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Presentation, progression, and predictors of ovarian insufficiency in classic galactosemia

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Summary

Classic galactosemia (CG) is an inherited metabolic disorder that affects about 1/50,000 live births in the United States and many other countries. With the benefit of early detection by newborn screening and rapid dietary restriction of galactose, generally achieved by removing dairy from the diet, most affected infants are spared the acute and potentially lethal symptoms of disease. Despite early detection and life-long dietary intervention, however, most patients grow to experience a constellation of long-term complications that include premature ovarian insufficiency in the vast majority of girls and young women. Our goal in the study reported here was to define the presentation, progression, and predictors of ovarian insufficiency in a cohort of 102 post-pubertal girls and women with CG. To our knowledge this is the largest cohort studied to date. We found that 68% of the girls and women in our study achieved spontaneous menarche while 32% achieved menarche only after starting hormone replacement therapy (HRT). Of those who achieved spontaneous menarche, fewer than 50% were still cycling regularly after 3 years, and fewer than 15% were still cycling regularly after 10 years. Of factors tested for possible association with spontaneous menarche, only detectable (0.04ng/mL) plasma Anti-Müllerian Hormone (AMH)

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Contributions of individual authors:

Allison Frederick and Grace Carlock assembled much of the data for this project, generated most of the figures, and contributed to editing of the manuscript.

Alison Zinsli and Karen Conneely performed all of the statistical analyses for this project and contributed to writing and editing of the manuscript.

Judy Fridovich-Keil designed the project, contributed to data collection, coordinated the activities of the other authors, and wrote most of the manuscript.

Competing interest statement:

Allison Frederick declares that she has no conflict of interest.

Alison Zinsli declares that she has no conflict of interest.

Grace Carlock declares that she has no conflict of interest.

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Ethics approval: The work was conducted with approval of the Emory University Institutional Review Board (Protocol 00024933, PI: JL Fridovich-Keil). Further, all procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Patient consent statement: All participants in this study were consented in accordance with Emory IRB policy.

Animal Rights (IACUC): This article does not contain any studies with animal subjects performed by the any of the authors.

level was significant. These results extend substantially from prior studies and confirm that detectable plasma AMH is a useful predictor of ovarian function in girls and women with CG.

Keywords

Ovarian insufficiency; POI; classic galactosemia; spontaneous menarche; HRT; AMH

Introduction

Primary ovarian insufficiency (POI) is one of the most common long-term complications associated with classic galactosemia (CG), impacting a large majority of girls and women with CG despite early detection (Fridovich-Keil et al 2011; Berry 2014) and careful lifelong dietary restriction of galactose (Frederick et al 2017). Details of the presentation and progression of POI in CG have remained unclear, however, as has clarification of a useful prognostic marker for this outcome (Welling et al 2017).

A literature trail extending back more than 35 years (Thakur et al 2018) documents the striking prevalence of POI in CG. For example, Kaufman and colleagues (Kaufman et al 1981) reported that of 15 girls and women in their study, ages 13–29, 12 (80%) showed signs of POI. Waggoner et al (Waggoner et al 1990) reported that 8 of 34, or almost 24% of women in their study who were at least 17 years old experienced primary amenorrhea, and many more experienced later signs of POI. Schweitzer et al (Schweitzer et al 1993) reported that 5 of 11, or about 45% of girls and women in their study who were >12 years old had primary amenorrhea. Spencer and colleagues (Spencer et al 2013) reported that 11 of 28, or 39% of post-pubertal women in their cohort had achieved menarche only after receiving hormone therapy (HRT). Finally, van Erven and colleagues (van Erven et al 2017) reported that >55% of their cohort of 85 women with CG and current POI had required HRT to achieve menarche. In all of these studies, POI is a strikingly common experience for girls and women with CG. The apparent differences in prevalence reported may reflect the small sample sizes of most of the studies, or differences in study population, ascertainment, age cut-offs, and/or other differences.

The course of longitudinal progression of POI among girls and women with CG who achieve spontaneous menarche has also remained unclear. For example, of the 12 women with abnormal gonadal function in the Kaufman study (Kaufman et al 1981), fewer than half experienced primary amenorrhea, and most developed secondary amenorrhea or oligomenorrhea in their teens or 20s. Of the 26 women who achieved spontaneous menarche in the Waggoner cohort (Waggoner et al 1990), most developed oligomenorrhea or secondary amenorrhea within a few years, and only 5 of 17 were still cycling regularly in their early 20s. The Schweitzer (Schweitzer et al 1993), Spencer (Spencer et al 2013), and van Erven (van Erven et al 2017) studies did not report on longitudinal progression of POI in their cohorts.

Finally, reliable predictors of POI in CG have remained unclear. For many decades follicle stimulating hormone (FSH) was considered the marker of choice (e.g. (Kaufman et al 1981)); however, studies have demonstrated that in young girls who are not yet peri-pubertal

FSH can be unreliable (Sanders et al 2009; Spencer et al 2013). An alternative emerged when Sanders and colleagues (Sanders et al 2009), and then Spencer and colleagues (Spencer et al 2013), reported that plasma Anti-Müllerian hormone (AMH), a glycoprotein produced by ovarian follicles at some but not all stages of development (La Marca and Volpe 2006; La Marca et al 2010) was strikingly deficient in most girls and women with CG. AMH is now a well-established biomarker of "ovarian reserve" among girls and women who do not have galactosemia (Hagen et al 2010; Hansen et al 2011) but how accurately plasma AMH predicts ovarian function among girls and women with CG has remained uncertain. Specifically, Spencer and colleagues (Spencer et al 2013) explored this question five years ago, but with relevant data from only 28 volunteers, they observed a compelling trend but lacked sufficient power to achieve a statistically significant result.

Here we report analyses of the presentation, progression, and markers of ovarian function for 102 post-pubertal girls and women with CG. To our knowledge, this is the largest cohort reported to date. Our results extend substantially from prior studies and provide a foundation of knowledge supporting evidence-based predictions of ovarian function for girls and women with CG.

Materials and Methods

Study volunteers

Cases for this study were selected from among volunteers already enrolled in our longitudinal protocol "Bases of Pathophysiology and Modifiers of Outcome in Galactosemia" (Emory IRB00024933; PI: JL Fridovich-Keil), which has been continuously approved by the Emory Institutional Review Board, or its predecessor the Human Investigations Committee, since 1992. Volunteers for the larger study have been recruited via a combination of self-referral, predominantly from members of the Galactosemia Foundation (www.galactosemia.org), and referral from metabolic clinics, predominantly in North America. Post-pubertal girls and women with CG enrolled in the larger study were selected for inclusion here based on availability of relevant marker and outcome information. Controls were post-pubertal unaffected siblings of cases in the larger study.

Outcomes quantified

Outcomes quantified for the current study included self reported (for adults) or parent reported (for children) information about age at menarche, whether menarche was spontaneous or HRT-assisted, and years of regular menstrual cycles following spontaneous menarche. We also collected information about HRT, where appropriate, including age at HRT initiation and termination, if relevant, and reasons for HRT initiation and termination, if relevant.

Markers and candidate covariates assessed

Markers tested for possible association with spontaneous menarche included Anti-Müllerian Hormone (AMH in ng/mL) and Follicle Stimulating Hormone (FSH in mIU/mL) quantified from plasma as described previously (Sanders et al 2009; Spencer et al 2013). Unless otherwise noted, AMH and FSH values used in this study were derived from blood samples

collected when the study volunteer was between 2–35 years old. Of note, while age does impact the normal range of AMH values (Johansen et al 2013), the lower limit of that range for 2–35 year olds is well above the 0.04 ng/mL threshold of detection used here. Exogenous hormone replacement therapy shows only a small impact on AMH levels (La Marca et al 2013), so AMH values used in this study were included whether or not the donor was on HRT at the time the blood was collected. In contrast, FSH level can be impacted dramatically by HRT so the FSH values included here were restricted to those derived from samples collected when the donor was not receiving HRT. Where we had more than one relevant AMH or FSH value from a given volunteer the appropriate values were averaged.

Covariates tested for potential association with spontaneous versus HRT-assisted menarche included race, year of birth, presence or absence of neonatal symptoms, family income, parent highest education level, and predicted residual GALT activity (Riehman et al 2001).

Statistical analyses

Associations between spontaneous versus HRT-assisted menarche and potential covariates, were tested through univariate logistic regression models. Spontaneous menarche was considered the event of interest. Year of birth was considered as a continuous variable whereas race, presence or absence of neonatal symptoms, family income, parent highest education level, and predicted residual GALT activity were considered as categorical variables. We used a likelihood ratio test to determine significance for all of the covariates; only year of birth was significant ($p < 0.05$). We then performed adjusted logistic regression models to test for association between spontaneous versus HRT-assisted menarche and AMH or FSH, with spontaneous menarche as the outcome of interest and continuous values of AMH or FSH as the independent variables, adjusting for year of birth.

To investigate years of continued spontaneous menstrual cycles beyond puberty, we created a Kaplan-Meier survival curve. The “event” quantified was cessation of spontaneous regular menstrual cycles. All calculations were conducted in SAS 9.4.

Results

Prevalence of spontaneous versus hormone replacement therapy (HRT)-assisted menarche among girls and women with CG

Our cases for this study included 102 post-pubertal girls and women with CG and 22 controls, recruited as described in Materials and Methods. Demographic characteristics of these volunteers are presented in Supplemental Table 1.

Of the cases in this study, 69 (68%) achieved spontaneous menarche and 33 (32%) achieved menarche only after starting HRT. The average age at spontaneous menarche in this cohort was 13.8 years (range 10 to 19), and the average age at HRT-assisted menarche was 14.2 years (range 11 to 18) (Figure 1). As expected, 22 of 22 controls achieved spontaneous menarche at an average age of 12.8 years (range 9–17).

Years of continued spontaneous menstrual cycles beyond puberty for young women with CG

As a simple measure of continued ovarian function beyond puberty, we asked study volunteers who had achieved spontaneous menarche about years of continued regular cycling, without HRT, following puberty; we used these data to create a Kaplan-Meier "survival" curve. As illustrated in Figure 2, only about half of those who achieved spontaneous menarche were still cycling regularly after 3 years; only about 35% were still cycling regularly after 5 years, and fewer than 15% were still cycling regularly after 10 years. Given that only 68% of study volunteers had achieved spontaneous menarche to begin with, this means that by their late teens only about one third of young women with CG in the study were cycling regularly without HRT, and by their mid-20s the number was down to about 10%. Of note, the final sample size of women contributing longitudinal data for this calculation was 48.

Plasma AMH but not FSH associates with spontaneous menarche in CG

We tested both plasma AMH and FSH for possible association with spontaneous menarche in our cohort. AMH was significant ($p=0.0009$) as was AMH converted to a log10 scale for sensitivity at the low end ($p=0.0220$). Of note, from box and whisker plots of the data (Figure 3, **panel A**) it is clear that undetectable AMH was the most common result for volunteers from *both* the HRT-assisted *and* spontaneous menarche cohorts. An undetectable AMH result is therefore not particularly predictive. However, a *detectable* AMH level ($>0.04\text{ng/mL}$) appears to be highly predictive as those scores were clearly skewed toward the spontaneous menarche group. Unlike AMH, FSH did not associate in a meaningful way ($p=0.7871$) with spontaneous versus HRT-assisted menarche in our cohort (Figure 3, **panel B**).

Starting and stopping hormone replacement therapy (HRT)

Of 55 girls and women with CG in the study who reported having taken HRT and who also gave a reason why HRT was initiated, 3 (5.4%) said they started HRT to stimulate growth and development, 27 (49.1%) said they started HRT to initiate or complete puberty, and 25 (45.5%) said they started HRT only after puberty to help manage irregular periods or other symptoms of menopause. The average age at initiation of HRT (Figure 4) for the first group was 12.3 years (range 11–14), for the second group was 12.8 years (range 9–17), and for the final group was 19.6 years (range 15–34). Nine additional women also reported taking HRT but for 2 the reason for initiation was "other" and for 7 no reason was given.

Of the 64 women in the study who reported having ever taken HRT, 58 shared if they had stopped treatment, and if so at what age and why. Of these 58 women, 47 (81%) said they were still on HRT. Of 11 women (19%) who had stopped, the most common reason given was concern about possible health risks or side effects. Other reasons included "unknown," "stopped working," "hysterectomy," and "elected not to use." The average age of stopping HRT was 30.8 years (range 16 – 51). Of volunteers who were still taking HRT the average age was 24.9 years (range 12.8 – 41.2).

Testing covariates for possible association with spontaneous menarche in CG

Finally, we tested each of 6 potential covariates for association with spontaneous menarche. These included presence of acute neonatal symptoms before diagnosis, year of birth, race, and two indicators of family socioeconomic status (family income and parent's highest level of education). Of these, only year of birth was even nominally significant ($p=0.0389$), with girls born earlier slightly more likely to achieve spontaneous menarche than girls born later. Specifically, for each year earlier a girl was born, she was 1.044 times as likely to have achieved spontaneous menarche. We assume this difference might reflect changing attitudes about how long it is appropriate to wait before initiating HRT for a girl with delayed puberty. In other words, some girls born more recently who started HRT at age 13 or 14 might have eventually achieved spontaneous menarche if they had waited.

The last potential covariate tested was residual GALT activity predicted from *GALT* genotype (Riehm et al 2001). Of 21 volunteers who reported HRT-assisted menarche and for whom we could predict GALT activity, only 2, or 9.5%, had at least 0.4% predicted residual GALT activity. In contrast, of 51 volunteers who reported spontaneous menarche, and for whom we could predict GALT activity, 12, or 23.5%, had at least 0.4% predicted residual GALT activity. While this >2-fold difference was notable, it was not statistically significant in our cohort ($p=0.1497$).

Discussion

The work presented here is important for two reasons. First, it both confirms and extends the results of prior, smaller studies documenting the prevalence and progression of POI in CG. Second, and contrary to current recommendation (Welling et al 2017), we found that detectable plasma AMH ($>0.04\text{ng/mL}$) was strongly positively associated with spontaneous menarche ($p=0.0009$), meaning in at least some cases it could be used to inform prediction. Finally, by testing and excluding a handful of possible covariates the results presented here help to rule out factors, such as family socioeconomic status and neonatal acute symptoms, as major contributors to POI in CG. In the search for factors that underlie outcomes, it is also useful to identify those that do not.

Predicting spontaneous menarche

One of the questions asked by families of girls with CG is: What is the chance my daughter will need help with puberty? From the results presented here we can say that with no further information the chance is about 32%. If plasma AMH is undetectable ($<0.04\text{ ng/mL}$), which is the most common result in CG, 32% is still our best estimate. When the AMH is detectable, however, we can do better. For example, when AMH is between 0.04 and 0.99ng/mL ($n=12$ in our cohort), the point estimate from a binomial exact test is 91.7% with a 95% confidence interval of 73.6% to 99.8%. If the AMH is 1 ng/mL or greater ($n=6$ in our cohort), the point estimate is 100% with a 95% confidence interval of 60.7% to 100%.

Of course, that undetectable AMH was the most common result for girls and women in *both* the spontaneous and HRT-assisted menarche groups while detectable AMH was clearly skewed toward spontaneous menarche raises the question: why? The most likely explanation

may be that the threshold of detection of the AMH assay used on our samples was limiting. Specifically, we hypothesize that some of the samples scored here as “undetectable AMH” actually had very low but non-zero levels of AMH that were below the threshold of detection of the assay applied, and the trace ovarian function producing those very low levels was sufficient to enable spontaneous menarche, at least in some girls. As higher sensitivity assays for AMH are developed this will become a testable hypothesis.

Experience with hormone replacement therapy

Our results presented here also give insight into the timing and reasons for HRT initiation and termination among girls and women with CG. For example, as presented in Results, a small number of girls in our study initiated HRT to promote growth, but the vast majority were split almost evenly between needing help beginning or completing puberty, and needing relief from peri-menopausal symptoms. As expected, the average age of girls in the first two groups was younger (12.4 years and 12.7 years, respectively) than of women in the third group (19.6 years). That said, in some cases girls as young as 15 started HRT to help control irregular periods, hot flashes, or other symptoms (Figure 4).

The decision of if and when to stop HRT has been a point of particular uncertainty and concern for many women with CG. Current recommendations (Berry 2014; Welling et al 2017) do not address this point, and indeed of the 65 women in our study cohort who had initiated HRT, the majority reported that treatment was still ongoing. The optimal duration of HRT for women with CG who have experienced POI remains unclear, but recommendations offered for other groups of young women with POI (e.g. women with Turner Syndrome or Fragile X pre-mutation carriers) may apply. In those cases the recommendation (Sullivan et al 2016) is to continue HRT at least until the normal age of menopause (e.g. around 50) unless contraindicated, for example, by a personal or family history of breast cancer.

Limitations of our study

While informative, this study also had a number of serious limitations. For example, our cohort size of 102 post-pubertal girls and women may be large for a rare disorder like CG, but it nonetheless limited our power to draw conclusions. Our cohort was also overwhelmingly north American, white non-Hispanic, well educated, and self-selected for families who chose to participate in a research study. How these results would compare in other geographies and demographic groups is unknown. Finally, because most of our study volunteers had not attempted pregnancy we could not make any meaningful statements about success rates in that regard. Specifically, of 15 women with CG in our cohort who reported attempting pregnancy, 14 had achieved spontaneous menarche, and 9 became pregnant. Of these 9, all of whom had achieved spontaneous menarche, only one received medical intervention before becoming pregnant. That 9/15 women with CG in our cohort who attempted pregnancy did become pregnant is encouraging and consistent with results reported recently by van Erven and colleagues (van Erven et al 2017); however, our sample was small and may not be representative of the larger population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Berry G. Classic Galactosemia and Clinical Variant Galactosemia. In: Pagon R, Adam M, Ardinger H. , et al., editors *GeneReviews*®. Seattle (WA): University of Washington, Seattle; 2014.
- Frederick A, Cutler D, Fridovich-Keil J. Rigor of non-dairy galactose restriction in early childhood, measured by retrospective survey, does not associate with severity of five long-term outcomes quantified in 231 children and adults with classic galactosemia. *J Inherit Metab Dis*. 2017; 40:813–821.
- Fridovich-Keil JL, Gubbels CS, Spencer JB, Sanders RD, Land JA, Rubio-Gozalbo E. Ovarian function in girls and women with GALT-deficiency galactosemia. *J Inherit Metab Dis*. 2011; 34:357–366. [PubMed: 20978943]
- Hagen CP, Aksglaede L, Sorensen K, et al. Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *The Journal of clinical endocrinology and metabolism*. 2010; 95:5003–5010. [PubMed: 20719830]
- Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertility and sterility*. 2011; 95:170–175. [PubMed: 20522327]
- Johansen M, Hagen C, Johannsen T, et al. Anti-Müllerian Hormone and Its Clinical Use in Pediatrics with Special Emphasis on Disorders of Sex Development. *International Journal of Endocrinology*. 2013; 2013:1–10.
- Kaufman FR, Kogut MD, Donnell GN, Goebelsmann U, March C, Koch R. Hypogonadotropic hypogonadism in female patients with galactosemia. *The New England journal of medicine*. 1981; 304:994–998. [PubMed: 6782485]
- La Marca A, Grisendi V, Griesinger G. How Much Does AMH Really Vary in Normal Women? *International Journal of Endocrinology*. 2013; 2013:1–8.
- La Marca A, Sighinolfi G, Radi D, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Human reproduction update*. 2010; 16:113–130. [PubMed: 19793843]
- La Marca A, Volpe A. Anti-Mullerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool? *Clinical endocrinology*. 2006; 64:603–610. [PubMed: 16712660]
- Riehman K, Crews C, Fridovich-Keil JL. Relationship between genotype, activity, and galactose sensitivity in yeast expressing patient alleles of human galactose-1-phosphate uridylyltransferase. *Journal of Biological Chemistry*. 2001; 276:10634–10640. [PubMed: 11152465]
- Sanders RD, Spencer JB, Epstein MP, et al. Biomarkers of ovarian function in girls and women with classic galactosemia. *Fertility and sterility*. 2009; 92:344–351. [PubMed: 18684449]
- Schweitzer S, Shin Y, Jakobs C, Brodehl J. Long-Term Outcome in 134 Patients with Galactosemia. *European journal of pediatrics*. 1993; 152:36–43. [PubMed: 8444204]
- Spencer JB, Badik JR, Ryan EL, et al. Modifiers of ovarian function in girls and women with classic galactosemia. *The Journal of clinical endocrinology and metabolism*. 2013; 98:E1257–1265. [PubMed: 23690308]

- Sullivan S, Sarrel P, Nelson L. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. *Fertility and sterility*. 2016; 106:1588–1599. [PubMed: 27912889]
- Thakur M, Feldman G, Puscheck EE. Primary ovarian insufficiency in classic galactosemia: current understanding and future research opportunities. *Journal of assisted reproduction and genetics*. 2018; 35:3–16. [PubMed: 28932969]
- van Erven B, Berry GT, Cassiman D, et al. Fertility in adult women with classic galactosemia and primary ovarian insufficiency. *Fertility and sterility*. 2017; 108:168–174. [PubMed: 28579413]
- Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. *J Inherit Metab Dis*. 1990; 13:802–818. [PubMed: 1706789]
- Welling L, Bernstein LE, Berry GT, et al. International clinical guideline for the management of classical galactosemia: diagnosis, treatment, and follow-up. *J Inherit Metab Dis*. 2017; 40:171–176. [PubMed: 27858262]

1 sentence take-home message

About 2/3 of girls with classic galactosemia (CG) achieve spontaneous menarche, but few cycle regularly into adulthood. Detectable plasma AMH (0.04ng/mL) is a significant predictor of spontaneous menarche in CG.

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Figure 1. Age at menarche of cases and controls in this study

Age at menarche, in years, was reported for each volunteer as a whole number (e.g.15, not 15.2). The average age at menarche for controls was 12.8 years, for cases with spontaneous menarche was 13.8 years, and for cases with HRT-assisted menarche was 14.2 years.

Figure 2. Years of regular menstrual cycles after spontaneous menarche

About half of young women with CG who achieved spontaneous menarche were still cycling regularly after 3 years; only about 35% were still cycling regularly after 5 years, and fewer than 15% were still cycling regularly after 10 years. In the Kaplan-Meier curve presented, the solid line represents the estimated "survival" function, and the dashed lines present the 95% confidence interval.

Figure 3. Average plasma AMH and FSH levels in girls and women with CG who achieved spontaneous versus HRT-assisted menarche

Plasma AMH 0.04ng/mL (**panel A**) associated strongly with spontaneous menarche ($p=0.0009$) but plasma FSH (**panel B**) did not ($p=0.7871$).

Figure 4. Age at initiation of HRT

The majority of girls and women in our study initiated HRT either to promote or complete puberty, or to manage peri-menopausal symptoms. As expected, we saw a range of ages in both groups, but as a whole those in the first group were pre-teens and teens, and those in the second group were in their older teens or 20s.