Pituitary-Specific Gata2 Knockout: Effects on Gonadotrope and Thyrotrope Function

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GATA2 is expressed in the pituitary during development and in adult gonadotropes and thyrotropes. It is proposed to be important for gonadotrope and thyrotrope cell fate choice and for TSH production. To test this idea, we produced a pituitary-specific knockout of Gata2, designed so that the DNA-binding zinc-finger region is deleted in the presence of a pituitary-specific recombinase transgene. These mice have reduced secretion of gonadotropins basally and in response to castration challenge, although the mice are fertile. GATA2 deficiency also compromises thyrotrope function. Mutants have fewer thyrotrope cells at birth, male Gata2-deficient mice exhibit growth delay from 3–9 wk of age, and adult mutants produce less TSH in response to severe hypothyroidism after radiothyroidectomy. Therefore, Gata2 appears to be dispensable for gonadotrope and thyrotrope cell fate and maintenance, but important for optimal gonadotrope and thyrotrope function. Gata2-deficient mice exhibit elevated levels of Gata3 transcripts in the pituitary gland, suggesting that GATA3 can compensate for GATA2. (Molecular Endocrinology 20: 1366–1377, 2006)

The SIX transcription factors of the GATA-binding family are critical for the development and function of many organ systems. Underscoring their importance is the embryonic lethality exhibited by mice disrupted with any GATA factor except Gata5 [Gene Expression Database (GXD) http://www.informatics.jax.org. Mouse Genome Informatics Web Site, The Jackson Laboratory, Bar Harbor, ME]. The amino acid sequences of mammalian GATA factors reveal two distinct motifs: most contain one or more transactivational domains in the N terminus, and all contain highly conserved zinc finger domains (1). Functional analysis of Gata1 revealed that the C-terminal zinc finger along with an adjacent basic region recognizes and binds the consensus sequence WGATAR, whereas the N-terminal zinc finger stabilizes the DNA interaction as well as binds other proteins (2, 3).

The functional role of Gata2 has been examined extensively in the hematopoietic system, where it is essential for the proliferation and survival of the stem cell pool (4, 5). Gata2 has also been implicated in regulating urogenital development (6), adipocyte differentiation (7), and in differentiated endothelial cell transcription (8–10). The role of Gata2 is context dependent because Gata2 can either lock cells in a precursor or stem state by repressing genes found in differentiated cells (4, 5), or it can activate genes characteristic of differentiated cells and maintain the function of the mature cell (8).

The spatial and temporal expression pattern of Gata2 suggests roles in pituitary gonadotrope and thyrotrope differentiation as well as potential downstream target genes. Both Gata2 mRNA and protein are detectable in the developing and adult anterior pituitary (11, 12), as well as in most gonadotropin or TSH-positive human adenomas (13), and mouse TtT97 thyrotropic tumors (14). Gata2 is transcribed in the developing anterior pituitary as early as embryonic d 10.5 (E10.5) and persists through birth and adulthood in an expression pattern coincident with the glycoprotein hormone subunit-α (known as αGSU or Cga) (12). GATA2 binds and transactivates the αGSU promoter (15) and it acts synergistically with PIT1 to activate transcription of the TSH β-subunit gene (Tshb) (12, 14, 16). Gata2 is genetically downstream of the transcription factor Pitx2, and Pitx2 can directly activate Gata2 transcription in cell culture (11). Gata2 transcription appears to be responsive to ventral bone morphogenetic protein signaling resulting in ventral localization of Gata2 transcripts (12, 17).

Expression studies in transgenic mice support a role for Gata2 in gonadotrope and thyrotrope cell fate. Gata2 overexpression under the control of the Pit1 promoter causes an apparent fate shift between the differentiated hormone producing cell types in trans-

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Abbreviations: CMV, Cytomegalovirus; E, embryonic day; ES, embryonic stem; αGSU, glycoprotein hormone subunit-α; IHC, immunohistochemistry; pitKO, pituitary-specific knockout of Gata2; SF1, steroidogenic factor 1; WT, wild type.

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genic mice (12). Gonadotropes are gained at the expense of the Pit1 lineage (somatotropes, thyrotropes, and lactotropes), indicating that Gata2 can promote gonadotrope specification. Consistent with this idea, Pitb2 ablation causes a loss of Gata2 expression and gonadotrope cell population (11). Similarly, expression of a dominant-negative Gata2 construct, in which the N-terminal transcriptional activation domain is replaced with the engrailed repressor domain causes a loss of gonadotropes and reduction in thyrotropes (12). However, studies of this nature are difficult to interpret, as the overexpressed, dominant-negative form of the protein may not be specific for Gata2, resulting in unpredictable and complicated nonphysiological interactions with other proteins. This is particularly important because Gata2 is almost universally coexpressed with other GATA-binding protein members (18).

Gata2 has been disrupted in mice by classical gene-targeting in embryonic stem (ES) cells (4) creating a null allele (Gata2−/−). Mice homozygous for this null allele (Gata2−/−) die during embryogenesis at E10.5 due to severe anemia (4, 6). Because Gata2 is critical for both primitive hematopoiesis in the yolk sac and for definitive hematopoiesis in the liver, the systemic null allele cannot be used to elucidate the role of Gata2 in the pituitary because the critical period for pituitary development begins at E9, when the mutants are beginning to die.

We used the site-specific recombinase system Cre-loxP to make a pituitary-specific knockout of Gata2 (pitKO) because of the early embryonic lethality of the Gata2−/− mice and the difficulty in interpreting over expression and dominant-negative studies. Deletion of the DNA-binding C-terminal zinc finger of Gata2 in the pituitary allows the role of Gata2 to be assessed without complications from other organ systems. This genetic ablation is efficient and effective at disrupting the function of GATA2. The pitKO mice have decreased thyrotrope cell population at birth and lower levels of circulating TSH and FSH in the serum of adults. Young male pitKO mice are significantly smaller than wild-type (WT) littermates; however, they ultimately reach full adult size and are fertile despite significantly smaller seminal vesicles. Castration and thyroid ablation studies reveal a decreased capacity of mutant gonadotropes and thyrotropes to mount the appropriate and sizeable response to the loss of negative feedback by steroid hormones and thyroid hormones, respectively. Gata3 transcripts are elevated in the pituitaries of Gata2 knockout mice, suggesting that these related genes may have overlapping functions in the pituitary gland.

RESULTS

Construction and Characterization of Novel Gata2 Alleles

Using homologous recombination in ES cells, we modified the Gata2 locus to create an allele that can be used for tissue-specific and inducible gene deletion. The targeting vector contained a neomycin selectable marker flanked by FRT sites (flrted) in the intron upstream of exon five, encoding the C-terminal zinc finger domain, and a pair of loxP sites: one site 5′ of the neomycin cassette and a second site 3′ of the fifth exon (Fig. 1A) that were in the same orientation. The combination of FRT sites and loxP sites allowed us to use a dual recombinase strategy. The targeting vector was electroporated into R1 ES cells and four of 960 ES cell clones underwent the desired homologous recombination event to produce the Gata2tm1Sac allele, referred to here as Gata2tm1, as determined by genomic DNA Southern blot analysis using a probe outside the targeting sequence. Of these four positive ES cell clones, two contributed to the germline of ES cell-mouse chimeras. Gata2tm1 heterozygotes were mated to F1pe recombinase transgenic mice (19) to remove the neomycin cassette and create the floxed allele (Gata2f). The neomycin cassette was removed because its presence can interfere with normal splicing and can create hypomorphic alleles as observed with Fgf8 (20) and Pitx2 (11). Genotypes were determined by PCR (Fig. 1B). Mice heterozygous for the Gata2 targeted mutation (Gata2f/f) or homozygous for the Gata2 floxed allele (Gata2ff) appear normal and are fertile. Each allele is transmitted in the expected Mendelian ratio (data not shown).

By mating mice with the floxed Gata2 allele (Gata2f) to mice carrying a Cre recombinase under the control of the universal cytomegalovirus promoter (CMV-Cre) (21), we obtained a systemic null allele (Gata2−/−). This allele lacks the fifth exon including the C-terminal zinc finger and is expected to produce a nonfunctional protein (2, 3, 22). Three lines of evidence confirmed that removal of the fifth exon produces a true null allele. First, an intercross of Gata2 heterozygous mice (Gata2f/f) produced WT and heterozygous mice in the expected 1:2 Mendelian ratio. No homozygous null pups (Gata2−/−) were obtained, which is significant (P = 000066) and agrees with lethality previously reported for the conventional Gata2 knockout mice (4, 6). Second, all genotypes were found at the expected 1:2:1 Mendelian ratios at E10.5, but Gata2−/− embryos display severe anemia, and their yolk sacs are completely devoid of detectable red blood cells (Fig. 2). By E11.5, all Gata2−/− embryos are undergoing resorption as expected (4, 6). Third, Western blot analysis reveals no evidence of a stable protein of the size expected from the Gata2−− allele, although a protein is produced from this allele in transfected cells (data not shown). Therefore, based on these criteria, a true Gata2 pituitary-specific knockout was generated for further analysis.

Creation of Mice with a Pituitary-Specific Knockout (pitKO) of Gata2

The mouse αGSU (or Cga) promoter drives Cre recombinase expression in the pituitary primordium leading
to efficient activation of Cre reporter gene expression in all five anterior pituitary hormone producing cell types (23). This well-characterized line [TgN(Cga-cre)S3Sac or \( /H9251\) GSU-Cre] efficiently converts a floxed allele of steroidogenic factor 1 (SF1 or \( \text{Nr5a1} \)) to a null allele by E14.5 (24). We crossed this \( /H9251\) GSU-Cre transgene to \( \text{Gata2f}/\text{H11001} \) mice and selected Cre transgenic, \( \text{Gata2f}/\text{H11001} \) mice to cross with \( \text{Gata2f}/\text{f} \) mice. We expected \( \text{Gata2f}/\text{f} \) progeny with the \( /H9251\) GSU-Cre transgene to have a pituitary-specific deletion of \( \text{Gata2} \). We extracted pituitary RNA from these mice and their WT littermates to determine the efficiency of \( \text{Gata2} \) deletion and quantified the amount of WT \( \text{Gata2} \) transcript using RT-PCR. A smaller, stable mutant \( \text{Gata2} \) transcript is detectable in pitKO mice. Sequencing revealed that this transcript is a novel splice variant of \( \text{Gata2} \) that lacks the fifth exon (\( \text{E5} \)) (Fig. 1C). This smaller \( \text{E5} \) transcript retains the final sixth exon in frame with the rest of the coding sequence, but no predicted \( \text{E5} \) \( \text{GATA2} \) protein is detectable in heterozygous or mutant mice by Western blot analysis (data not shown). All pitKO pituitaries contained this \( \text{E5} \) transcript and very low levels of WT \( \text{Gata2} \) transcript. By comparing the amounts of WT and \( \text{E5} \) \( \text{Gata2} \) transcript to known amounts of WT and \( \text{E5} \) cDNA we determined the efficiency of conversion of the \( \text{Gata2} \) WT allele to the \( \text{Gata2} \) null allele at the mRNA level to be between 92 and 98% (Fig. 1C).

Pituitaries of Neonatal \( \text{Gata2} \) pitKO Mice Contain Fewer Thyrotropes and Gonadotropes

To determine whether there is a change in the size of any cell population in the pituitaries of \( \text{Gata2} \) pitKO mice, we used immunohistochemistry (IHC) with antibodies against pituitary hormones. The number of pro-opiomelanocortin- and GH-immunoreactive cells is indistinguishable between mutant and WT pituitaries of newborn pitKO mice relative to their normal littermates (n = 3 per genotype) (Fig. 3). There appeared to be fewer FSH-positive cells in the mutants, although the SF1 immunostaining looked similar in mutant and WT (data not shown). There are clearly fewer TSH-positive cells in the mutants, suggesting that thyrotropes are underrepresented in the \( \text{Gata2} \) pitKO mice. The reduction in TSH staining could be due either to a failure of
cell specification or to a profound decrease in hormone production such that the cells are not detectable by hormone IHC. Interestingly, IHC analysis of adult pituitaries revealed a recovery of the thyrotrope population (Fig. 3).

**TSH Is Reduced in Gata2 pitKO Mice**

Because of the dramatically decreased number of detectable thyrotropes in neonatal pitKO pituitaries, we expected less circulating TSH. We measured circulating TSH levels by collecting serum and performing RIA. As expected, we found decreased TSH levels in male pitKO mice that are 56% the levels in WT littermates \( (P = 1.6 \times 10^{-6}) \) (Fig. 3). This level of TSH is sufficient for normal thyroid histology in adult Gata2-deficient mice. The follicular size and height of the follicular epithelia are indistinguishable in pitKO and WT mice.

**Circulating FSH Is Reduced in Gata2 pitKO Mice**

To determine whether gonadotropes were functioning normally, we measured circulating levels FSH and examined the testes and testosterone sensitive organs. We measured FSH by collecting serum and performing RIA. The circulating level of FSH in Gata2 pitKO mice is 28% of that found in WT littermates \( (P = 1.6 \times 10^{-6}) \) (Fig. 3).

Seminal vesicle weight is a sensitive indicator of testosterone production \( (25, 26) \). Gata2 pitKO males have small seminal vesicles with weights approximately 55% that of their WT littermates \( (P = 0.0053) \) (Fig. 4, A and E). Internal cellular morphology and maturity of the seminal vesicles are unchanged (Fig. 4, B and F). Surprisingly, given the dramatic reduction in seminal vesicle weight, testicular weight is identical in WT and Gata2-deficient mice \( (n = 5 \text{ per genotype}) \). Testicular histology of the Gata2-deficient mice is normal with all cell types readily apparent including mature sperm (Fig. 4, C, G, I, and J). Indeed, male pitKO are fertile. Thus, the reduced level of gonadotropins in mutant mice is sufficient for fertility.

**Male Gata2 pitKO Mice Are Smaller than WT Littermates**

Defects in the production or secretion of either GH or TSH can lead to growth insufficiencies or delays \( (27–29) \). After following the weights of the Gata2 pitKO mice we found that male, but not female, pitKO mice had significant growth delay relative to their WT litter-
mates (Fig. 5). Beginning at 3 wk of age, the male pitKO mice begin to show growth insufficiency. Their weights are intermediate between WT male and female littermates (90% the weight of WT male littermates) until wk 9 when the weight difference between normal and pitKO males becomes insignificant.

**Thyrotropes Have a Defective Response to Thyroid Ablation in Gata2 pitKO Mice**

We challenged the pituitary-thyroid axis to determine whether the Gata2 pitKO thyrotropes could mount a normal physiologic response to a loss of negative feedback by thyroid hormone. We treated normal and pitKO mice with $^{131}$I to ablate the thyroid gland (30). The mice had virtually undetectable $T_4$ levels ($<0.1 \mu g/dl$) 6 wk after radioiodination. Without the negative feedback of thyroid hormone, normal mice increase $Tshb$ transcription and TSH secretion by approximately 15× and 100×, respectively. Gata2 pitKO mice had a severely blunted response to thyroid ablation, showing only 22% of WT hypothyroid $Tshb$ RNA levels ($P = 1.1 e^{-5}$) and only 15% of the TSH protein levels in serum ($P = 5.9 e^{-6}$) (Fig. 6, B and C). This dramatic
inability of the Gata2 pitKO mice to increase TSH production and secretion in the context of exceedingly low T4 levels reveals a severe defect in thyrotrope function not apparent under normal physiological conditions.

**Gata2 pitKOs Exhibit Elevated Levels of Gata3 Transcripts**

The GATA family of transcription factors is hypothesized to have overlapping functions in organogenesis of several tissues. For example, loss of Gata1 results in elevated levels of Gata2 transcripts in the hematopoietic system, and expression of Gata2 or Gata3 from the Gata1 locus control region can rescue most of the Gata1-deficient phenotype (31, 32). Gata2 is the most abundantly expressed GATA family member in the pituitary gland (data not shown), but low levels of Gata3 transcripts are detectable in developing and adult pituitary tissue as well as in α-TSH cells and TtT97 thyrotropic tumors (12, 14). To determine whether Gata2 deficiency affects Gata3 transcription, we used quantitative PCR to measure Gata3 levels in pituitaries of normal and Gata2 pitKO mutants after radiothyroidectomy. Gata3-specific PCR was carried out in duplicate on RNA prepared from four pitKO and four control pituitaries and normalized to ribosomal RNA levels (Fig. 6). Gata3 levels are significantly elevated, more than 15-fold in mutants relative to normal radiothyroidectomized mice (P/H11005 0.010). Gata3 levels appeared slightly elevated in euthyroid pitKO pituitaries relative to euthyroid controls, but the effect in euthyroid mice was less significant (data not shown).

**Gonadotropes of Gata2 pitKOs Produce Lower Levels of FSH**

To assess maximal functional reserve of the gonadotropes in Gata2 pitKO mice we castrated 2-month-old male pitKO mice along with their WT littermates. Castration eliminates testosterone production and its negative feedback on the hypothalamus and the anterior pituitary, which normally results in a striking increase in both FSH and LH secretion as the pituitary attempts to compensate. Increased gonadotropin levels are normally detectable 5 wk after castration and continue to increase 3–5 months after castration (33). Some gonadotropes undergo both hypertrophy and hyper-

**Fig. 4. Gata2 pitKO Male Mice Have Drastically Smaller Seminal Vesicles, but Normal Testes and Thyroid Morphology**

Seminal vesicles of Gata2 pitKO male mice (E) are approximately 55% the weight of WT littermates (A); however the internal morphology is identical between pitKO (F) and WT (B) mice. Testes size and morphology is identical between pitKO (low power, G; high power, J) and WT mice (low power, C; high power, I). Mature sperm and histologically normal Leydig cells are visible (C, G, I, and J). Thyroid follicle size is also similar between WT (D) and pitKO (H) littermates. Six-micrometer histological sections were stained with hematoxylin and eosin (B, C, D, F, G, H, I, and J).
plasia that is detectable histologically as signet ring cells (also referred to as castration cells) (34).

We found the gonadotropes in castrated Gata2 pitKO mice are responsive to decreased testosterone feedback. Three months after castration, we assayed FSH serum levels. Normal mice increase FSH serum levels approximately two times in response to castration. Castrated Gata2 pitKO mice are capable of increasing their basal FSH serum levels 3-fold ($P < 0.018$), which is a larger relative increase than their WT littermates; however, the FSH levels at this increased capacity only reach the basal level of normal intact males (Fig. 6A). Thus, the gonadotropes of Gata2 pitKO are capable of increasing gonadotropin levels in response to a change in feedback; however, the absolute level of circulating gonadotropin is significantly less than their WT littermates.

**DISCUSSION**

Mice with a loss of Gata2 in the anterior pituitary have a reduction in thyrotripe number at birth and reduced function of both gonadotropes and thyrotropes in adult mice. Eliminating the negative feedback from the thyroid gland or testes reveals the full extent of the functional insufficiency. The pituitary defects cause a transient growth insufficiency in males as well as reduced seminal vesicle weight; however, the FSH levels at this increased capacity only reach the basal level of normal intact males (Fig. 6A). Thus, the gonadotropes of Gata2 pitKO are capable of increasing gonadotropin levels in response to a change in feedback; however, the absolute level of circulating gonadotropin is significantly less than their WT littermates.

The transient reduction in body weight is apparent in male pitKO mice but is not significant in female pitKO mice. The sex-specific nature of the growth delay may be due to the gonadotrope functional insufficiency, but it is also consistent with marginal thyrotripe function that only has obvious consequences during the pubertal growth spurt in males. Reduction in gonadotropes or decreased gonadotropin secretion leads to a decrease in testosterone production by the Leydig cells in the testes. Although there is no decrease in the weight of the mutant testes, we observe a profound decrease in the weight of the seminal vesicles, which is indicative of inadequate testosterone levels (25, 26). Because testosterone action is responsible for the male-specific pattern of growth both in muscle and bone, testosterone deficiencies in males can cause a growth pattern that closely resembles that of female mice, and a final adult weight equivalent to females. This is usually accompanied by failure to induce the male-specific pattern of liver gene expression (35). The male-specific pattern of major urinary protein expression is induced at the same time in normal and pitKO males, however (data not shown). This suggests that the most likely cause of the transient growth insufficiency in the Gata2 pitKO mice is inadequate thyrotripe function.

The recovery of the thyrotripe population size, gonadotrope function, and body weight of the pitKO mice may be due to several factors. Firstly, Gata2 may be important, but not critical for basal function of the adult pituitary. The transient growth insufficiency indicates a role in early pituitary development and/or function, but other factors may compensate for loss of Gata2 in the adult animal. We considered the possibility that some targeted cells escaped recombination.

**Fig. 5.** Loss of Gata2 in the Pituitary Causes Growth Insufficiency

Male pitKOs are smaller than normal littermates from 3 wk of age through 9 wk of age. Male pitKO mice are approximately 90% the weight of normal littermates during this time period. Fifteen to 35 mice were weighed for each category: (□), WT males; (○), WT females; (■), pitKO males; and (●), pitKO females. Weights were averaged for 3-d spans for each category and se (bars) were calculated for each time point. $P$ values for male WT compared with male pitKO were determined using an unpaired Student’s $t$ test for each time point: *, $< 0.05$; **, $< 0.001$. 

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and that these cells could proliferate in response to selection pressure to partially replenish the cell population. This scenario is ruled out by the observation that the residual levels of normal Gata2 transcripts are nearly identical in euthyroid and hypothyroid pitKO mice, a state with intense selection pressure for thyrotrope proliferation. The recovery of pituitary cell numbers is better explained by a more limited requirement for Gata2 than originally proposed or functional compensation by other factors in the pituitary, either as part of normal expression dynamics, or induced by the loss of Gata2. A candidate for compensation is Gata3, which is expressed in αTSH cells, TtT97 thyrotropic tumors, and the developing pituitary gland (12, 14). Compensation between transcription factors that are closely related is common and is seen in the pituitary between PITX genes (37) and with GATA genes themselves in other tissues (31, 32). We detected dramatic elevation of Gata3 transcripts in pituitaries of the Gata2 pitKO mice challenged by thyroidectomy, providing support for the idea that Gata3 partially substitutes for Gata2 in pituitary thyrotropes.

Although pituitary cell numbers are normalized in the adult Gata2 pitKO mice by 9 wk of age, the pituitary cells do not have normal hormone-producing capacity. Challenges to both the gonadotropes and thyrotropes reveal that cells that lack Gata2 are not able to secrete hormones at appropriate levels. This is especially evident in the thyrotropes, which show a profound deficiency in TSH production, both basally and in response to thyroid ablation, despite the profound elevation of Gata3 transcription. The gonadotrope and thyrotrope deficiencies may be due to the role of Gata2 in directly binding the promoters of the hormone genes, as observed in cell culture with Tshb (12, 14, 16), or from direct or indirect effects of Gata2 on cellular function, cell expansion and/or maintenance. The phenotype of the Gata2 pitKO mice is less dramatic than previous ectopic expression and dominant-negative experiments predicted. Gata2 pitKO mice

Fig. 6. Thyrotropes and Gonadotropes of Gata2 pitKO Mice Fail to Mount Appropriate Response to Thyroid Ablation and Castration

A, Thyroid-ablated Gata2 pitKO mice have dramatically lower levels of Tshb mRNA than WT littermates. B, Secreted TSH protein is also found at lower levels in the serum of thyro-ablated Gata2 pitKO mice as compared with WT thyro-ablated littermates. C, Gata3 mRNA levels are elevated in thyroid-ablated Gata2 pitKO mice; P = 0.010. D, FSH levels in pitKO mice are lower than WT both before and after castration. Levels of FSH increase in both WT and Gata2 pitKO mouse serum with the loss of testosterone-negative feedback. P values = 1.1e^-5 (*), 5.9e^-6 (**), and 0.018 (**).
have defects in the same pituitary cell populations as the dominant-negative Gata2 transgenic mice (12), but the extent of the defect is much less severe. This difference in severity is likely due to the dominant-negative protein causing unpredicted protein-protein or DNA-protein interactions in pituitary cells or interfering with the function of other GATA family members in the pituitary, such as GATA3. We cannot rule out the possibility that the αGSU-Cre transgene converts the Gata2 allele to the Gata2 allele after critical cell specification events have occurred, and a small population of committed cells expands to populate the organ. The αGSU-Cre line used for this study is the same one that has been shown to recombine the floxed reporter gene TnfrsflacZ and a floxed allele of SF1 with nearly 100% efficiency by at least E14.5 (23, 24). There is some variability in timing and penetrance of cre-mediated excision with the Rosa26 floxed reporter strain, however (data not shown). The αGSU promoter drives expression of reporters such as LacZ by E9.5, which is 4–5 d before Gata2 transcripts are detectable in the region of the pituitary from which the thyrotropes emerge (38, 39). Thus, the modest phenotype of Gata2 pitKO mice most likely reflects the fact that Gata2 is not absolutely necessary for many basal gonadotrope and thyrotrope functions.

Based on the phenotype of the Gata2 pitKO mouse and the previously described expression pattern of GATA2, it is likely that the role of Gata2 in the pituitary is unlike the role it has in the hematopoietic system (4, 5). In the hematopoietic system, Gata2 is found only in undifferentiated cells and is responsible for maintaining the hematopoietic progenitors in a precursor state. In adipose tissue, Gata2 has a similar role and represses genes found in differentiated adipocyte cells (7). In the anterior pituitary, GATA2 is expressed during the period of cellular specification but is also found in two fully differentiated cell types: the thyrotropes and gonadotropes. This is reminiscent of the proposed role of Gata2 in differentiated endothelial cells (8). Based on expression patterns and transfection studies in cell culture, it is likely that Gata2 is responsible for positive regulation of the differentiated state. The inability of thyrotropes and gonadotropes in Gata2 pitKO mice to produce normal levels of TSH and gonadotropins strengthens this hypothesis.

Gata2 likely has a dual role in the anterior pituitary gland. The defects found in neonatal Gata2 pitKO mice are consistent with an initial role in the specification and/or expansion of thyrotropes because the number of differentiated cells is reduced at birth. Gata2 likely has an additional role in the maintenance of hormone production in both gonadotropes and thyrotropes. Whether the maintenance function is a direct effect of Gata2 binding to the Tshb promoter and perhaps the Lhb or Fshb promoters, or from indirect effects mediated through other transcription factors, is not immediately evident. However, it is clear that Gata2 is not acting alone in this capacity, and that other factors, like Gata3, are likely cooperating to maintain thyrotrope and gonadotrope function.

MATERIALS AND METHODS

Construction of Gata2 Targeting Vector

A 6.5-kb fragment of mouse Gata2 was amplified by PCR from 500 ng ES cell genomic DNA (strain R1) using 200 ng each of a sense strand oligonucleotide within the intron/exon 3 junction (5′-TACGGCGCGCCTGACAGCCGCTCTACGGAGGCGAGAGAC-3′) and an antisense strand oligonucleotide in the 3′ untranslated region (5′-TAGGGGCCGCCAGCACAATGCTCTCCGTGGGCTACGGCG-3′), each containing a NotI site (underlined). The amplification reaction was performed using 5 U Takara La Taq polymerase with an initial denaturation step of 94 C for 2 min followed by 32 cycles of 98 C for 20 sec, 65 C for 1 min, and 68 C for 8 min. The product was agarose gel purified using Qiagen II resin (QIA-GEN, Valencia, CA), digested with NotI, ligated into pGem13, and all exons sequences and intron/exon junctions were verified by DNA sequencing. A 1.8-kb HindIII-HindIII fragment containing pGK-Neo with a Flp recombinase site (FRT) on both sides and in the same orientation (kindly provided by Dr. Steven Fiering, Dartmouth Medical School, Hanover, NH) was ligated into the PvuII site of pSP72. The construct was linearized with SalI and ligated with a nonphosphorylated oligonucleotide duplex containing the pGK-neo-FRT cassette was excised with SalI and XhoI in the same orientation. The modified Gata2 genomic fragment that was located within the intron between exons 4 and 5. The orientation of the cassette was established by DNA sequencing. Similarly, a duplex oligonucleotide containing a single loxP site with KpnI compatible termini and an additional XhoI site was ligated into the unique KpnI site within the intron between exons 5 and 6 and a recombinant selected with both loxp sites in the same orientation. The modified Gata2 fragment was excised completely by digestion with NotI, gel purified, and electroporated into the R1 strain of mouse ES cells.

Breeding Genotyping and Care of Mice

Mice were housed in ventilated cages under 14-h light, 10-h dark cycles. They were fed Purina Mills (St. Louis, MO) 5020 mouse chow ad libitum. C57BL/6J mice were obtained from The Jackson Laboratory and CD-1 mice were obtained from Charles River (Wilmington, MA). Timed pregnancies were generated using mice naturally cycling in estrus, and the morning after mating was designated as E0.5. The Committee on Use and Care of Animals from the University of Michigan and the University of Colorado Health Sciences Center approved all procedures using mice. All experiments were conducted in accord with the principles and procedures outlined in the National Institutes of Health Guidelines for the Care and Use of Experimental Animals.

Quantitative, Real-Time PCR

RNA was isolated from neonatal and adult mouse pituitaries using an RNAqueous Micro kit (Ambion, Austin, TX) and Ultra-Turrax T8 homogenizer (IKA, Wilmington, NC). RNA isolation from larger organs was prepared using the RNAqueous kit (Ambion). RNA concentrations were determined in TE
buffer by using a DU 530 spectrophotometer (Beckman Coulter, Fullerton, CA). cDNA was synthesized using SuperScript First-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA).

Gata3 transcripts were quantified by TaqMan assay. The primers for the TaqMan assay are as follows: the forward primer AGGATCCTACGCCGGTGTCGGATGTGAATTGCGG-3 and the reverse primer GTGTCGGGCCCAAGGCGTCACAGCAGACGAAGAGCCAGGAGTGGTGCAGAAC. The reaction contained 5 μM of template DNA and 50 μM of primer DNA in 50 mM NaCl and 1 mM MgCl2. A three-step PCR was performed for 35 cycles. Denaturation was at 94°C for 20 sec. Annealing was performed at 55°C for 20 sec. Extension was performed at 72°C for 30 sec.

Full-length transcripts for both WT and JES forms of Gata2 were amplified from 2 μg pituitary RNA from a Gata2f/f mouse expressing α-GSU-Cre using the following primer set that also contains an XbaI site or a NotI site (underlined): sense 5′-GGCTTAGACCTGGCTGGAGGCTTGGCCTGAG-3′ and antisense GCGGAGGCCCAAGGCACGATCCAGCACAGAAGGG-AGTTGTGTAAC. The reaction contained 5 μM of template DNA and 50 μM of primer DNA in 50 mM NaCl and 1 mM MgCl2. A three-step PCR was performed for 35 cycles. Denaturation was at 94°C for 20 sec. Annealing was performed at 55°C for 20 sec. Extension was performed at 72°C for 30 sec.

Growth Curve

Mice were weighed at various intervals from 2 wk of age to 3 months of age. Approximately 15–35 mice were weighed in each of the following categories: male WT, female WT, male pitKO, female pitKO. Weights were averaged for every 3-d time span for each category and standard errors were calculated for each time point. P values for male WT compared with male pitKO were determined using an unpaired Student’s t test for each time point.

Serum Analysis

Whole blood was collected either with glass pipettes from retro-orbital bleeds or with insulin needles from the hearts of anesthetized mice. The blood was allowed to clot for 1.5 h at room temperature and then spun in a Spin-X (Costar, Fullerton, CA) at room temperature. The serum was collected as a supernatant and frozen at −20°C before analysis. Serum was shipped under dry ice to the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core UVA and Dr. A. F. Parlow at the National Hormone and Peptide Program for RIAs for the levels of various circulating hormones including LH, FSH, TSH, GH, T4, and testosterone.

Castration Challenge

Mice were gonadectomized via a single scrotal incision after sodium pentobarbital anesthesia (50 mg/kg body weight) as previously described (40). Sham-operated males underwent pentobarbital anesthesia, scrotal incisions, and suture placement. Three months after castration, serum was collected and analyzed with RIA as described above.

Thyroid Ablation Study

Mice were kept on a low iodine diet for 10 d followed by ip injections of 150 μCi Na131I per mouse diluted in 200 μl sterile PBS (IV grade) as described (30). Mice were killed 6 wk after radiothyroidectomy by cervical dislocation and serum collected and stored at −20°C until assayed for T4 and TSH. Pituitaries were collected into 300 μl RNA-later (Ambion) and kept at −20°C until total RNA was extracted using a Qiagen RN-Easy microcolumn system, which included a deoxyribonuclease I step before elution with 50 μl sterile water.

IHC

Tissues were fixed in 4% paraformaldehyde in PBS (pH 7.2) on ice. Embryos at d 10.5, 11.5, 12.5, 14.5, 18.5, and 20 C were fixed with 10% buffered formalin (pH 7.4) at room temperature and then spun for 15 min at 2000 g. Retro-orbital bleeds or with insulin needles from the hearts of male Wts and female pitKOs were determined using an unpaired Student’s t test for each time point.

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