Gene-Environment Interactions, Not Neonatal Growth Hormone Deficiency, Time Puberty in Female Rhesus Monkeys

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Abstract

The factors that influence the timing of puberty and the onset of adult fertility are poorly understood. While focus on the juvenile period has provided insights into how growth-related cues affect pubertal timing, growth velocity during infancy that is sustained into the juvenile period may be important. On the other hand, social factors, specifically exposure to psychosocial stressors, can delay sexual maturation, possibly by altering growth velocities during development. Using female rhesus monkeys, the present study used a prospective analysis to determine how neonatal growth hormone (GH) inhibition with a sandostatin analog or suppression of the pituitary – gonadal axis with a GnRH analog affected growth and sexual maturation. Secondly, a separate retrospective analysis was done assessing the effects of social dominance status during development on pubertal timing. Because a specific polymorphism in the gene encoding the serotonin (5HT) reuptake transporter increases vulnerability to psychosocial stressors, females were also genotyped and were then classified as socially dominant having both alleles for the long promoter variant or having at least one allele for the short promoter variant or classified as socially subordinate having the long variant or subordinate having the short variant. Neonatal treatments were not balanced for social status or genotype so analyses were performed separately. Although the neonatal treatments reduced GH secretion postnatally and through the juvenile period, neither growth nor sexual maturation were affected. In contrast, the retrospective analysis showed sexual maturation was delayed significantly in subordinate females carrying at least one allele of the short promoter variant in the gene encoding the serotonin reuptake transporter and this delay was associated with reduced GH and leptin secretion during the juvenile phase but not with differences in growth velocities from birth. These data suggest that decreased neonatal GH secretion does not adversely affect sexual maturation but that polymorphisms in the gene encoding the 5HT transporter modulate the adverse consequences of social subordination on the timing of puberty in female rhesus monkeys.

Keywords

neonate; puberty; social status; 5HTTLPR; Lupron; GH deficiency; rhesus monkey

Introduction

While the understanding of the common neural pathways [1–3] that drive the appropriate release of GnRH from the medial basal hypothalamus to initiate puberty are well accepted [4–7], the factors that influence the variation in pubertal timing are less clear. Prepubertal growth or factors that regulate growth have long been considered important for timing

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maturation of the reproductive axis. Indeed, changes in hormones that either stimulate skeletal or muscular growth [8–12] or increase in response to accumulation of body fat [13–15] explain some of the variation in puberty timing, suggesting these can act centrally to influence systems regulating developmental increases in GnRH [16,17].

While focus on the juvenile period has provided insights into how growth-related cues affect pubertal timing, birth weight and postnatal growth may also be important. Clinical data indicate that low birth weight followed by rapid catch-up growth is associated with increases insulin-like growth factor (IGF) I secretion and early puberty [18,19]. These children are taller, heavier, and have more fat than age match normal birth weight children [20]. Other epidemiological data indicate that girls heavier at birth have early puberty compared with normal birth weight girls showing similar patterns of growth during infancy [21]. In an analysis of birth weight and growth patterns of female monkeys, low birth weight is associated with blunted postnatal and juvenile growth and later age at fertility [22]. The key variable in these studies appears to be the growth velocity during infancy that is sustained into the juvenile period. Additional support for the notion that growth during infancy may be important is provided by studies showing the experimental manipulation of the neonatal hypothalamic – pituitary – gonadal (HPG) axis in male monkeys affects growth and puberty. Males treated with a GnRH analog during the neonatal period have blunted growth and delayed puberty [23,24]. The mechanism by which GnRH analog treatment during the neonatal period reduces growth is not clear but could be due to altered gonadal steroid stimulation of the growth hormone (GH) – IGF1 axis.

For children, social factors can also influence growth and, thus, puberty timing. Again, epidemiological data on adolescents living in urbanized settings indicate girls in families with higher socio-economical status (SES) have earlier menarche preceded by accelerated growth [19,25–27]. While access to better nutrition may explain the positive relationship between higher SES and early puberty [19], SES and caloric intake is complex. Indeed, other data indicate that in industrial societies, children from families of low SES may consume high caloric, inexpensive foods that accelerates weight gain and body fat accumulation [28–31], advancing the age of puberty [32]. In these cases, indices of body fat, such as BMI, are better predictors of puberty timing than SES [33]. However, adolescent girls who show an increased vulnerability to psychosocial stress have an increased incidence of anovulation [34]. Similarly, studies of socially-living rhesus monkeys, where diets are limited to standard low fat, high fiber meals, indicate social status is a robust predictor of puberty timing [35] and reproductive success [36,37]. Male rhesus monkeys from socially subordinate matrilines have delayed puberty [23,38] while females from subordinate matrilines show a significantly later age at first ovulation [35,39–41]. Although weight at first ovulation is similar between the later maturing subordinates compared with the earlier maturing dominant females, it is not known whether these differences are predicted from growth during infancy.

Although social status accounts for a significant amount of the variance in puberty timing in rhesus monkey groups, there are nevertheless differences in the tempo of maturation between females of similar social dominance ranks. For example, 42% of dominant and 88% of subordinate females show a later age at first ovulation [35]. These data suggest that factors that modify an individual's response to social status may further explain variance in pubertal timing. In this regard, the variable number tandem repeat polymorphisms in the length of the promoter region of the *SLC6A4* gene that encodes the serotonin reuptake transporter could be important. The short (*s*) promoter length 5HTTLPR has diminished transcriptional activity compared to the long (*l*) promoter length variant [42], and when present in humans, increases the incidence of affective disorders in response to life stressors [43–46]. Homologous promoter length variations in the *SLC6A4 gene* with reduced transcriptional activity are also present in rhesus monkeys [42,47]. The presence of the *s* 5HTTLPR is associated with greater emotional reactivity in juvenile monkeys [48–50] and potentiates the adverse consequence of social

subordination on metabolic and anthropometric measures in adult females [51]. Previous studies of rhesus monkeys also indicate that females with the *s* variant 5HTTLPR show reduced ovulatory frequency, lower body weights, and reduced serum levels of leptin compared with those with homozygous for the long promoter variant [52] and the 5HTTLPR accounts for some variation in the timing of reproductive output in males [53]. Because the 5HTTLPR is associated with differences in reactivity to social environments, it is possible that these polymorphisms interact with social status to influence the timing of reproductive maturation.

Using a rhesus monkey model, the present analysis combines both a prospective study to determine how birth weight and growth during infancy predict the timing of pubertal events and a separate retrospective study to determine how social status and *SCL6A4* polymorphisms influence pubertal timing. The neonatal period was manipulated in one of two ways compared to control females. One group was treated continuously with a somatostatin analog to decrease GH secretion while the other was treated continuously with a GnRH analog to block the neonatal activation of the pituitary-gonadal axis. Although not as robust as occurs in males, the HPG axis is active during the postnatal period in females [54,55]. Because postnatal treatment with a GnRH analog blunts growth in male monkeys [23,24], we tested the hypothesis that such treatment would slow growth in females in a manner similar to that as the inhibition of GH secretion. In addition, given the importance of social status as a predictor of age at first ovulation and the possibility that this may be influenced by 5HTTLPR polymorphisms, data were analyzed separately to determine if growth and puberty is slowed significantly in subordinate females with the s-variant 5HTTLPR genotype.

Methods

Female rhesus monkeys (Macaca mulatta) were subjects. All females were members of established social groups maintained at the Yerkes National Primate Research Center Field Station. These groups varied in size from 25 to 85 animals and each contained one to two adult males, multiple adult females and their juvenile or infant offspring. Animals were fed a standard monkey diet (Purina Mills Lab Diet #5037, containing 13% of calories from fat, 18% from protein, and 69% from carbohydrates) and received seasonal fruit and vegetables daily. The Emory University Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals" approved the protocol.

On the day of birth, animals were randomly assigned to one of three treatment groups: control (Con), depot Lupron treated (Lup), or Sandostatin LAR treated (GHx). Control animals (n = 6) were weighed and injected SC were saline (0.25 ml) that continued every 25 days until the last treatment at 225 days of age. Lup females (n = 6) were weighed and injected with 750 µg/kg SC Depot Lupron (Tap Pharmaceuticals) every 25 days until the last treatment at 225 days of age. This dose effectively blocks activation of the pituitary – gonadal axis in juvenile female monkeys [56,57] and arrests precocious puberty in girls [58]. GHx females (n = 6) were weighed and injected with 625 µg/kg, IM of Sandostatin LAR (Sandoz Pharmaceuticals) every 25 days until the last treatment at 225 days of age. This dose produces GH deficiency in juvenile female monkeys [56]. Animals received their respective treatments every 25 days with the last treatment given at 225 days or 7.4 mo of age. Because both Depot Lupron and Sandostatin LAR sustain serum levels of the drug for 28 days, treatments effectively ended at 8.3 mo of age (252 days of age).

The relative dominance status of each female in her social group was defined by the outcome of dyadic interactions observed opportunistically throughout the study period [59]. Social status is determined by membership in a matriline, as infants acquire the rank of their mothers [59]. However, as the animals age and begin to engage in more agonistic interactions, their

respective dominance positions become evident. Because the specific number of animals within a group varied amongst the subjects, it was not possible to assign individual numerical ranks to females. Consequently, females were classified as dominant or subordinate depending whether their position was in the top half or bottom half of the group. It was not possible to further categorize females as high, middle, or low ranking as the number of animals in some cells in the social status by genotype matrix would have had one subject, precluding any meaningful analysis.

5HTTLPR polymorphisms were determined as described previously for rhesus monkeys [42]. Specifically, DNA was extracted from whole blood using the Pure Gene Blood Kit (Gentra, D-4000). Polymorphisms in the promoter region of the *SCL6A4* gene were identified following amplification of the relevant gene segments by polymerase chain reaction using the oligonucleotide primers fwd (cag ggg aga tcc tgg gag gga) and rev (ggc gtt gcc gct ctg aat gc) based on the protocol described previously. The "s" amplicon (398bps) and the "l" amplicon (419bps) were separated on an agarose gel containing ethidium bromide and identified by direct visualization. Because the *l/s* genotype produces a similar phenotype as the *s/s* genotype on most measures [47,49], females carrying at least one s allele were categorized as having an "s-variant" genotype. Because subjects were not selected *a priori* on the basis of social status and 5HTTLPR polymorphisms, social status classification and 5HTTLPR were not balanced across neonatal treatments and the effects of each were analyzed separately (see below). A description of the subjects is contained in Table 1.

Females were studied from birth through first ovulation

The primary outcome measures were reproductive and anthropometric. Puberty was characterized in several ways. In addition to menarche, assessed from daily observations, the age at the initial perineal swelling and coloration was recorded. For rhesus monkeys, this typically proceeds menarche and is an external indication estradiol secretion has commenced [60]. First ovulation was determined in all females and the interval from menarche to first ovulation was considered as the tempo of puberty [61]. Body weights were obtained at birth and monthly thereafter. Heights were obtained every 90 days while the females were anesthetized (10 mg/kg ketamine hydrochloride). Females were placed in a supine position with the legs straightened on a long piece of paper and a mark was made at the top of the head and at bottom of the heel. Two individuals measured the distance using MHC vernier calipers and the mean of the two measurements was calculated for an animal's height. Body mass index was calculated as body weight in kg divided height in m^2.

Serum samples were obtained by femoral venipuncture of infants following removal from her mother. By 4 mo of age, females had become habituated to allow venipuncture of the saphenous vein. Conscious venipuncture of captive acclimated rhesus monkeys does not adversely affect limbic – HP – adrenal (LHPA) arousal, puberty, or reproductive performance [62–64]. Morning, non-fasted samples were obtained once a month from birth through 18 months of age, once weekly until menarche, and twice weekly until first ovulation was confirmed. Samples obtained at 6 and 7 mo were assayed for GH to determine whether either of the neonatal treatments affected GH secretion. Nocturnal samples, collected at 18 and 24 mo of age on each subjected were also assayed for GH. To better estimate GH secretion at these ages, samples were obtained at 2200 ans 2230 hr and the average GH value between these two time points was used for analysis. Other hormone analyses were focused on the interval from 24 through ~32 mo of age, as this represents the age range from pre-menarche through the earliest age of first ovulation in outdoor housed rhesus monkeys at our facility [64]. Daytime samples obtained from 24 through ~32 mo of age were also assayed for leptin and cortisol. Following menarche, all samples were assayed for progesterone. First ovulation was inferred from a sustained rise in serum progesterone (>1 ng/ml for >5 days). Short luteal phase cycles were

identified by a blunted rise in progesterone (1 to 3 ng/ml) for 4 to 6 days followed by the appearance of menstruation [8].

All assays were performed in the YNPRC Biomarkers Core Lab. Progesterone was measured using a previously described radioimmunoassay (RIA) that employs a commercially available kit from Diagnostic Products Corporation (Los Angeles CA) [52]. The assay has a sensitivity of 0.10 ng/ml with an inter- and intra-assay CV of 8.14% and 7.73%, respectively. Serum GH was determined by a commercially available ELISA (Diagnostic Systems Laboratory (DSL), Webster TX) having a sensitivity of 0.10 ng/ml using 20 μl of serum with an inter and intra assay CV of 9.13% and 6.33%, respectively. Serum leptin were measured by RIA using a commercially available kit validated for nonhuman primates (Linco, St. Louis MO). Assaying 100 μl, the assay has a range of 0.5 to 100 ng/ml. Intra-assay CVs were 5.5% and inter-assay were 8.8%. Serum levels of cortisol were measured by RIA with a kit from DSL. The assay has a sensitivity of 0.10 ng/ml with an inter- and intra-assay coefficient of variation (CV) of 6.3% and 9.1%, respectively. Data were summarized as the mean ± sem. As noted above, neither social status nor 5HTTLPR genotype was balanced across the neonatal treatment groups (Con, Lup, or GHx) so data were analyzed separately. The first analysis tested the hypothesis that neonatal treatments with Lurpon or Sandostatin were significantly slow growth and delay puberty. The separate retrospective analysis tested the hypothesis that social subordination, exacerbated by the presence of the s-variant allele in the *SCL6A4* gene would significantly slow growth and delay puberty. Data were analyzed with ANOVA models. Tests having a p < 0.05 were considered significant. A Bonferroni adjustment (p = .05/n) was used for n pair wise comparisons if interaction terms are significant. Data were transformed, as needed, to produce homogeneity of variance. Because sample size for the social status by 5HTTLPR analyses was small, we also calculated effects size that measures the magnitude of a treatment effect independent of sample size. Effect sizes >0.8 are considered large, with percentage of overlap in the distribution of measures between two populations becoming smaller as effect size increases [65].

Results

Consequences of neonatal treatments

As shown in Table 2, neonatal concentrations of GH were significantly affected by the treatments ($F_{2, 15} = 3.85$, $p = 0.04$). Serum levels of morning GH were significantly higher in Con compared to GHx females, with concentrations in Lup females being intermediate. While birth weight did not vary significantly between treatment groups ($F_{2, 15} = 3.15$, $p = 0.06$), weight gain during the 8 mo treatment interval was significantly greater in Con compared to Lup females, with GHx females being intermediated ($F_{1, 15} = p = 0.03$). In contrast, neither height at birth ($F_{2, 15} = 0.62$, $p = 0.53$) nor the increase in height during the 8-month treatment period ($F_{2, 15} = 0.49$, $p = 0.62$) varied significantly between groups.

As can be seen in Table 2, the neonatal treatments had no effect on any of these same parameters of puberty. Age at the appearance of secondary sexual characteristics, measured by perineal swelling and coloration, did not vary among the three groups ($F_{2, 15} = 0.18$, $p = 0.84$). In addition, neither age at menarche ($F_{2, 15} = 0.68$, $p = 0.68$) nor was age at first ovulation ($F_{2, 15} = 0.21$, $p = 0.82$) differed significantly as a function of the neonatal treatments. Consequently, the tempo of maturation, defined as the interval from menarche to first ovulation, was also not affected by the neonatal treatments ($F_{2, 15} = 0.61$, $p = 56$).

Body growth from the end of treatment at 8 mo through 30 mo of age was not affected by neonatal treatments, as the increase in body weight ($F_{2, 15} = 0.18$, $p = 0.84$) and height ($F_{2, 15} = 0.25$, $p = 0.78$) was not significantly different among the groups. In contrast, a significant neonatal treatment age interaction was observed for serum GH ($F_{2, 15} = 8.36$, $p <$

0.01). At 18 mo of age, nocturnal GH levels were significantly higher in Con compared with GHx females that, in turn, were significantly higher than Lup-treated females. At 24 mo of age, nocturnal GH concentrations were still significantly higher in Con compared with GHx females, with animals treated neonatally with Lupron being intermediate. Serum leptin in the months prior to and following menarche (25 through 30 mo of age) also did not vary significantly between the three neonatally treated groups ($F_{2, 15} = 0.43$, $p = 0.66$, data not shown). Finally, serum levels of cortisol did not vary significantly between the three groups during the neonatal treatment period (Table 2; $F_{2, 15} = 0.52$, $p = 0.61$). Serum levels did increase significantly in all females during this period ($F_{5, 75} = 7.58$, $p < 0.05$), from average concentrations at birth of 18.66 ± 2.22 µg/dl to 29.46 ± 2.63 µg/dl at the end of the neonatal treatment period.

Importance of social status – 5HTTLPR

Reproductive maturation was significantly influenced by the interaction of social status and 5HTTLPR genotype (Figure 1). The age at the onset of perineal swelling was not influenced by a female's social status during development ($F_{1, 14} = 0.32$, $p = 0.58$), genotype ($F_{1, 14} = 0.96$, $p = 0.35$), nor the interaction of status and genotype ($F_{1, 14} = 1.67$, $p = 0.22$). Similarly, age at menarche was not influenced by social status ($F_{1, 14} = 1.02$, $p = 0.33$), genotype ($F_{1, 14} = 0.30$, $p = 0.59$), or a status by genotype interaction ($F_{1, 14} = 0.46$, $p = 0.51$). In contrast, age at first ovulation occurred significantly earlier in dominant females ($F_{1, 14} = 13.40$, $p < 0.01$) and those with an *l/l* genotype ($F_{1, 14} = 13.82$, $p < 0.01$). However, the variance in the age at first ovulation was best explained by a significant status by genotype interaction ($F_{1, 14} = 32.04$, $p < 0.01$), as subordinate females with the s-variant genotype had first ovulation at a significantly older age than all other females. The computation of effect size for age at first ovulation for these subordinate females compared to all other females was 1.81. Consequently, the tempo of puberty was significantly longer ($F_{1, 14} = 7.72$, $p = 0.02$) in subordinate s-variant females (11.2 ± 1.3 mo) compared subordinate females with an *l/l* genotype (3.6 ± 1.8 mo), or dominant *l/l* (5.2 ± 1.8 mo) or s-variant females (3.1 ± 2.0 mo). The effect size for the tempo of maturation between subordinate, s-variant females and all other females was also large (1.47).

Further analyses revealed that hormonal but not anthropometric measures were also affected by status and 5HRRLPR. As illustrated in Table 3, birth weights and heights were not significantly influenced by status (weight: $F_{1, 14} = 0.46$, $p = 0.51$; height: $F_{1, 14} = 0.40$, $p = 0.54$), genotype (weight: $F_{1, 14} = 1.79$, $p = 0.20$; height: $F_{1, 14} = 0.29$, $p = 0.60$), or status by genotype interaction (weight: $F_{1, 14} = 0.10$, $p = 0.99$; height: $F_{1, 14} = 3.36$, $p = 0.09$). Similarly, neither body weight gain nor accumulation of height through 30 mo of age was influenced by status (weight: $F_{1, 14} = 0.04$, $p = 0.84$; height: $F_{1, 14} = 1.34$, $p = 0.27$), genotype (weight: $F_{1, 14} = 0.08$, $p = 0.78$; height: $F_{1, 14} = 0.90$, $p = 0.36$), or by a status - genotype interaction (weight: $F_{1, 14} = 1.49$, $p = 0.24$; height: $F_{1, 14} = 1.03$, $p = 0.33$). Effect sizes comparing subordinate, s-variant females to all other females was also small for the gain in both weight (0.21) and height (0.34).

In contrast to the anthropometric data, nocturnal concentrations of GH (Figure 2) were significantly influenced a status by 5HTTLPR interaction ($F_{1, 14} = 9.32$, $p = 0.01$) that did not vary with advancing age ($F_{1, 14} = 0.16$, $p = 0.70$). As can be seen, the main effect of genotype is significant ($F_{1, 14} = 33.31$, $p < 0.01$), with levels higher in females homozygous for the *l*-allele. However, GH is significantly lower in subordinate s-variant females compared to all other females and this was reflected in a large effect size of 1.25 for these females compared to all others. Serum leptin also varied significantly between dominant and subordinate females between 24 and 30 months of age (Figure 3; $F_{6, 84} = 2.69$, $p = 0.02$). Post hoc analyses revealed

that serum concentrations were significantly lower in subordinate, s-variant females between 24 and 27 mo of age compared with all other females, again reflected in an effect size of 0.98.

Analysis of developmental patterns of morning cortisol concentrations indicated that the significantly age dependent increase during the adolescent period (Figure 4, $F_{13, 182} = 11.90$, $p < 0.05$) was not affect by social status ($F_{2, 15} = 1.00$, $p = 0.34$) or 5HTTLPR ($F_{2, 15} = 0.34$, $p = 0.57$) nor their interaction with age ($F_{13, 182} = 0.98$, $p = 0.48$).

Because the neonatal treatment groups were not formed on the basis of social status and 5HTTLPR, the interactive effects of these treatments with social status and genotype cannot be statistically evaluated. However, it appeared that neonatal treatments would have had little additive effect. Of the seven subordinate females with the s-variant genotype who ovulated significantly later than all other females, two were control, three were Lupron treated, and two were Sandostatin-treated.

Discussion

The prospective analysis revealed the neonatal suppression of the HPG or GH axis decreased the developmental secretion of GH but had no adverse effects on growth or the timing of puberty. However, reanalyzing the data to determine whether social status interacted with polymorphisms in the gene encoding the 5HT transporter influenced development indicated the socially subordinate females carrying the s-variant allele in the *SCL6A4* gene have delayed puberty and this is associated with reduced concentrations of GH and leptin during the prepubertal period. While these analyses do not support the hypothesis that perturbations in neonatal GH secretion influence pubertal timing, they do show how social factors and genetic polymorphisms can interact to influence the occurrence of first ovulation in female rhesus monkeys and this effect is associated with significant differences in growth-related signals.. The rationale for manipulating the HPG and GH axes neonatally was based on data from children [20,21] and monkeys [22] that postnatal growth velocities appeared to influence the timing of puberty possibly, due to changes in GH and IGF1 [18,19]. Furthermore, suppression of the neonatal HPG axis in male monkeys slowed growth and delayed puberty [23,24]. Thus, our intent was to disrupt postnatal growth in females by suppressing the either the HPG or the GH axis. However, we saw no effect of either the GnRH analog or somatostatin analog treatments during the neonatal interval on the eventual parameters of growth or puberty in female monkeys. While GH secretion was lowered significantly neonatally and during prepuberty by both treatments compared to saline, no other effects were evident. Given the sex differences in the neonatal activation of the HPG axis in rhesus monkeys [54,55], perhaps it is not surprising that the neonatal administration of Lupron had no effect on eventual puberty as the axis shows much greater activity in males compared with females. What the data do indicate that the lower serum GH concentrations throughout development produced by Lupron and Sandostatin postnatal do not adversely affect growth or eventual puberty. Although significantly lower than controls, these GH concentrations were not suppressed to the degree of those produced in other monkey models of juvenile GH deficiency that does result in a slowing of growth and a delay in first ovulation [10,56]. Taken together, these data suggest there may be a critical lower limit below of GH secretion below which normal growth and puberty is compromised. Nevertheless, the data from the present study clearly indicate that perturbations of the GH and HPG axes during the neonatal period have no adverse effects on maturation in female monkeys.

In contrast, the retrospective analyses not only confirms previous reports on male [23,38] and female monkeys [35,39–41] that social subordination delays puberty but extends these findings to indicate that this effect of subordination occurs predominantly in those subordinate females having at least on s-allele in the encoding 5HT transporter. These data are consistent with a

recent report of an increased incidence of functional hypothalamic amenorrhea is girls experienced an increased incidence of stress-induced psychopathology [34]. In macaque groups, social subordination is largely maintained through continual harassment and the threat of aggression from dominant towards subordinate animals [59]. As a result, socially subordinate adult females are characteristically hypercortisolemic secondary to a dysregulation of the LHPA axis [66–68]. Because the presence of the s allele is associated with the increased response to psychosocial stressors in rhesus monkeys [47–50,69], we expected that morning cortisol concentrations would be highest in subordinate females with the s-variant genotype. Such a finding would have supported that hypothesis that activation of the LHPA axis accounted for the delayed puberty in this cohort [70,71]. However, morning cortisol during the juvenile period did not vary significantly between the groups. Recognizing that this measure is an inadequate marker of LHPA activity, other assessments such as a dexamethasone suppression test to determine glucocorticoid negative feedback, may have better differentiated the groups [66]. Although anthropometric measures also did not differ as a function of social status and 5HTTLPR, morning GH and leptin concentrations were significantly lower in these slowly maturing females. It is possible that increased psychosocial stress accounted for these differences. CSF concentrations of somatostatin are higher [72] and the GH response to clonidine blunted [73] in macaques raised in an unpredictable environment, possibly due to a CRH increasing somatostatin [74–76]. CRH inhibits pulsatile GH release [77] and GHRH-induced GH secretion in rats [78,79] while a CRH antagonist increases GH secretion and hypothalamic GHRH mRNA [80]. While the lower concentrations of serum leptin could be the result of stress-induced attenuation of food intake, lowering body fat stores [81], no differences in body weight or BMI scores were observed among the animals. Given similarities in body weight, it would seem differences in food intake and adequate nutrition would not explain the delayed puberty in the subordinate, s-variant females. However, the present analysis also cannot determine whether the lower levels of GH and leptin during in juvenile subordinates with the s-variant genotype played any role in the delayed occurrence of first ovulation. As noted above, experimentally induced deficits in juvenile concentrations of serum GH account for variation in pubertal timing [10,56] and leptin administration advances puberty in female [82] but not male monkeys [83]. Furthermore, leptin administration restores LH secretion and ovulatory function in some women with functional hypothalamic anovulation [84]. Obviously further studies are needed to identify what signals mediate the slowing of puberty in these subordinate monkeys.

This retrospective analysis must be interpreted cautiously as it was severely under-powered. Furthermore, we must emphasize that our sample size is not adequate to determine the genetic contributions to behavior and physiology and it is entirely likely that the reproductive phenotypes examined in this study are influenced by many genes [85]. Indeed, recent analyses have begun to show variations in puberty timing are explained by polymorphisms in genes whose protein products affect GnRH secretion, including the insulin receptor subtype 1 (*IRS1*) [86], the gene encoding leptin (*LEP*) [87], as well the gene encoding kisspeptin (*KISS1*) [88] and its receptor (*KISS1R*) [89]. Furthermore, a polymorphism in the SHBG gene (*SHBG)*, that may regulate bioavailability of estradiol, is associated with the timing of menarche [90]. In contrast, attempts to find associations between puberty timing and polymorphisms in the gene encoding the GnRH receptor (*GnRHR*) have not been successful [91]. The data from the present study show that a gene polymorphism that affects individual reactivity to socio-environmental stressors can also affect puberty, presumably through activation of the LHPA axis. It is likely that this polymorphism could act independently or synergistically with other gene polymorphisms to influence the timing of puberty by acting at multiple levels of the HPG axis. These present data can best serve as the foundation for broader linkage and association analyses to understand how social context and genes interact to time the onset of puberty [92]. Nevertheless, the data confirm the critical importance of the

psychosocial environment for development and illustrate the potential of using socially housed macaques to understand gene by environment effects on behavior and physiology [93].

Acknowledgements

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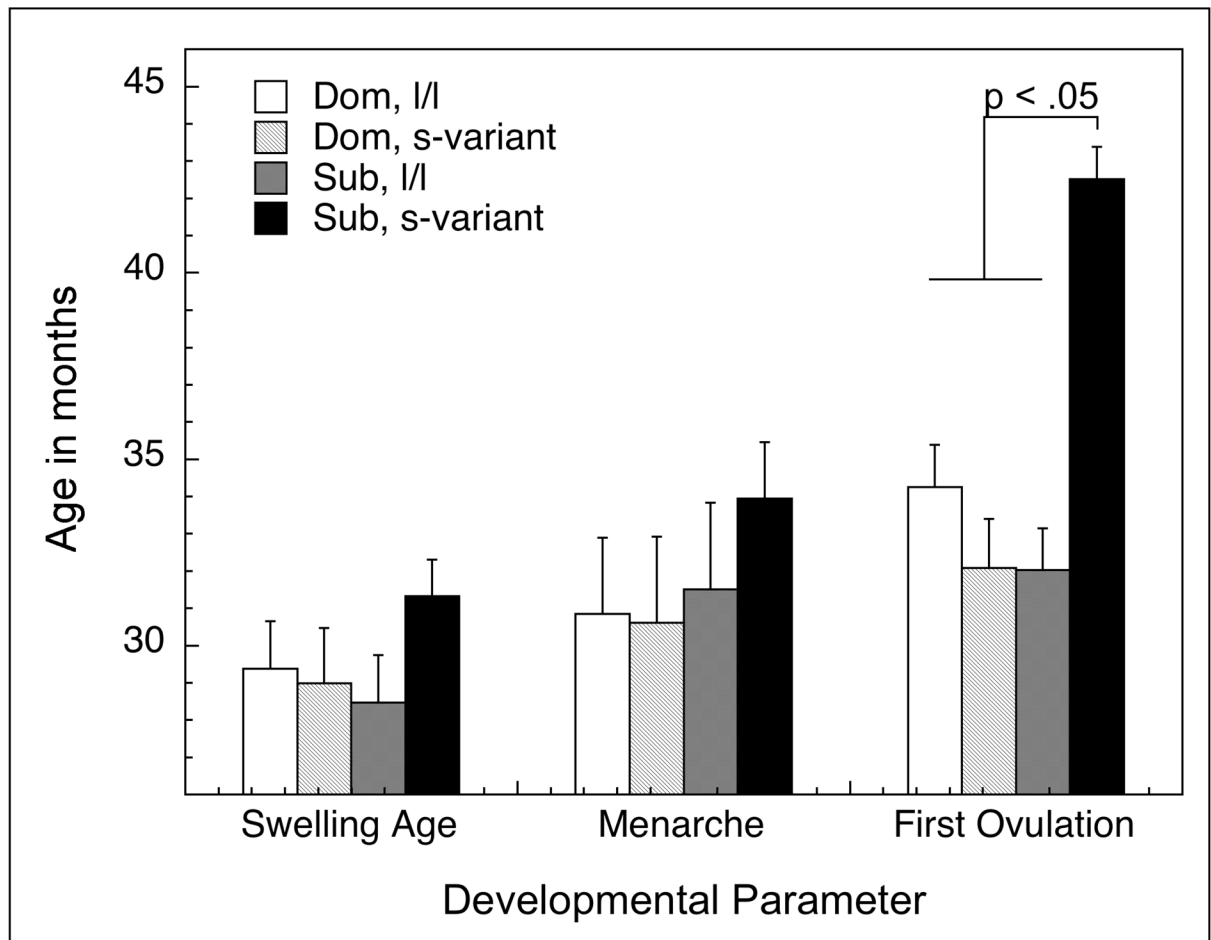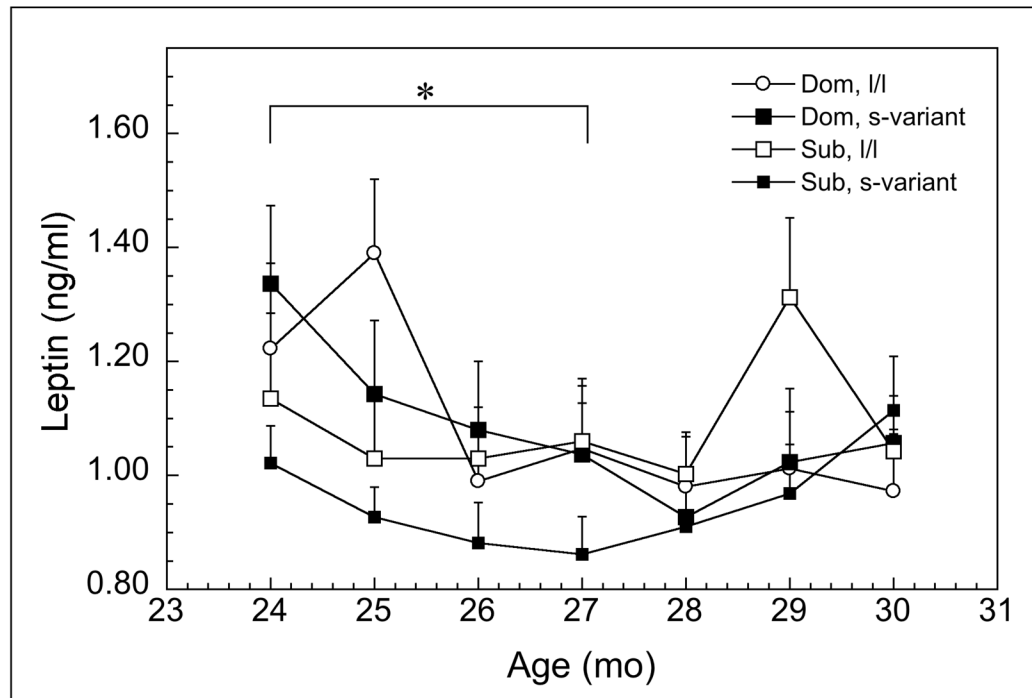
Figure 1.
Distribution of the mean ± SEM age of initial perineal swelling, menarche, and first ovulation
in females categorized as dominant (dom) or subordinate (sub) being homogenous for the long
promoter length variant or (*l/l*) or having at least one short promoter length allele (s-variant)
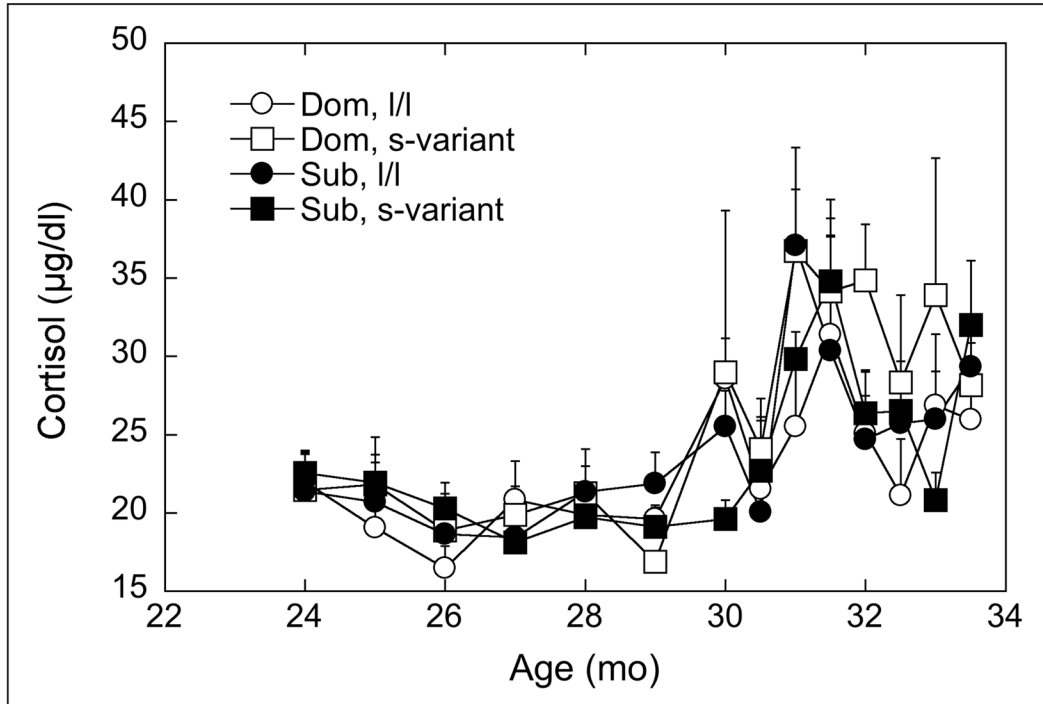polymorphism in the *SCL6A4* gene.
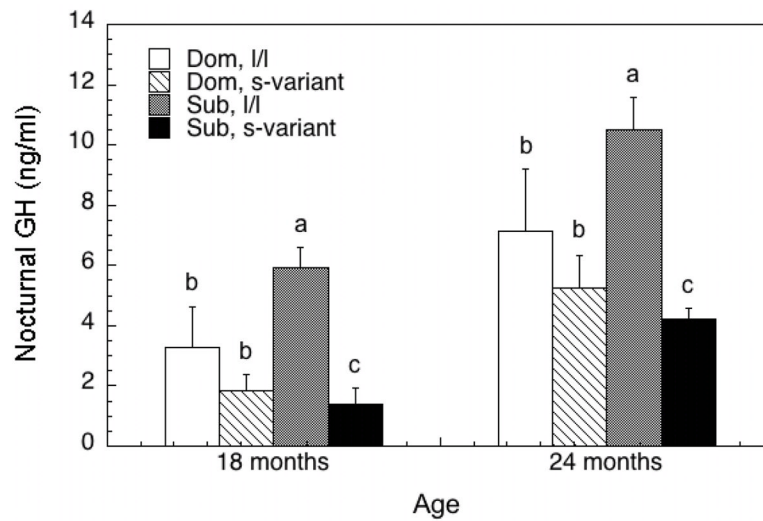
Figure 2.
Mean ± SEM serum concentrations of nocturnal GH (ng/ml) at 18 and 24 months of age for dominant (dom) or subordinate (sub) being homogenous for the long promoter length variant or (*l/l*) or having at least one short promoter length allele (s-variant) polymorphism in the *SCL6A4* gene. Different letters at each age indicate groups are significantly different (p < 0.05).

Figure 3.
Mean ± SEM serum concentrations of morning leptin (ng/ml) from 24 through 30 months af
age for dominant (dom) or subordinate (sub) being homogenous for the long promoter length
variant or (*l/l*) or having at least one short promoter length allele (s-variant) polymorphism in
the *SCL6A4* gene. Asterisks indicate subordinate, s-variant females had significantly lower
concentrations than other groups (p < 0.05).

Figure 4.
Mean ± SEM serum concentrations of morning cortisol (μg/dl) from 24 through 33.5 months of age for dominant (dom) or subordinate (sub) being homogenous for the long promoter length variant or (*l/l*) or having at least one short promoter length allele (s-variant) polymorphism in the *SCL6A4* gene. Different letters at each age indicate groups are significantly different (p < 0.05).

Table 1

Samples sizes for the prospective analysis of neonatal treatments and the retrospective analysis of social status – genotype effects on pubertal timing.

		Social Status X 5HTTLPR			
		Dominant		Subordinate	
Neonatal Treatment	Sample Size	l/l	s-variant	l/l	s-variant
Control	6	1	0	3	2
Lupron–treated	6	1	2	0	3
Sandostatin Treated	6	2	1	1	2
Sample sizes	**18**	4	3	4	7

Table 2

Developmental parameters (mean ± sem) for females treated neonatally with saline (Con), depot Lupron (Lup), or Sandostatin LAR (GHx). For a given measure, groups with different letters are significantly different from one another ($p < 0.05$).

Parameter	Con n = 6	Lup n = 6	GHx n = 6
Birth weight (kg)	0.45 ± 0.02	0.43 ± 0.03	0.40 ± 0.01
Birth Height (cm)	29.9 ± 0.5	30.1 ± 0.4	29.0 ± 0.4
Weight gain			
Birth – 8 mo	1.12 ± 0.05[2]	0.89 ± 0.07[b]	1.05 ± 0.06[a, b]
8 – 30 mo	2.57 ± 0.10	2.49 ± 0.15	2.58 ± 0.10
Height Gain			
Birth – 8 mo	18.5 ± 0.4	17.5 ± 0.5	18.4 ± 1.1
8 – 30 mo	46.6 ± 0.5	47.1 ± 1.2	47.4 ± 0.6
GH (ng/ml): birth – 8 mo	3.43 ± 1.06[a]	1.25 ± 0.42[a, b]	0.67 ± 0.23[b]
Nocturnal GH (ng/ml)			
18 mo	4.42 ± 1.01[a]	1.30 ± 0.40[c]	1.89 ± 0.49[b]
24 mo	8.16 ± 1.19[a]	5.82 ± 1.51[a, b]	4.38 ± 0.41[b]
Cortisol (μg/dl): birth – 8 mo	25.1 ± 3.6	29.3 ± 3.89	24.6 ± 3.84
Puberty			
Age at swelling (mo)	30.42 ± 1.10	29.34 ± 0.49	29.64 ± 1.55
Menarche age (mo)	32.81 ± 0.81	33.04 ± 2.22	31.01 ± 1.85
First ovulation age (mo)	35.54 ± 2.14	37.62 ± 2.57	36.71 ± 2.13
Tempo (mo)	5.12 ± 1.78	8.27 ± 2.26	7.07 ± 2.05

Table 3

Mean ± sem anthropometric measures of females classified as dominant or subordinate having either the *l/l* or s-variant *SCL6A4* genotype. For a given measure, groups with different letters are significantly different from one another (p < 0.05).

Parameter	Dominant		Subordinate	
	l/l (n = 4)	*s*-variant (n = 3)	*l/l* (n = 4)	*s*-variant (n = 7)
Birth weight (kg)	0.43 ± 0.02	0.40 ± 0.02	0.44 ± 0.02	0.41 ± 0.01
Birth Height (cm)	29.2 ± 0.5	29.8 ± 0.6	30.5 ± 0.5	29.2 ± 0.4
Weight gain				
Birth – 30 mo	3.49 ± 0.20	3.67 ± 0.23	3.76 ± 0.20	3.47 ± 0.15
Height Gain				
Birth – 30 mo	35.5 ± 0.9	37.1 ± 0.9	35.3 ± 0.9	35.3 ± 0.6