ARTICLE

Subfertility and growth restriction in a new galactose-1 phosphate uridylyltransferase (GALT) - deficient mouse model

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The first *GalT* gene knockout (KO) mouse model for Classic Galactosemia (OMIM 230400) accumulated some galactose and its metabolites upon galactose challenge, but was seemingly fertile and symptom free. Here we constructed a new *GalT* gene-trapped mouse model by injecting *GalT* gene-trapped mouse embryonic stem cells into blastocysts, which were later implanted into *pseudo*-pregnant females. High percentage *GalT* gene-trapped chimera obtained were used to generate heterozygous and subsequently, homozygous *GalT* gene-trapped mice. Biochemical assays confirmed total absence of galactose-1 phosphate uridylyltransferase (GALT) activity in the homozygotes. Although the homozygous *GalT* gene-trapped females could conceive and give birth when fed with normal chow, they had smaller litter size (*P* = 0.02) and longer time-to-pregnancy (*P* = 0.013) than their wild-type littermates. Follicle-stimulating hormone levels of the mutant female mice were not significantly different from the age-matched, wild-type females, but histological examination of the ovaries revealed fewer follicles in the homozygous mutants (*P* = 0.007). Administration of a high-galactose (40% w/w) diet to lactating homozygous *GalT* gene-trapped females led to lethality in over 70% of the homozygous *GalT* gene-trapped pups before weaning. Cerebral edema, abnormal changes in the Purkinje and the outer granular cell layers of the cerebellum, as well as lower blood GSH/GSSG ratio were identified in the galactose-intoxicated pups. Finally, reduced growth was observed in *GalT* gene-trapped pups fed with normal chow and all pups fed with high-galactose (20% w/w) diet. This new mouse model presents several of the complications of Classic Galactosemia and will be useful to investigate pathogenesis and new therapies.

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INTRODUCTION

Classic Galactosemia (OMIM 230400) is an autosomal recessive disorder caused by deficiency of galactose-1 phosphate uridylyl-transferase (GALT, EC 2.7.7.12) activity (Supplementary Figure 1).[1–5] GALT is the second enzyme in the evolutionarily conserved galactose metabolic pathway, and facilitates the simultaneous conversion of uridine diphosphoglucose and galactose-1 phosphate (gal-1P) to uridine diphosphogalactose (UDP-galactose) and glucose-1 phosphate (Supplementary Figure 1).[6] GALT deficiency leads to accumulation of gal-1P, deficiency of UDP-galactose and other metabolic derangements.[7,8] If untreated, Classic Galactosemia can be lethal for the affected newborns.[1,5] Since inclusion of this disease in the newborn screening panel in the US, neonatal mortality has decreased.[9] The mainstay of treatment is the withdrawal of galactose from the diet.[5] Despite early dietary management, chronic complications such as intellectual deficits, ataxia, speech dyspraxia, premature ovarian insufficiency (POI) and decreased bone mineralization occur in many adults.[10–19]

Different cell and animal models have been utilized to understand the pathogenic mechanisms of the acute neonatal lethality and the long-term complications associated with Classic Galactosemia. Early genetic studies in the yeast *Saccharomyces cerevisiae* showed that GALT-deficient mutant yeasts are sensitive to galactose in growth medium; but disruption of galactokinase (GALK) function in these yeasts reversed their galactose sensitivity.[20,21] This seminal work re-affirmed the suspected pathogenic role of gal-1P, product of GALK, in GALT-deficiency, but did not reveal the mechanism of the *in vivo* toxicity of gal-1P leading to speech dyspraxia and POI. The first *GalT* gene-knockout (KO) mouse model for Classic Galactosemia[22–24] had moderate accumulation of galactose and gal-1P upon galactose challenge, but no overt human disease phenotypes.[22–24] The first *dGALT*-deficient *Drosophila melanogaster* model had arrested larval development with a high-galactose diet.[25] Impaired geotaxic response was also seen in these flies despite dietary restriction of galactose.[25] However, there were no reports on reduced fertility in these fruit flies and more importantly, the metamorphic changes in the life cycle of this insect are non-existent in humans, making it difficult to study any potential pre-natal effects of galactose toxicity in human patients.

In this study, we constructed a new *GalT* gene-trapped mouse model. These new *GalT* gene-trapped mice, similar to the previous

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GalT-KO mice, showed some degrees of resistance to galactose toxicity, but expanded characterization revealed subtle phenotypic differences between the mutant mice and their wild-type (ie, normal) littermates, which could shed new insights into the pathophysiology of the disease.

MATERIALS AND METHODS

Targeting GalT gene in murine embryonic stem (ES) cells and construction of the GalT gene-trapped mice

E285B04 gene trap ES cells with GalT gene trap were produced by the German Genetrap Consortium (GGTC) and were obtained under an official Materials Transfer Agreement between GGTC and the University of Utah. The GalT gene was trapped using VelociGene's COMP Definitive Null Allele Design, which deletes the GalT gene by replacing critical exons with a reporter/selection cassette. Gene-trapped ES cells are heterozygous for the null mutation. ES cells were grown, prepared and injected at the University of Utah Transgenic and Gene Targeting Mouse Core. In all, 12–15 ES cells, derived from agouti 129/Ola mice, were injected into the blastocoel of black C57Bl/6J e3.5 day blastocysts to generate agouti and black chimeras. Injected blastocysts were surgically implanted into e2.5 day pseudo-pregnant CBA × C57Bl/6J F1 females and allowed to progress naturally to birth and weaning. High percent chimeric males were crossed to C57Bl/6J females to ascertain germline transmission. All agouti offspring were genotyped to determine the presence of the heterozygous allele. As expected, approximately 50% of the agouti offspring were heterozygous for the gene trap allele (Figure 1a). Heterozygous mice were intercrossed to produce offspring that were homozygous for the mutant allele, hence were deficient in GalT. Expected Mendelian ratios of wild-type, heterozygotes and homozygous null mutants were obtained. All mice are maintained in accordance with Association for Assessment and Accreditation of Laboratory Animal Care guidelines, and protocols approved by the Institutional Animal Care and Use committee are strictly followed at all times.

Molecular genotyping of the GalT gene trap allele

DNA from tail clips was harvested by alkaline hydrolysis and the presence of the GalT gene trap was confirmed using PCR primer Splirev2 (5′-GCCAA ACCTACAGGTGGGGTCTTT-3′) and E285 (5′-CCAAGCTCAGGTCTTC GTCT-3′). Wild-type GalT allele was amplified with PCR primers SU (5′-CGGTGTGCAGGAGAATCATC-3′) and E285 (CCAAGCTCAGGTCTTCG TCT-3′). PCR conditions for both wild-type and gene-trapped alleles were set as follows: (1) initial denaturing step: 94 °C for 2 min; (2) 36 cycles of amplification steps comprising of denaturing at 94 °C for 30 s, annealing at 56 °C for 30 s and elongation at 72 °C for 90 s; and (3) final elongation step at 72 °C for 5 min.

Galactose challenge of mice

Modified RMH with 40% (or 20%) galactose diet was manufactured by Test Diet Inc. (Richmond, IN, USA).

Biochemical studies

Determination of gal-1P was performed using methods described previously.[8] GALT activity was determined according to procedure published by Li et al,[26] which used a direct method with LC-MS/MS detection of the product of the reaction. Waters XBridge BEH amide column (2.1 mm × 100 mm, 3.5 μm) and AB Sciex API 4000 system (AB Sciex IS, Framingham, MA, USA) equipped with Shimadzu LC units was used in this study.

Determination of glutathione levels

Blood GSH and GSSG levels were determined by the 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) assay according to the previous reported method[27] with a minor modification. Briefly, whole blood was kept in cold metaphosphoric acid (5%) buffer after the blood draw. Red blood cells were lysed by vigorous vortexing and then the mixture was centrifuged at $10\,000 \times g$ for 10 min before the supernatant was transferred to new tubes for further assay. The reaction mixture contained 1 mM EDTA, NADPH (0.24 mM), glutathione reductase (0.06 U), DTNB (86 μM) and samples. Yellow 5-thio-2-nitrobenzoic acid formation was monitored at 412 nm. GSSG was measured by masking the GSH in the metaphosphoric acid (5%) buffer with 10 mM 4-vinylpyridine in the presence of 6% triethanolamine and derivatized at 37 °C overnight.

Histological studies

Organs harvested immediately from killed animals were fixed in 10% formalin for at least 48 h before sending off to the histology research core at ARUP Laboratories (Salt Lake City, UT, USA) for tissue processing and slide
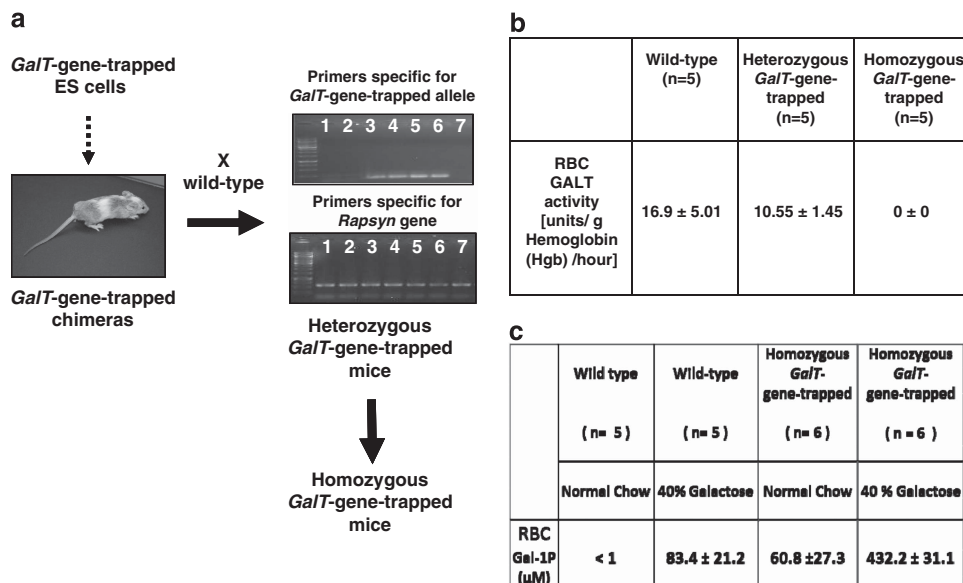

a

GalT-gene-trapped ES cells

↓

GalT-gene-trapped chimeras

X wild-type

Primers specific for GalT-gene-trapped allele
1 2 3 4 5 6 7

Primers specific for Rapsyn gene
1 2 3 4 5 6 7

Heterozygous GalT-gene-trapped mice

↓

Homozygous GalT-gene-trapped mice

b

	Wild-type (n=5)	Heterozygous GalT-gene-trapped (n=5)	Homozygous GalT-gene-trapped (n=5)
RBC GALT activity [units/ g Hemoglobin (Hgb) /hour]	16.9 ± 5.01	10.55 ± 1.45	0 ± 0

c

	Wild type (n= 5)	Wild-type (n= 5)	Homozygous GalT-gene-trapped (n = 6)	Homozygous GalT-gene-trapped (n = 6)
	Normal Chow	40% Galactose	Normal Chow	40 % Galactose
RBC Gal-1P (μM)	< 1	83.4 ± 21.2	60.8 ±27.3	432.2 ± 31.1

Figure 1 Construction and biochemical characterization of a new GalT gene-trapped mouse model. (**a**) Schematic of GalT gene-trapped mouse model construction and molecular genotyping of the GalT gene trap allele. (**b**) Red blood cell GALT activity was measured in 10-day-old mice with various GALT genotypes by a LC-MS/MS method described by Li et al.[26] (**c**) Gal-1P contents in RBC lysate of mice fed with normal chow/high-galactose diet were measured by enzymatic method previously described.[8]

preparations. H&E-stained sections prepared were sent off to board-certified pathologists at University of Utah and University of Florida, respectively, for analysis in a single-blinded manner at which the pathologists were unaware of the genotypes of the mice until the results were reported. For estimation of the number of ovarian follicles, at least three slide sections (50 μM apart) were prepared from each ovary from each female mouse.

Statistical analysis
If required and appropriate, Student's T-test (two-tailed) was performed to evaluate whether differences between two groups are significant.

Hematocrit determination
Volumes of red blood cells were determined from 40 to 45 μl of blood drawn from WT and homozygous $GalT$ gene-trapped females at different time points after galactose (40% w/w) challenge. Values obtained were expressed as percentages of the total blood volumes in the respective samples.

RESULTS
In this study, we developed a new $GalT$ gene-trapped mouse model to test the in $vivo$ efficacy of a novel therapy aimed to reduce the accumulation of toxic level of gal-1P in Classic Galactosemia.

Molecular genotypes of the new $GalT$ gene-trapped mouse models
Using PCR-based methods, we confirmed the presence of the $GalT$ gene trap alleles in mice, which are either heterozygous or homozygous for the allele (Figure 1a).

Biochemical phenotypes of the new $GalT$ gene-trapped mouse model
Using the LC-MS/MS procedure published by Li et al,[26] we confirmed the total absence of GALT activity in the homozygous $GalT$ gene-trapped mice (Figure 1b). Also, the homozygous $GalT$ gene-trapped mice accumulated up to 60.8 ± 27.3 μM of gal-1P in RBC even when they were unchallenged (ie, fed with normal chow; Figure 1c). However, when these animals were fed with high-galactose (40% w/w) diet (Test Diet Inc.), their blood gal-1P increased only to 432 ± 31 μM, which was lower than that reported in the untreated/undiagnosed human newborns.[5]

Galactose challenge in $GalT$ gene-trapped lactating females led to lethality in newborn $GalT$ gene-trapped mice
Newborns with Classic Galactosemia usually die shortly after birth if they receive normal human milk. Mouse milk contains 66% less lactose than human milk,[28] but the previous $GalT$-KO pups were never challenged with excess galactose before they were weaned to mimic the high galactose load of human milk.[22] Therefore, those mice could have survived because of the minimal challenge received. It is technically difficult and invasive to force feed the newborn pups with (mouse) milk containing added galactose, but it is reasonable to assume that the excess dietary galactose, which cannot be properly metabolized by nursing homozygous $GalT$ gene-trapped mothers, be passed to their newborn pups through milk.[29–33] Therefore, we fed the lactating homozygous $GalT$ gene-trapped mothers with high-galactose (40% w/w) diets as soon as the homozygous $GalT$ gene-trapped pups were born. Over 70% of the newborn pups of the galactose-challenged mothers died before weaning (row 3, Table 1). Such high levels of lethality were not seen when wild-type mothers were fed with excess dietary galactose (row 5, Table 1). Neither did we observe the same extent of lethality resulting from heterozygous pups nursed by homozygous $GalT$ gene-trapped mothers fed with high galactose (row 4, Table 1). Biochemical analysis of livers harvested from the succumbed $GalT$ gene-trapped newborns confirmed the presence of large amounts of gal-1P ($\sim$20 μg gal-1P per mg liver cell protein), and histological examination excluded signs of starvation in the liver, such as accumulation of periportal fat. Thus, our observations confirmed that GALT deficiency in both the mother and the newborns was necessary to observe the acute lethal phenotype induced by galactose challenge of the mother.

Galactose challenge in $GalT$ gene-trapped lactating females affects the brains of the newborn $GalT$ gene-trapped mice
Histological examination of the succumbed pups reveal mild inflammatory responses in the livers (data not shown), but there were no overt signs of sepsis or bleeding disorder. Yet, the brains of the intoxicated pups showed increased inflammatory response in the cerebrum (Figure 2a). The Purkinje cells appeared smaller, more acidophilic and the cell layer seemed to be punctate (Figures 2b and c). The outer granular cell layer of the cerebellum was also thicker in the intoxicated animals (Figure 2d).

Galactose challenge in $GalT$ gene-trapped lactating females causes oxidative stress in newborn $GalT$ gene-trapped mice
A recent study using $Drosophila$ as an animal model of GALT deficiency suggested that oxidative stress has a significant role in the toxicity syndrome seen in this model.[34] To assess oxidative stress in the galactose-intoxicated pups, we measured the GSH/GSSG ratio in the blood of the animals (Supplementary Figure 2). In the absence of galactose challenge of the mothers, there was no difference in the blood GSH/GSSG ratio between wild-type and homozygous $GalT$ gene-trapped pups. However, the mutant pups exhibited a lower GSH/GSSG ratio when they were challenged via the mothers' milk, suggesting that excess galactose led to manifestation of oxidative stress in these pups.

Growth restriction in $GalT$ gene-trapped mice
If the intoxicated pups survived the toxic insult of galactose, they manifested severe growth restriction later in life, even when normal chow resumed after the weaning period ended (Figures 3a and b).[35] As 40% (w/w) galactose diet induced significant deaths in newborn

Table 1 Acute lethality induced in newborn pups in nursing mothers fed with high-galactose diet *post-partum*

Male parents	Female parents	Galactose-rich diet fed to nursing mothers	# of crosses	# of live pups born	% Alive by weaning (# living/total)
Homozygous $GalT$ gene-trapped	Homozygous $GalT$ gene-trapped	No	3	23	95.7% (22/23)
Homozygous $GalT$ gene-trapped	Homozygous $GalT$ gene-trapped	Yes[a]	9	63	28.6% (18/63)
Wild-type	Homozygous $GalT$ gene-trapped	Yes[a]	2	17	94.11% (16/17)
Wild-type	Wild-type	Yes[a]	3	25	100% (25/25)

[a]High-galactose (40% w/w) diet was fed to the lactating mothers only after the pups were born. Before that, normal chow was fed to all adult parents as they were weaned.
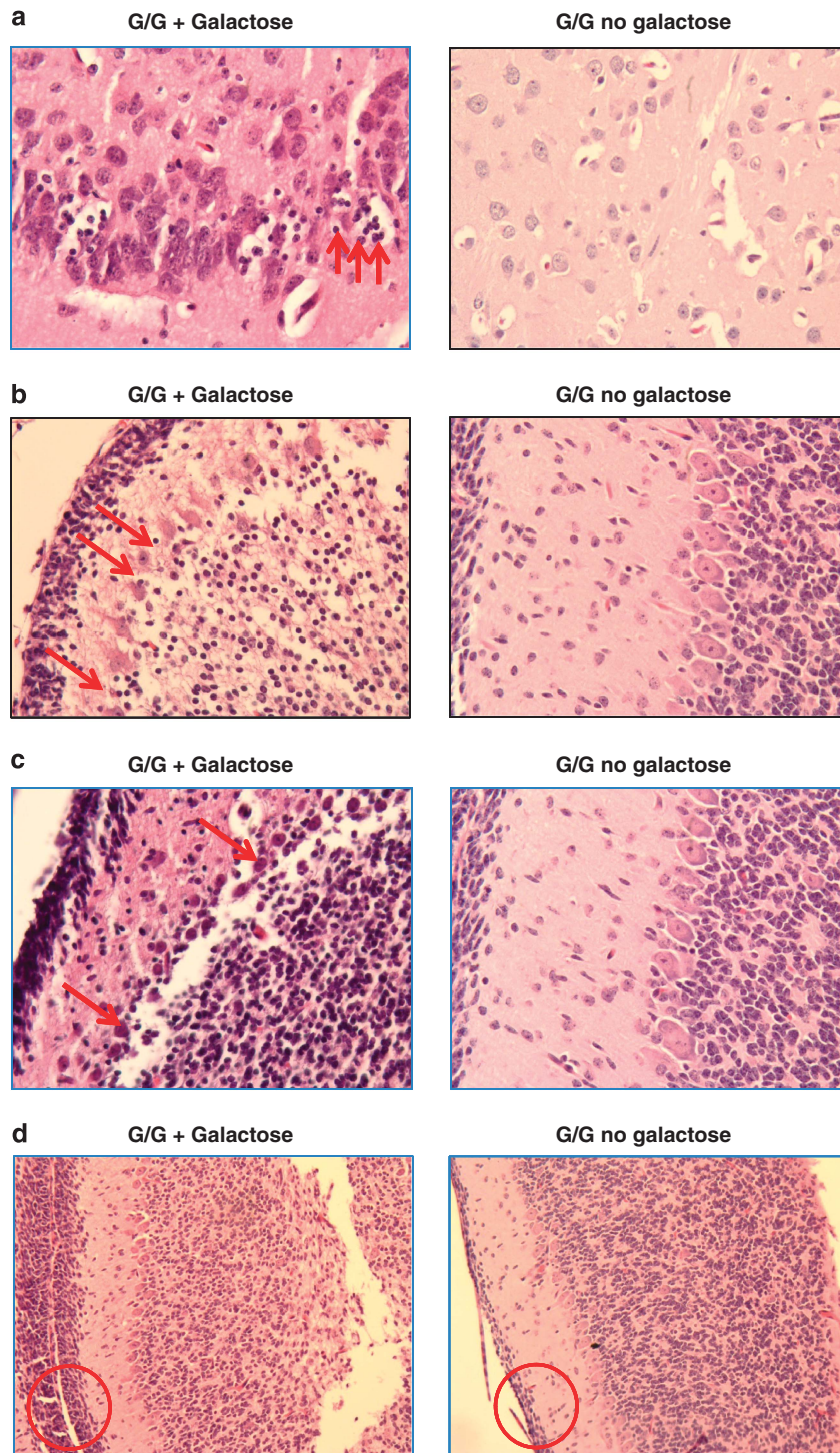
Figure 2 Histological examination of brains of galactose-intoxicated homozygous *GalT* gene-trapped (G/G) mice. Left panels showed the brain sections of the galactose-intoxicated homozygous *GalT* gene-trapped (G/G) mice and the right panel showed the same area of the brain obtained from G/G mice, which were not intoxicated. (**a**) Infiltration of lymphocytes in cerebrum (arrows, left panel; H&E, × 40). (**b**) Smaller Purkinje cells with larger gaps (arrows) were seen in the cerebellum (left panel; H&E, × 40). (**c**) Extensive acidophilic changes in the Purkinje cells (arrows, left panel; H&E, × 40). (**d**) Thicker outer granular cell layers were seen in the cerebellum (circled, left panel; H&E, × 40).

homozygous *GalT* gene-trapped pups, it was difficult for us to study the long-term effect of galactose challenge on the growth of these mice. When galactose levels in the diet of the nursing mothers were reduced to 20% (w/w), the survival of the pups increased to 100%.

Figure 3c shows the growth of normal (wild-type) and homozygous *GalT* gene-trapped mice up to 10 weeks of age. Both groups of mice were exposed to either normal chow or 20% galactose diet. The normal (wild-type) mice attained the higher average growth rate and
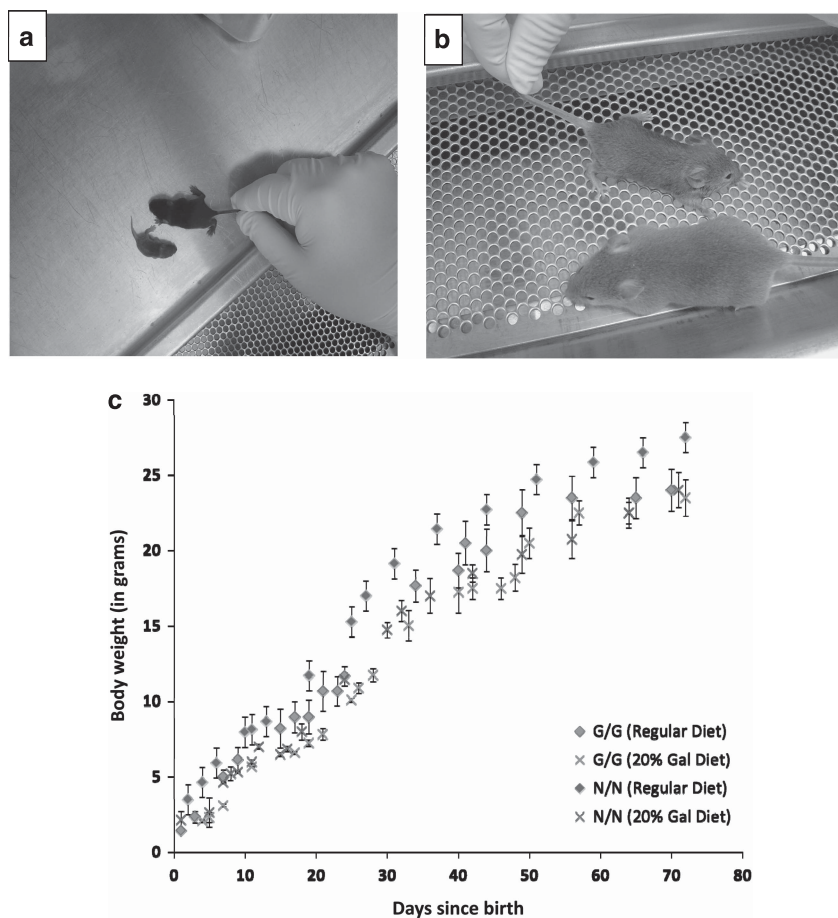
Figure 3 Effect of galactose on growth in mice with different genotypes. (**a**) A 9-day-old (smaller) homozygous *GalT* gene-trapped pup from a galactose-challenged lactating mother posed next to a 9-day-old homozygous *GalT* gene-trapped pup (bigger) whose mother was not fed with a high-galactose diet. (**b**) A 46-day-old homozygous *GalT* gene-trapped mouse (smaller) who survived galactose challenge during the nursing period posed next to an age-matched homozygous *GalT* gene-trapped pup mouse whose mother did not receive any galactose challenge. Galactose challenge of the surviving mouse stopped after day 21, the end of the weaning period. (**c**) Body weights of normal, wild-type (N/N) and homozygous *GalT* gene-trapped (G/G) mice were measured for a period of about 10 weeks since birth. Before weaning, half of the G/G mice were nursed by homozygous *GalT* gene-trapped mothers (red crosses) challenged with 20% galactose-diet and half were nursed by homozygous *GalT* gene-trapped mothers fed with normal chow (red diamonds). After weaning, the pups exposed to 20% galactose were fed with the same diet while the pups exposed to normal chow continued to be fed with normal diet. Similar treatments were rendered for the wild-type (N/N) mice nursed by N/N mothers. Each data points represented the average of six mice and the error bars represented ±1 SD.

maximum weight when compared with their homozygous *GalT* gene-trapped counterparts, even when both groups were unchallenged and fed with regular diets.

Surprisingly, the growth rate and weight of both the wild-type and the homozygous *GalT* gene-trapped mice were reduced when they were exposed to 20% galactose diets since birth. These results show that postnatal exposure to high galactose can induce growth restriction in the homozygous *GalT* gene-trapped mice. In addition, since growth reduction also occurred in the wild-type mice, which were able to utilize galactose, suggesting that growth reduction in the homozygous *GalT* gene-trapped mice cannot solely be explained by their inability to utilize the galactose in the food and thus, received less calories/nutrients.

Although the homozygous *GalT* gene-trapped fed with 20% galactose initially trailed slightly behind the homozygous *GalT* gene-trapped mice fed with normal chow in their rate of growth, they finally caught up at about day 50, which marked the onset of puberty. It appears that these GALT-deficient mice significantly

overcame their growth sensitivity to galactose at this stage of their life cycle. However, such reversal of growth sensitivity was not immediately seen in the wild-type mice fed with 20% galactose.

Reduced fertility in the *GalT* gene-trapped female mice

In sharp contrast to human female patients with Classic Galactosemia who are on a galactose-restricted diet, the female homozygous *GalT* gene-trapped mice appear to be fertile when they were fed with normal chow. However, when we examined these mice over a long period of time, we observed that these mice had significantly smaller litter size ($P = 0.02$; Figure 4a) and significantly longer time-to-pregnancy ($P = 0.013$; Figure 4b) than their wild-type littermates, suggesting diminished reproductive capacity, also known as subfertility in the homozygotes.[36] For instance, in spite of normal chow, the GALT-deficient mice had a maximum of three litters throughout their life (Figure 4b). Levels of follicle-stimulating hormone were not different between age-matched mutant and wild-type females (data

not shown). Yet, histological examination of ovaries dissected from age-matched mutant females fed with normal chow consistently showed fewer numbers of follicles per ovarian section than their wild-type counterparts (Figures 4c and d; $P = 0.0073$), despite follicles at different stages of development were seen. Ovaries of the homozygous *GalT* gene-trapped mice also had more *corpora lutea* of larger size than the ovaries of wild-type females (Figure 4c).

DISCUSSION

Originally constructed for the purpose of assessing the *in vivo* efficacy of novel therapies for Classic Galactosemia, the new *GalT* gene-trapped mouse model described here exhibited phenotypes not reported in the original *GalT*-KO mouse model:[22]

(a) galactose challenge of the homozygous *GalT* gene-trapped newborns led to high mortality rate (Table 1), insults in brain tissues (Figure 2) and altered GSH/GSSG ratio in red blood cells (Supplementary Figure 2);

(b) growth restriction in non-challenged homozygous *GalT* gene-trapped mice (Figure 3); and

(c) reduced fertility in non-challenged homozygous *GalT* gene-trapped females (Figure 4).

How do these findings compare with other cell/animal models of GALT deficiency and patients with Classic Galactosemia? The excess mortality among the intoxicated mouse pups is in-line with the galactose sensitivity phenotype seen in galactose-intoxicated *gal7*-deleted (GALT-deficient) yeast,[37,38] *dGALT-null* (GALT-deficient) fruitflies and human neonates.[25] Of course, it does not necessarily mean that the toxicity mechanisms involved are identical in all cases. At the moment, the cause of death for the intoxicated pups remains unclear, despite the fact that oxidative stress had a pathogenic role in the galactose-induced lethality in the GALT-less *Drosophila* model of Classic Galactosemia.[34] Without further studies involving anti-oxidants supplementation, we cannot be certain whether the degree of oxidative stress experienced by these pups led to the lethal
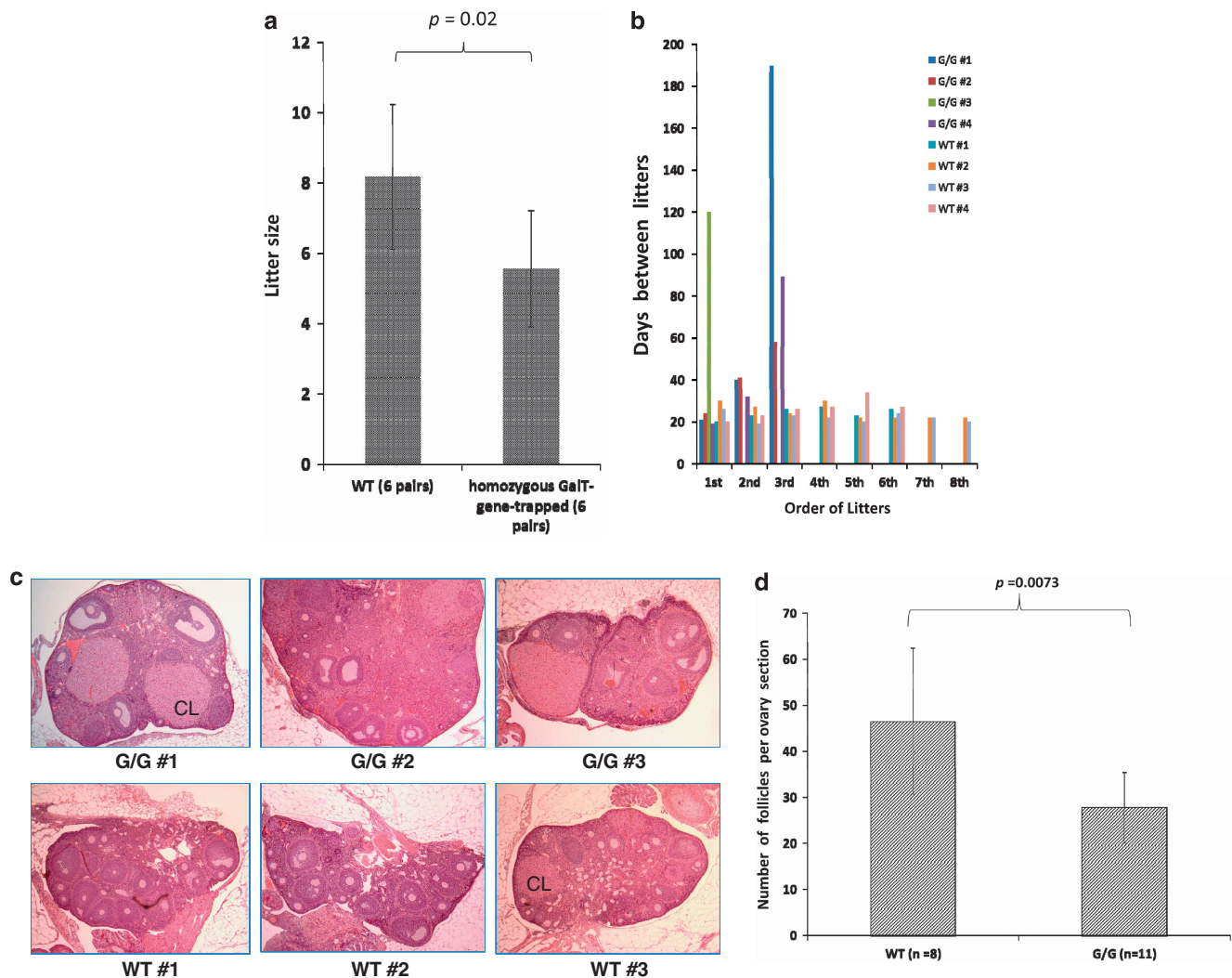

Figure 4 Subfertility phenotypes in unchallenged homozygous *GalT* gene-trapped (G/G) female mice. (a) Average litter size was calculated for females with different genotypes. Error bars represent ±1 SD. (b) Time to pregnancy (TTP) of GALT-deficient (G/G) mice and wild-type mice ($n = 4$ for both) were compared. TTP was counted from the birth date of the previous litter to the birth date of the next litter. G/G #1 did not conceive for >180 days after her third pregnancy and G/G #3 had only one pregnancy throughout the study period. (c) H&E sections of ovaries from three G/G mice of 6 months of age (upper panel) and three age-matched wild-type mice (lower panel) (×5 magnification) (CL, *corpus luteum*). (d) Follicle counts from 8 wild-type and 11 G/G females fed with normal chow.

consequences, nor do we know how excess galactose caused oxidative stress in these pups. Alternatively, high concentration of galactose contained in the dam's milk could also lead to osmotic stress, but histological examination of the tissues of the dead pups did not support this hypothesis (data not shown). Moreover, we had monitored the hematocrit values of galactose-challenged homozygous *GalT* gene-trapped female mice and found that short term (up to 18 days) of galactose challenge did not result in dehydration (ie, increase in hematocrit; Supplementary Figure 3).

As we did not feed the newborn pups directly with galactose, we were concerned if galactose challenge of the lactating females, especially those homozygous for the *GalT* gene-trapped alleles, may render them sick and change their feeding behavior. But similar to what Leslie et al[22] reported, galactose challenge of the adult homozygous *GalT* gene-trapped mice did not have short-term overt adverse effects, nor did we observe any changes in (nursing) behavior that may have contributed to the negligence or starvation of the newborn pups. In fact, we noticed that the stomachs of the succumbed homozygous *GalT* gene-trapped pups were often full of milk (Supplementary Figure 4), emphasizing that it was not the lack of nursing that caused their deaths. The accumulation of high level of galactose metabolites, as well as absence of signs of starvation in the liver tissues of the succumbed pups, was another indication that the pups were well-fed and galactose present in the mother's diet was passed to the pups via milk.

The brain insult seen in the succumbed pups has never been reported among all animal models and could have significant implications in the pathophysiology of the ataxia phenotype seen in some human patients with galactosemia. The changes in Purkinje cells, which occurred only in the galactose-intoxicated pups, also suggested a post-natal role of galactose in the central nervous system pathology.

The non-challenged homozygous *GalT* gene-trapped mice had growth restriction (Figure 3) and reduced fertility (Figure 4), phenotypes that resemble those seen in human patients. Our mouse model thus confirms the importance of GALT activity in ovarian function, independently from other genes or exogenous factors that confounded the previous experimental approaches of experimental hypergalactosemia[39,40] and artificial activation of the prolactin short form receptor in the ovary.[41]

As for the absence of sepsis and bleeding disorder in the intoxicated newborn pups, there is serotype specificity of the *Escherichia coli* involved in human sepsis[42,43] and this specific bacterial species may be absent in the normal flora of the murine species. The lack of bleeding disorder, which was previously thought to be predominantly due to liver failure, may also be explained by inter-species differences in glycosylation between humans and mice.[44–46] Nevertheless, most animal models of human diseases do not recapitulate all aspects of the human pathology. For example, the early mouse model of cystic fibrosis never acquired spontaneous and chronic lung infections, but manifested meconium ileus, alteration of mucous and serous glands, and obstruction of gland-like structures.[47] Using the strategy of conditional KO, Hodges et al[48] finally confirmed the role played by cystic fibrosis transmembrane conductance regulator in lung inflammation in mice.

Despite the above-mentioned differences, there are similarities between the old *GalT*-KO and the new *GalT* gene-trapped mouse models. Female mice were able to conceive and give birth at young age; and the adult mice were relatively resistant to galactose toxicity in both cases. In addition, like the original *GalT*-KO model, the new mouse model accumulated only modest level of gal-1P in red blood cells, even under galactose challenge (Figure 1c). The molecular mechanisms that underlie the lower accumulation of gal-1P in these GALT-deficient mice are unknown, but they may explain the higher galactose resistance in these rodents compared with humans.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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