical galactosemia patients (4 girls and one boy) diagnosed during a selective screening program from 1996 to 2003 in Estonia [3]. One of them was homozygous for the p.Q188R mutation, three patients were compound heterozygotes for the p.Q188R/p.R272C mutation and one patient had the p.Q188R/p.H114P compound heterozygosity. All were older than 6 years at the time of data collection.

The Ethics Review Committee on Human Research of the University of Tartu approved this study. Informed consent was obtained from the children’s parents.

2.2. Clinical data

The clinical data was collected from case histories from two centralized hospitals, Tartu University Hospital and Tallinn Children’s Hospital. Their growth and also psychological, speech, neurological and sexual development and ophthalmological status were assessed on a regular basis by specialists. Mental development was carefully tested at the age of 6 years (before school) in the youngest child and at the age of 14 years in the oldest children. The last physical examination was carried out in 2010.

2.3. Diet

Two 14-year-old patients diagnosed in 1996 are the first living galactosemia patients in Estonia. We had no experience of how to perform the lactose free-diet in galactosemia patients or precise information about the galactose content of different food products. We introduced a lactose-free diet due to the suspicion of a classical galactosemia diagnosis in all patients, and it was based on the few literature sources that were available in a country of the former Soviet Union. Our diet eliminates the lactose present in dairy foods, but we did not restrict the consumption of mature cheeses, fruits and vegetables (Table 1). In the infant period, all patients used soya-based or lactose-free milk formulas for infants. Secondly, a strict lactose- and galactose-free diet was not used in everyday practice due to the lack of possibilities of objective measurement of the effectiveness of the diet up to 2008 in Estonia (including galactose and galactitol measurement in body fluids and RBC Gal-1-P). The average daily galactose intake was retrospectively estimated to be at least 50 mg in all cases. This was based on the fact that a lactose-free diet, which is enriched with galactose-rich fruit and vegetables, results in a daily galactose intake of approximately 50 mg [11]. Our patients showed quite a significant variation in galactose exposures over time, but we had no registered information regarding that fact.

2.4. Biochemical follow-up

Laboratory studies were performed retrospectively with urine samples that were preserved during the years 1996–2009. Urinary galactose and galactitol content were evaluated. The method of choice was high-performance liquid chromatography (Shimadzu). Refractive index (RI) and ultraviolet–visible spectrophotometrical detectors were used in parallel in order to distinguish sugars from other organic compounds. A BioRad HPX-87 H column (300 × 7.8 mm × 9 μm) was used. Isocratic elution, 0.6 ml/min with 4 mM H2SO4 (Penta) on ambient temperature, was applied. Galactose and galactitol standards were obtained from Sigma. Stock solutions of 10 mM were preserved at −20 °C, and the calibration was performed using 62.5, 125, 250 and 5000 μM standard samples (coefficient of correlation 0.9999). Retention values for galactose and galactitol are around 10 min. Urine needs no pre-treatment except filtration through a 0.45 μm filter. 100 μl of the sample was injected. The results were calculated as a mean of 3 parallel tests. The advantages of this method are speed and simplicity, but its drawback is low sensitivity. Since the concentrations of galactose and galactitol in urine of classical galactosemia patients are, however, high enough for the RI detector to detect, we found the use of this method to be suitable.

3. Results

3.1. Clinical data

The clinical data and genotype of the patients is given in Table 2. The diagnosis of galactosemia was confirmed in one case prenatally and in four cases postnatally (at 8 days, and 2, 4 and 6 weeks respectively). All patients had normal height and weight parameters, except for patient 1, who was overweight (BMI 25.2).

In three patients mental and speech development was normal at their last evaluation. In one of them (patient 5) the galactosemia was only diagnosed at 4 weeks of age. Patient 1, who was also diagnosed late (at 6 weeks of age), had moderate mental retardation, a speech disorder (verbal dyspraxia) and extrapyramidal signs with ataxia and stereotypical movements. She was also the only patient with bilateral cataracts (now stabilized). Patient 3 has a mild speech delay and cognitive function deficit, with focal epilepsy diagnosed at four years of age. In both patients with developmental problems, a brain MRI was performed, and this showed bilateral subcortical changes in the cerebral white matter — typical findings in galactosemia patients.

Of the four females, premature ovarian insufficiency with markedly increased FSH levels at the age of 12 years was only diagnosed in patient 4 (p.Q188R homozygote), and therefore she receives hormone replacement therapy.

3.2. Biochemical follow-up

We found 23 preserved urine samples from our galactosemia patients (4.6 samples per patient). The concentrations of urinary galactose (Fig. 1) and galactitol (Fig. 2) varied from one sample to
another. Galactose ranges from 60 to 600 mmol/mol creatinine (normal 4–6; pathological value >10) [12], which is 10–100 times higher than the reference range. Galactitol ranged from 70 to 1400 mmol/mol creatinine (normal 2–4, pathological value >10) [12], which is also 17–300 times higher than the reference range.

### 4. Discussion

Patients with galactosemia are monitored by regular measurement of the galactitol in their urine, which is a product of the alternate pathway for galactose metabolism. The second metabolite to be monitored is their RBC Gal-1-P [1]. We do not yet have the ability to measure RBC Gal-1-P regularly in Estonia. In our study we retrospectively measured galactose and galactitol excretion in our galactosemia patients. During the treatment, galactose excretion was at least 10 to 100 times higher than normal. Galactitol excretion was also markedly increased, with levels exceeding 600–800 μmol/mmol of creatinine, i.e. up to 300 times above the reference value (see Fig. 2). However, increased excretion of galactitol had great inter-individual variability and was not correlated with the long-term outcome of our classical galactosemia patients. It has previously been shown that in the case of classical galactosemia the urinary excretion range of galactitol exceeds 1000 μmol/mmol of creatinine at newborn age and persists with a diet of between 100 and 400 μmol/mmol of creatinine [13]. We believe that the markedly increased excretion of galactose and galactitol in our patients is due to their relatively variable and relaxed diet, which also included mature hardened cheeses such as Gouda and Emmentaler. These cheeses usually contain no galactose because of the action of fermenting microorganisms [14]. We freely permitted these types of cheese, as they are an excellent source of calcium. Bosch et al. [9] also gave high oral galactose (up to 600 mg per day) to classical galactosemia patients, and also found markedly increased excretion of galactitol, i.e. up to 1000 mmol/mol creatinine, which was similar to our patients. They did not notice significant changes in clinical symptoms. Therefore urinary galactose and galactitol analyses are of questionable value in the follow-up of patients on a regular diet, and can only be useful in detecting severe non-compliance. It has also been recognized by other authors that other than detecting very significant galactose intoxication, RBC Gal-1-P and urine galactitol are not reliable measures of individual galactose tolerance and mild to moderate deviations from a galactose restricted diet [2,11,15,16]. This is due to the fact that all of these parameters show large intra- and interindividual variations, and therefore the clinical implications of these measurements are unclear [17]. More sensitive biomarkers are needed.

Our retrospective study group was small and quite heterogeneous. There were a number of substantial confounders according to the following criteria: the onset of dietary therapy and genotype. The initiation of treatment was very variable among patients. Patient 2 was diagnosed prenatally and had a good outcome, although her elder sister (patient 1) with the same genotype was diagnosed later, i.e. at 6 weeks of age, and has marked speech and neurological complications.
and cataracts. As a result, one is tempted to conclude that earlier diagnosis ensures a better outcome. Nevertheless, patient 5 was also diagnosed quite late (at 4 weeks of age) and also has a good outcome. This therefore raises the question of whether or not one really can conclude that earlier diagnosis ensures a better outcome. Many earlier studies have also revealed that, except for diagnosis after 2 months of age, neither the age at the time of diagnosis nor the severity of clinical illness at the time of diagnosis correlates with the presence and severity of later complications [16,18–23]. Screened patients might be expected to have fewer neonatal complications [24]. To date, most of the data suggest that cognitive impairment and speech defects, as well as premature ovarian insufficiency, originate in prenatal life; neonatal lactose exposure may only magnify these toxicities [13].

The final answer will be given after carefully planned prospective studies of patient outcome [20].

In our group of patients, one was homozygous and four were heterozygous for the p.Q188R mutation (p.R272C and p.H114P mutation in the second allele, see Table 2). Neither of the other mutations were previously mentioned in the international database of GALT mutations [6]. Therefore it is difficult to make a genotype–phenotype correlation in these compound heterozygote patients, except for one p.Q188R homozygote. The literature shows that verbal dyspraxia and premature ovarian failure have the worst outcome in p. Q188R homozygotes [18,23,25–27]. Moreover, children who were homoallelic for the p.Q188R mutation had significantly lower IQ scores than those who were heteroallelic [23]. Our single homozygote for the p.Q188R mutation (patient 4) demonstrated premature ovarian insufficiency, but studies well in normal school and has no speech problems. The other three females with the p.Q188R/p.R272C genotype had normal ovarian function at the time of their last evaluation at the ages of 14, 12 and 11 years respectively.

Patient 5 (p.Q188R/p.H114P genotype) was diagnosed relatively late, at 4 weeks of life, but had normal growth and development at his last evaluation, at 12 years of age. The p.H114P mutation was evaluated to be a neutral mutation [3]. In this case we can say that the p. Q188R/p.H114P genotype has a better outcome, probably due to residual GALT activity.

In three out of five patients, mental and speech development was normal on their last evaluation at the ages of 14, 12 and 11 years respectively. Two large long-term outcome studies showed IQ decline with increasing age [16,21,22]. There was also a high degree of microcephaly, partly in conjunction with intention tremor, and mild to severe ataxia that appeared at ages 9–14 [22]. Therefore it is possible that later deterioration has not been ruled out in these three patients. In contrast, Schadewaldt et al. [28] recently showed evidence for an absence of substantial galactosemia-induced aggravation of reduced cognitive ability with increasing age, at least in patients from 4 to 40 years of age. They suggested that a reduction of cognitive function in galactosemia may be initiated by an in utero toxicity of endogenously formed galactose that is later maintained throughout life. A prenatal deficiency of myo-inositol due to an accumulation of both galactose-1-phosphate and galactitol may play a role in the rise of the postnatal central nervous system dysfunction [29].

It has been debated how stringent diet should be after the first year of life, as endogenous galactose production is an order of magnitude higher than that ingested from foods other than milk [8–10]. The endogenous production of galactose reaches 1 g per day in adults [30]. There are also studies suggesting that the over-restriction of galactose could contribute to ongoing pathophysiology [31,32]. Many European metabolic centers, including those in the Netherlands, long recommended a very strict diet with restriction of galactose-containing fruits and vegetables, thus further complicating the lives of patients with galactosemia [9,33]. Other centers, including those in the UK, Germany and the USA, have been more liberal, advising only a lactose-free diet and placing no strict restrictions on other components [33–35]. To our knowledge there have been two studies that assessed short-term increased galactose intake, suggesting no considerable effect on biochemical markers [9,15]. Both studies showed no significant changes in clinical outcome and monitoring levels. The patient reported by Lee et al. [36], who discontinued galactose restriction at 3 years of age, had an outcome no worse than that seen in many treated individuals. Now some physicians are concerned that we have transformed galactosemia into a progressive disease through the very use of a chronic strict diet therapy that limits galactose intake and thus creates further deficiency of uridine diphosphate (UDP)-galactose and UDP-glucose in some target tissues [13,37].

Finally, we conclude that a less strict lactose-free diet and metabolic control in Estonian classical galactosemia patients does not change long-term outcome compared to previously published studies.

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References


