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Duarte (DG) galactosemia: A pilot study of biochemical and neurodevelopmental assessment in children detected by newborn screening

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ABSTRACT

Newborn screening for galactosemia has shown a high prevalence of partial galactose uridyl transferase deficiencies such as Duarte (DG) galactosemia.

Study objective: To determine whether (a) there is any clinical impact of DG galactosemia on development (b) there is a relationship between outcome and biochemical parameters in patients who receive no treatment.

Study population: Twenty-eight children with DG galactosemia. Group-I—17 children had a lactose restricted diet in the first year of life. Group-II—11 children had a regular diet since birth.

Methods: Developmental, physical, and ophthalmologic assessments were completed on both DG groups. RBC gal-1-p and urine galactitol were monitored during the follow-up visits in every child with DG galactosemia. Gal-1-p, urine galactitol, liver function tests, and FSH were tested at the time of study visit.

Results: The groups had statistically significant differences on RBC gal-1-p and urine galactitol at the 2 week, 1 month, 6 month, and 1 year time points. There was no statistical difference of gal-1-p or urine galactitol in group-I and -II at the time of study. The groups had statistically significant differences on adaptive scores, but not on language or IQ. None of the DG subjects had abnormal liver function at the time of diagnosis or the study visit. The FSH levels were normal. There were no statistically significant relationships between the first year metabolic values and developmental outcomes.

Conclusions: The data presented here indicate that clinical and developmental outcomes in DG galactosemics are good regardless of any diet changes.

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Introduction

Classical galactosemia (1/40,000–60,000 live births) is an inborn error of metabolism caused by a profound or complete deficiency of galactose-1-phosphate uridyltransferase (GALT)[1] [1]. Hundreds of mutations of the GALT gene have been reported [2–4]. The most common mutations are associated with milder clinical variants and are considerably more prevalent than those leading to the classical form. The most frequent GALT mutation is called Duarte (N314D), which can exist in two different forms: Duarte-1 and Duarte-2. The Duarte allele is defined biochemically by isoforms distinguishable by native gel electrophoresis and isoelectric focusing. Duarte-1, the Los Angeles variant, is associated with increased activity compared to Duarte-2 [1]. Both variants exhibit the same electrophoretic pattern but are distinguishable at the genetic level by additional base changes.

The Duarte-2 (N314D) mutation is found in about 5% of the US population. Many infants (1/4000 in Caucasians) are compound heterozygotes for the Duarte-2 variant and classical galactosemia, so-called DG variants. These individuals typically manifest 14–25% of normal GALT activity [1,5]. In DG galactosemia there is increased red blood cell galactose-1-phosphate, galactitol, galactonate, and increased urinary excretion of galactitol during early infancy [6].

Many developed countries screen all newborns for galactosemia by analyzing blood total galactose (gal-1-p) and /or determining

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[1] *Abbreviations used:* DG, Duarte-classical galactosemia; RBC, red blood cell; Gal-1-P, galactose-1-phosphate; GALT, galactose-1-phosphate uridyltransferase.

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the activity of GALT enzyme. The residual activity of GALT enzyme correlates with the loci of mutations in the GALT gene. Some screening laboratories perform mutation analysis using DNA derived from the original newborn dried blood specimen. The four most frequently encountered classical galactosemia alleles (Q188R, S135L, K285N, and L195P) and the N314D Duarte variant mutation are surveyed. Many newborns with variant forms of galactosemia such as DG or DD are detected through newborn screening.

Ever since DG babies have been discerned in newborn screening programs, a vexing question has been whether the reduction of RBC GALT to approximately 14–25% of normal places an infant at risk for having any aspect of the classic galactosemia syndrome. The need to restrict dietary galactose in these babies is uncertain, and a variety of recommendations have been made. Some metabolic physicians restrict lactose in the diet for approximately 1 year and then gradually introduce this sugar and follow the clinical and biochemical response. Some physicians do not restrict lactose at all.

There are no data to predict the likelihood that medical or developmental complications can result from untreated DG galactosemia. In this study we compare the clinical, biochemical, and developmental outcomes of DG galactosemics who received a galactose free diet in the first year of life with those on an unrestricted diet, up to 6 years of age.

Materials and methods

Study population

Twenty-eight children (13 male and 15 female) with DG galactosemia participated in the study. Participants ranged in age from 1 to 6 years (2.96 ± 1.31 years), and were divided into two groups based on history of lactose restriction during the first year of life. Group-I—17 DG children (age range: 1.3–6 years, median 3.5 years) were on a lactose restricted diet in the first year of life. A lactose restricted diet was initiated during the first 10 days of life for all participants. Group-II—11 DG children (age range: 1.1–5 years, median 2.28 years) were on regular diet since birth. At the time of the study all children were on a regular diet. Twenty-five out of 28 children were compound heterozygotes for Q188R and N314D mutations. Three other patients were compound heterozygotes for S135L and N314D, K285N and N314D, and N314D and deletion, respectively. All participants spoke English as their primary language. The ethnic composition of the participants was 97% Caucasian, and 3% Hispanic. This breakdown by ethnicity reflects the distribution of DG across populations and its rarity among African, Americans and Asians.

None of the participants had a history of significant unrelated medical problems (e.g., extreme prematurity, nonmetabolic liver disease, cardiac or respiratory arrest, acquired traumatic brain injury or other genetic disorders).

Evaluations at the time of the study visit

A general physical and neurological exam was completed for each subject. History included questions related to neonatal health, particularly the presence of jaundice, feeding difficulties, sepsis, or other illnesses, surgeries, hospitalizations, and developmental progress. A slit lamp exam for cataracts and neurodevelopmental test were completed on each subject.

Diet history

A metabolic dietician obtained detailed diet information on current (based on a 3-day diet record) and past diets. For the purposes of this study a participant was considered "treated" if he/she received lactose restricted formula during the first year of life.

Laboratory studies

RBC gal-1-p, and urine galactitol levels were monitored closely in every patient since their newborn period. This biochemical information was included in the study. At the time of study visit each participant also had blood tests for comprehensive blood metabolic panel and coagulation studies, and FSH levels (females only). RBC gal-1-p and urine galactitol levels were used to assess the metabolic control in participants.

Sample preparation and GC/MS assay

The sample preparation and derivatization of RBC gal-1-p has been described previously [7]. The peak of RBC gal-1-p was identified chromatographically and by its fragmentation pattern. Quantitation was determined from standard curves for each metabolite constructed with each group of specimens analyzed as described previously using the *m/z* ratio for gal-1-p of 204/205 [7]. The gal-1-p is expressed as mg/dL. Urine galactitol was quantitated by the GC method reported previously [8]. All standards, chemicals and materials used were as described previously [6].

Neurodevelopmental battery

A brief neurodevelopmental battery was designed to assess general cognitive ability, language development, and adaptive functioning in study participants.

Individuals aged 12–35 months completed the Bayley Scales of Infant Development—Third Edition (BSID-III), a standardized and individually administered assessment of developmental functioning in infants and young children. The administered test produces three scales—a cognitive, language, and motor scale. Two additional scales of social-emotional and adaptive behavior were calculated, based on parent report.

Individuals aged 36 months or older completed the Wechsler Preschool and Primary Scale of Intelligence—Third Edition (WPPSI-III), a standardized and individually administered assessment of cognitive functioning for toddlers and preschoolers. The measure provides a full-scale intelligence quotient as well as scales of verbal abilities, nonverbal reasoning abilities, and general language ability. Individuals aged 36 months or older also completed the Adaptive Behavior Assessment Scale—Second Edition (ABAS-II), a parent report measure of behavioral and adaptive functioning that is directly analogous to the social-emotional and adaptive behavior questionnaire administered in the infant battery. Adaptive scores reflect parents' ratings of the child's real-life functioning in multiple domains, including communication skills, community use skills, functional pre-academic skills, home living skills, health and safety behaviors, leisure activities, self-care skills, self-direction skills, and social skills.

This study was performed with the approval of the Children's Hospital of Philadelphia Institutional Review Board (#2005-8-4364).

Statistical analysis

The distributions of all outcome variables were examined using histograms and one-sample Kolmogorov–Smirnov tests.

When outcomes had normal distribution, or when a logarithmic transformation resulted in a normal distribution, differences between the two groups were examined using *t*-tests for independent samples. For outcomes that could not be normalized, nonparametric Mann–Whitney tests were used.

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The relationships between (a) first year metabolic levels (RBC gal-1-p and urine galactitol) and developmental outcomes, and (b) metabolic levels (RBC gal-1-p and urine galactitol) at the study visit and developmental outcomes were examined using Pearson parametric or Spearman rank order correlation coefficients.

Change in RBC gal-1-p and urinary galactitol as measured repeatedly across time during the first year of life was analyzed using generalized estimating equations (GEE) [9]. To account for the possibility of a curvilinear aspect in the data, quadratic and cubic time effects were included in the model fitting gal-1-p, along with linear time. Dependent variables were the RBC gal-1-p and urine galactitol levels. Each of these metabolic outcomes was analyzed separately. Independent variables were group membership, age, time of measurement, and the interaction of group and time of measurement.

Results

RBC gal-1-p and urine galactitol levels

During the first year of life

Table 1 shows RBC gal-1-p levels at 2 weeks, 1 month, 6 months and 1 year in group-I ($n = 17$) and group-II ($n = 11$) DG galactosemics. RBC gal-1-p levels were more frequently monitored in group-II subjects, who were not on lactose restricted diet, and their gal-1-p levels at 3 and 4 months of age are also shown in Table 1.

Urine galactitol levels, measured at 2 weeks and 1 year in group-I subjects ($n = 17$), and at 2 weeks, 1 month, 6 months, and 1 year in group-II subjects ($n = 11$), are shown in Table 1.

Longitudinal analyses indicate that during the first year of life, RBC gal-1-p and urine galactitol levels were significantly changing across time. Gal-1-p was changing across time in both a linear and a complex curvilinear manner ($p < 0.0005$ for linear, quadratic, and cubic time effects). The main effect of group (restricted diet in first year-group-I versus regular diet since birth-group-II) was not statistically significant ($p = 0.38$). However, the group * time effects were all statistically significant ($p = 0.030$, 0.009, and 0.006, for linear, quadratic, and cubic interaction effects, respectively), indicating that gal-1-p is changing at different rates and in different manners in the two groups. The fact that urine galactitol was measured only twice in the restricted diet group prevented an examination of curvilinear changes. The main effects of group ($p = 0.001$) and of linear time ($p < 0.0001$) on urine galactitol were both highly significant, indicating differences between the groups and among the different time points of measurements. The group * time interaction effect was statistically significant ($p = 0.027$), indicating that urine galactitol was changing at different rates in the two groups. Box plots of the data are shown in Figs. 1 and 2.

Since longitudinal models indicate that the effect of group differs depending on the time of measurement, the following results were obtained based on non-longitudinal analyses.

The groups had statistically significant differences on gal-1-p levels at 2 week ($p = 0.007$), 1 month ($p < 0.0005$), 6 month ($p < 0.0005$), and 1 year time points ($p = 0.005$).

The groups had statistically significant differences on urine galactitol at the 2 week ($p = 0.013$) and 1 year time points ($p = 0.037$).

In addition, the groups had statistically significant differences on maximum change in gal-1-p ($p = 0.008$) and maximum change in urine galactitol ($p = 0.047$). The groups had no statistically significant differences on GALT enzyme values ($p = 0.38$).

At study visit

All DG subjects in both groups were on a regular diet at the time of the study visit. The amount of galactose consumed by each individual is shown in Table 2. The average galactose intake for patients on a lactose restricted diet in the first year of life (group-I) ($n = 17$) was 11,996 mg galactose/day. The average galactose intake for patients who were unrestricted in the first year of life (group-II) ($n = 11$) was 18,067 mg galactose/day. There was a statistically significant difference in galactose intake between the two groups ($p = 0.019$). There was a marginal relationship between galactose intake and age ($r = -0.32$, $p = 0.098$, $n = 28$), not statistically significant.

There was no relationship between galactose intake and study visit's gal-1-p (Pearson $r = 0.45$, $p = 0.015$, $n = 28$, using the log-transform of gal-1-p). There was no significant relationship between galactose intake and urine galactitol levels (Spearman $r = 0.20$, $p = 0.30$, $n = 28$).

There were no statistically significant relationships between galactose intake and adaptive (Pearson $r = 0.17$, $p = 0.38$, $n = 28$), language ($r = -0.24$, $p = 0.22$, $n = 28$), and full-scale IQ scores ($r = -0.12$, $p = 0.53$, $n = 28$).

The mean values of RBC gal-1-p were 0.16 ± 0.21 and 0.41 ± 0.71 in group-I and -II, respectively (Table 2). The mean values for urine galactitol were 33.94 ± 72.70 and 32.36 ± 45.59 in group-I and -II, respectively (Table 2). There was no statistical difference of RBC gal-1-p ($p = 0.44$) or urine galactitol ($p = 0.71$) in group-I and -II.

Comprehensive blood metabolic panel, coagulation studies, and FSH levels

None of the DG subjects had abnormal liver function at the time of diagnosis or the study visit. They all had normal PT and PTT values.

The FSH levels ranged between 1.2 and 6.2 mIU/mL in female subjects (reference range 0–8) at the time of study visit (Table 2). There was no statistically significant difference in FSH levels between group-I and -II subjects ($p = 0.84$).

Ophthalmologic exam

Complete eye exam including a slit lamp exam for cataracts did not show any abnormalities in group-I and -II patients at the time of study visit.

Neurodevelopmental tests

The distributions of the three outcome variables (adaptive, language, and full-scale IQ) were found to be normal.

The values of adaptive, language and full IQ tests for group-I and -II subjects are shown in Table 2.

The groups had statistically significant differences on adaptive scores ($p = 0.027$), but not on language ($p = 0.99$) or overall IQ scores ($p = 0.16$).

There were no statistically significant correlations between maximum change in first year metabolic levels (RBC gal-1-p and urine galactitol) and developmental outcomes. Similarly, there were no correlations between metabolic levels (RBC gal-1-p and urine galactitol) at the study visit and developmental outcomes.

Discussions

Although the biochemical profile of children with DG galactosemia suggests a metabolic problem, very little clinical evidence supports the value of galactose restriction. There have been no formal studies of older DG subjects, but anecdotal assessment of known older DG subjects suggests that they are normal and that the condition is benign [10]. The decision to treat with lactose

Table 1
RBC gal-1-p and urine galactitol levels in subjects with the D/G genotype in the first year of life

Subject	GALT activity*	Genotype	Gal-1-p mg/dL** 2 weeks	Gal-1-p mg/dL** 1 month	Gal-1-p mg/dL** 3 months	Gal-1-p mg/dL** 4 months	Gal-1-p mg/dL** 6 monhts	Gal-1-p mg/dL** 1 year	Urine galactitol mmol/mol Cr*** 2 weeks	Urine galactitol mmol/mol Cr*** 6 months	Urine galactitol mmol/mol Cr*** 1 year
Group-I: lactose restricted diet											
1	3.99	Q188R/ N314D	2.31	0.08			0.02	0.03	74		65
2	4.74	Q188R/ N314D	16.04	0.00			0.03	0.01	49		25
3	3.85	K285N/ N314D	10.30	0.09			0.01	0.01	124		2
4	4.2	Q188R/ N314D	0.99	0.05			0.04	0.02	60		2
5	4.9	Q188R/ N314D	0.23	0.10			0.02	0.04	2		2
6	3.89	Q188R/ N314D	29.20	0.28			0.06	0.09	110		134
7	4.65	Q188R/ N314D	26.50	0.06			0.10	0.19	114		74
8	5.27	Q188R/ N314D	0.76	0.06			0.10	0.93	2		26
9	4.29	Q188R/ N314D	1.79	0.01			0.06	0.02	2		2
10	4.41	Q188R/ N314D	0.45	0.30			0.20	0.38	38		38
11	7.9	Q188R/ N314D	0.01	0.01			0.05	0.20	2		2
12	4.28	N314D/ deletion	24.09	0.06			0.04	0.08	129		14
13	4.2	Q188R/ N314D	0.41	0.25			0.02	0.00	2		2
14	4.47	Q188R/ N314D	0.01	0.01			0.04	0.00	4		29
15	7	Q188R/ N314D	10.00	0.11			0.02	0.07	77		26
16	4.5	Q188R/ N314D	28.13	0.06			0.10	0.29	112		16
17	3.9	Q188R/ N314D	0.71	0.05			0.10	0.64	10		58
Mean ± SD	4.73 ± 1.10		8.93 ± 11.30	0.09 ± 0.02			0.05 ± 0.04	0.17 ± 0.25	53.59 ± 49.89		30.41 ± 35.38
Group-II: lactose containing diet											
1	3.69	Q188R/ N314D	21.82	18.06	10.06	6.60	4.99	1.32	58	44	53
2	3.89	Q188R/ N314D	15.87	14.89	5.97	4.96	2.35	0.06	92	120	46
3	6.42	Q188R/ N314D	33.80	30.06	8.63	6.20	2.35	0.50	172	72	32
4	4.18	Q188R/ N314D	14.67	15.33	7.34	6.56	3.38	1.60	133	81	36
5	5.64	S135L/ N314D	21.40	13.95	9.82	6.90	2.93	0.38	120	78	56
6	4.27	Q188R/ N314D	17.23	12.94	8.99	5.99	2.94	1.30	82	46	51
7	4.01	Q188R/ N314D	26.41	14.23	9.20	5.20	2.26	0.26	137	78	36
8	3.17	Q188R/ N314D	29.18	18.90	10.16	6.90	5.17	0.46	110	78	71
9	4.78	Q188R/ N314D	19.36	17.80	9.96	6.20	4.80	0.53	54	55	62
10	3.99	Q188R/ N314D	26.24	17.10	11.01	5.54	4.50	0.27	111	48	44
11	4.67	Q188R/ N314D	21.63	16.80	9.11	5.20	4.70	0.04	94	99	23
Mean ± SD	4.42 ± 0.92		22.5 ± 5.88	17.27 ± 4.64	9.11 ± 1.41	6.08 ± 0.70	3.67 ± 1.16	0.61 ± 0.54	105.73 ± 34.93	72.64 ± 23.54	46.36 ± 14.08

* GALT enzyme activity in μmol UDP galactose per hour per gram of hemoglobin. The normal range is 15.9–26.4.
** 1 mg/dL = 38.4 μmol/L.
*** Cr indicates creatinine.

restriction for only 1 year has been based on (a) higher than normal level of gal-1-p in the newborn, and (b) the assumption that impaired galactose metabolism reflected by the elevated gal-1-p would correct itself with time.

There is no uniformly accepted standard of treatment for patients with DG in the United States. While some clinics suggest 1 year of lactose free diet, others do not put any restrictions on lactose. Gitzelmann and Bosshard [11] advocated adopting a middle ground with lactose restricted diet for 4 months in subjects where the initial RBC gal-1-p was greater than 10 mg/dL. After 4 months, a lactose containing diet (here called "regular diet") was initiated. If the gal-1-p was less than 2 mg/dL on this diet, it was maintained.

With such a wide range of treatment practices, it is important to verify that children with DG do not have inherent differences in

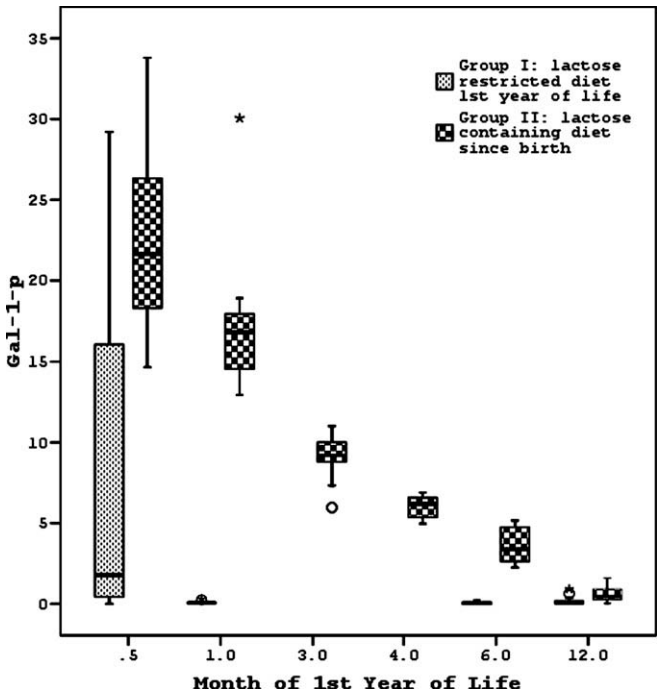

Fig. 1. RBC gal-1-p levels in DG galactosemics on lactose restricted (group-I) and lactose containing (group-II) diets in the first year of life. The box represents the interquartile range which contains 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. Outliers (o) are defined as cases with values between 1.5 and 3 box lengths from either end of the box. Extreme outliers (∗) are cases with values greater than 3 box lengths from either end of the box.

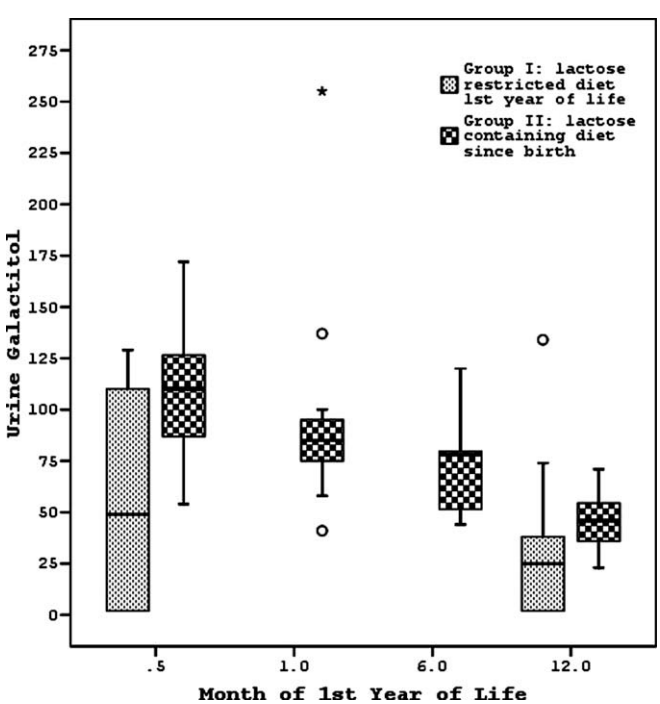

Fig. 2. Urine galactitol levels in DG galactosemics on lactose restricted (group-I) and lactose containing (group-II) diets in the first year of life. The box represents the interquartile range which contains 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. Outliers (o) are defined as cases with values between 1.5 and 3 box lengths from either end of the box. Extreme outliers (∗) are cases with values greater than 3 box lengths from either end of the box.

clinical and developmental outcomes that could ultimately be attributed to lack of diet restriction. In this study, we examined the biochemical and developmental outcomes and nutritional status of DG children, and compared the outcomes in treated and untreated children hoping to shed light on whether such children benefit from lactose restricted diet after diagnosis.

Biochemical outcomes

We showed that DG newborns on regular diet had significantly higher levels of RBC gal-1-P, and urine galactitol than those of DG newborns on diet treatment. Very high levels of RBC gal-1-P gradually decreased without any dietary interventions during the first year of life. At 1 year of age, untreated DG galactosemics had near normal levels of RBC gal-1-P and urine galactitol.

DG children on a lactose restricted diet in the first year of life had normal RBC gal-1-p and urine galactitol levels during infancy. DG children, who had started a lactose restricted diet before the confirmatory tests were done, experienced only slightly higher levels of RBC gal-1-p and urine galactitol. However, all children in group-I, including those who had been on a regular diet for one or two weeks before starting a lactose restricted diet, had normal levels of RBC gal-1-p and urine galactitol levels by 1 month of age. This suggests that these children responded very quickly to diet treatment compared to patients with classical galactosemia whose RBC gal-1-p and urine galactitol levels remain higher than normal regardless of diet. At 1 year of age group-I DG galactosemics were started on a regular diet and their RBC gal-1-p and urine galactitol levels were found in the normal range while on a regular diet. This suggests that by age one both group-I and -II DG galactosemics were able to metabolize galactose more efficiently.

As our data show, group-II-DG children (those on regular diet) have elevated galactose metabolites especially during the first 6 months of life (ranging from 22.5 ± 5.8 at 2 weeks to 3.67 ± 1.16 at 6 months) but none developed any symptoms or signs that are associated with classical galactosemia. In classical galactosemia, the levels of RBC gal-1-p are usually above 80 mg/dL at the diagnosis and, if untreated, are accompanied by severe and sometimes irreversible damage including liver failure, mental retardation, and sometimes even death. For years, it has been a puzzle why patients with classical galactosemia develop long term complications such as learning disability, low IQ, verbal apraxia, difficulties in word retrieval, mathematics, handwriting and reading even when they are started on a lactose restricted diet immediately after birth [12–15]. While untreated DG children and classical galactosemics both have elevated levels of gal-1-p during the first year of life, it is not known why their long term outcomes are so different.

Our data indicate that RBC gal-1-p levels of 30–40 mg/dL measured during early infancy in DG children may not be as toxic as previously thought. This suggests that RBC gal-1-p levels alone are not sensitive indicators of metabolic dysfunction and cannot explain why classical galactosemics develop such severe sequelae. Classical galactosemia still remains an enigmatic disease.

Developmental outcomes

The developmental tests indicate that all DG children (group-I and -II) function within the normal IQ range. There were no significant differences in language and IQ scores between all DG children (group -I and -II) at the time they were tested (ranging from 1 to 6 years). This suggests that the initial difference in RBC gal-1-p levels not only disappears by age one but also has no impact on childhood development.

Adaptive scores in untreated DG galactosemics were significantly higher than those treated in the first year. The difference in adaptive but not IQ/developmental or language functioning in

Table 2
Metabolic, developmental, FSH values and galactose intake in subjects with the D/G genotype at study visit

Subject	Age	Gal-1-p mg/dL*	Urine galactitol mmol/mol Cr**	FSH***	Total galactose intake/day	Adaptive scores	Language scores	IQ scores
Group-I: lactose restricted diet								
1	3.0	0.038	21	6.2	13173	98	88	101
2	2.5	0.046	25	3.3	18033	100	124	110
3	2.3	0.221	56	1.7	15010	108	129	110
4	3.9	0.036	12	2.2	10599	101	118	120
5	6.0	0.097	11		897	110	118	101
6	3.5	0.275	311		17453	106	100	101
7	3.1	0.135	41		13693	98	116	109
8	4.3	0.041	11		23967	111	100	117
9	4.0	0.250	15		7719	114	128	134
10	3.5	0.151	14	2.2	8935	85	104	105
11	1.3	0.541	11	2.7	20666	124	83	105
12	3.5	0.034	12		1797	97	94	101
13	4.0	0.010	12		7996	87	110	113
14	2.0	0.810	0		18750	74	103	105
15	3.5	0.07	0		7838	81	121	110
16	5.5	0	16		8115	89	116	105
17	4.0	0.020	9		9295	119	108	93
Mean ± SD	3.5 ± 1.16	0.16 ± 0.21	33.94 ± 72.70	2.96 ± 1.49	11996.24 ± 6403.76	100.12 ± 13.74	109.41 ± 13.58	108.24 ± 9.3
Group-II: lactose containing diet								
1	5	0.389	123	6.2	25157	106	118	105
2	1.3	2.510	106	3.2	18484	137	121	105
3	2.5	0.436	67		19000	127	86	105
4	2.1	0.010	0		3750	118	112	95
5	1.6	0.48	0	2.6	24079	97	94	105
6	1.0	0.234	0		23511	108	97	90
7	1.0	0.142	13		10504	105	94	115
8	4.0	0.340	33		25000	100	96	100
9	3.0	0.076	0	1.2	15357	94	125	81
10	2.5	0.000	0	2.6	10899	126	136	122
11	1.1	0.000	14		23000	122	124	105
Mean ± SD	2.28 ± 1.3	0.41 ± 0.71	32.36 ± 45.59	3.16 ± 1.85	18067 ± 7144.19	112.41 ± 14.03	109.36 ± 16.53	102.55 ± 11.17

* 1 mg/dL = 38.4 μmol/L.
** Cr indicates creatinine.
*** Only female patients have FSH levels.

these groups may reflect the influence of other factors (e.g. attention, impulsivity) that affect real-life functioning but cannot be assessed through standardized testing until a child is older. This difference may also reflect parent bias in reporting adaptive functioning based on their knowledge of dietary treatment.

Nutritional status

We estimate that the average galactose intake for a typical American toddler is at least 15,000 mg per day [16]. Study patients who followed a lactose restricted diet in their first year consumed less galactose than the average American toddler.

How can we explain why DG galactosemic children who had been on a lactose restricted diet during their first year of life continue to have markedly lower levels of galactose consumption when they were no longer on a restricted diet? We hypothesize that the explanation is behavioral. It is possible that parents of DG galactosemics treated with a lactose restricted diet continue to monitor and limit the galactose intake of their children in the belief that normal galactose consumption may harm their children. The very process of labeling children as having DG galactosemia may shape parents' long term perceptions of their child's health and optimum diet even as our data suggest that there are no long term benefits of restricted diet even during the first year of life.

At the time of the study visit, some DG galactosemics had slightly higher levels of RBC gal-1p than normal reference range but there was no statistically significant difference between two groups and no significant correlation was found between galactose intake and RBC gal-1-p levels. Since DG galactosemics have decreased GALT enzyme activity, it is possible levels of RBC gal-1-p would be higher if they consume too much galactose. Nevertheless, Schwarz et al. performed galactose loading tests (1 g of galactose per kg) on a group of DG children and showed that all had an abnormal rise in gal-1-p and increased excretion of urinary galactitol [17]. They concluded that DG infants and children continue to have impaired galactose metabolism and they stated from their studies "it seems possible, therefore, that normally fed Duarte/galactosemic variant infants may have elevated galactose-1-phosphate levels over much of their first months of life."

Clinical outcomes

None of the DG galactosemics had abnormal liver function tests at birth or at the time of the study visit. All female patients had a normal FSH and there was no difference between treated and untreated subjects. None of the DG children had cataracts at the time of the study. There is concern that DG galactosemics may develop early cataracts but no evidence supports this. In 2003, we reported a 38 year old DG galactosemic female who developed bilateral presenile cataracts but this could be coincidental since the frequency of DG genotype is common in the population [18].

Karas et al. reported an association between presenile cataracts and decreased GALT enzyme activity in individuals who are carrier for K285N mutation [19]. No reports show that presenile cataracts are common in DG galactosemics.

In conclusion, our results indicate that the outcomes for DG galactosemics are good. Lactose restriction in the first year of life does not appear to make any difference in DG galactosemics' bio-

chemical, clinical, and developmental outcomes up to 5 years. This suggests (1) the need for future studies in older DG individuals to assess their cognitive and behavioral skills, for which current tests in young children could not assess subtle differences, and (2) The need to consider adjusting the cutoff levels of galactose and GALT enzyme in order not to detect newborns with DG galactosemia in newborn screening programs. A future study would determine the appropriate cutoff levels so as to ensure the efficacy of newborn screening in detecting classical galactosemia.

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References

[1] J.B. Holton, J.H. Walter, L.A. Tyfield, Galactosemia, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, eight ed., McGraw-Hill, New York, 2001, pp. 1553–1588.
[2] S. Greber-Platzer, P. Guldberg, S. Scheibenreiter, C. Item, E. Schuller, N. Patel, W. Strobl, Molecular heterogeneity of classical and Duarte galactosemia: mutation analysis by denaturing gradient gel electrophoresis, Hum. Mutat. 10 (1997) 49–57.
[3] L.J. Elsas II, K. Lai, The molecular biology of galactosemia, Genet. Med. 1 (1998) 40–48.
[4] F.R. Calderon, A.R. Phansalkar, D.K. Crockett, M. Miller, R. Mao, Mutation database for the galactose-1-phosphate uridyltransferase (GALT) gene, Hum. Mutat. 28 (2007) 939–943.
[5] S.D. Langley, K. Lai, P.P. Dembure, L.N. Hjelm, L.J. Elsas, Molecular basis for Duarte and Los Angeles variant galactosemia, Am. J. Hum. Genet. 60 (1997) 366–372.
[6] C. Ficicioglu, C. Yager, S. Segal, Galactitol and galactonate in red blood cells of children with the Duarte/galactosemia genotype, Mol. Genet. Metab. 84 (2) (2005) 152–159.
[7] J. Chen, C. Yager, R. Reynolds, M. Palmieri, S. Segal, Erythrocyte galactose-1-phosphate quantified by isotope-dilution gas chromatography-mass spectrometry, Clin. Chem. 48 (2002) 604–612.
[8] M. Palmieri, A. Mazur, G.T. Berry, C. Ning, S. Wehrli, C. Yager, R. Reynolds, R. Singh, K. Muralidharan, S. Langley, L. Elsas, S. Segal, Urine and plasma galactitol in patients with galactose-1-phosphate uridyltransferase deficiency galactosemia, Metabolism 48 (1999) 1294–1302.
[9] S.L. Zeger, K.Y. Liang, P.S. Albert, Models for longitudinal data: a generalized estimating equation approach, Biometrics 44 (1988) 1049–1060.
[10] H.L. Levy, S.J. Sepe, D.S. Walton, V.E. Shih, G. Hammersen, S. Houghton, E. Beutler, Galactose-1-phosphate uridyl transferase deficiency due to Duarte/galactosemia combined variation: clinical and biochemical studies, J. Pediatr. 92 (1978) 390–393.
[11] R. Gitzelmann, N.U. Bosshard, Partial deficiency of galactose-1-phosphate uridyltransferase, Eur. J. Pediatr. 154 (7 Suppl. 2) (1995) S40–S44.
[12] S. Schweitzer, Y. Shin, C. Jakobs, J. Brodehl, Long-term outcome in 134 patients with galactosaemia, Eur. J. Pediatr. 152 (1) (1993) 36–43.
[13] F.R. Kaufman, J.K. Reichardt, W.G. Ng, Y.K. Xu, F.R. Manis, C. McBride-Chang, J.A. Wolff, Correlation of cognitive, neurologic, and ovarian outcome with the Q188R mutation of the galactose-1-phosphate uridyltransferase gene, J. Pediatr. 125 (2) (1994) 225–227.
[14] M.A. Cleary, L.E. Heptinstall, J.E. Wraith, J.H. Walter, Galactosaemia: relationship of IQ to biochemical control and genotype, J. Inherit. Metab. Dis. 18 (2) (1995) 151–152.
[15] S.E. Waisbren, T.R. Norman, R.R. Schnell, H.L. Levy, Speech and language deficits in early-treated children with galactosemia, J. Pediatr. 102 (1983) 75–77.
[16] S. Kranz, P.J. Lin, D.A. Wagstaff, Children's dairy intake in the United States: too little too fat?, J Pediatr. 151 (6) (2007) 642–646.
[17] H.P. Schwarz, K.A. Zuppinger, A. Zimmerman, H. Dauwalder, R. Scherz, D.M. Bier, Galactose intolerance in individuals with double heterozygosity for Duarte variant and galactosemia, J. Pediatr. 100 (1982) 704–709.
[18] C. Ficicioglu, P. Kaplan, S. Segal, G.T. Berry, Bilateral Cataracts in a 38-year-old woman with D/G galactosemia: A Case Report Society for Inherited Metabolic Disorders (2004), Annual meeting, Orlando, Florida March 7–10, 2004.
[19] N. Karas, L. Gobec, V. Pfeifer, B. Mlinar, T. Battelino, J. Lukac-Bajalo, Mutations in galactose-1-phosphate uridyltransferase gene in patients with idiopathic presenile cataract, J. Inherit. Metab. Dis. 26 (7) (2003) 699–704.