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No pubertal growth spurt, rapid bone maturation, and menarche post GnRHa treatment in girls with precocious puberty

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No Pubertal Growth Spurt, Rapid Bone Maturation, and Menarche Post GnRHa Treatment in Girls with Precocious Puberty

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Abstract:	Objective To study total growth, rate of bone maturation and menarche after discontinuation of Gonadotropin releasing hormone agonist (GnRHa) treatment for central precocious puberty (CPP). Methods Twenty girls with CPP on treatment with GnRHa were followed from discontinuation of treatment to final height (FH). Height, height velocity (HV), and bone age were measured every 6 months. Age at menarche was collected. Results Once treatment is discontinued, rate of bone maturation (bone age [BA]/chronological [CA]) accelerated from 0.7 ± 0.3 at end of treatment to 1.2 ± 0.8 post treatment, similar to BA/CA prior to treatment. BA at treatment discontinuation ranged from 11 – 14 years. On average, treatment was stopped when CA was within 9 months of BA. All girls continued to grow from end of treatment to menarche averaging an increase of 4.7 ± 3.7 cm, with HV 3.2 ± 2.0 cm/y. Post-menarche they grew an additional 4.6 ± 2.1 cm, with HV 2.4 ± 1.9 cm/y. Acceleration of HV was not seen post treatment. The younger the BA at initiation or completion of treatment, the longer time to menarche. No one had menarche prior to a BA of 12.5 y. Conclusion A pubertal growth spurt does not usually occur after treatment with GnRHa in girls with CPP. Rate of bone maturation accelerates post treatment. These factors are important in assessing optimal height outcome and decisions regarding cessation of treatment. This study will help clinicians give patients and families better estimates of growth and onset of menarche post treatment.

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Thank you for your review of our manuscript entitled "No Pubertal Growth Spurt, Rapid Bone Maturation, and Variable Menarche Post GnRHa Treatment in girls with Central Precocious Puberty" for publication in *The Journal of Pediatric Endocrinology & Metabolism*.

We are submitting a revised version per the reviewers' comments. A point by point rebuttal is as follows:

Reviewer: 1

"This is an outstanding manuscript reporting results from a study that validates what many in this field believed but had insufficient data to confirm. While the study is relatively small, it is extremely well designed, conducted and analyzed. This paper will provide the community with important information on the management of this population of patients. The only, very minor, suggestion I have is to go back and have the bone age films read by a single, blinded individual (if this was not already done). This will further strengthen the already excellent data presented in this manuscript."

Authors' response: The bone age readings were by a single, blinded individual. The methods contained the statement of the single individual, so we added the word, "blinded" which was how the readings were done. We also added that 2 investigators read the images, as AB completed her fellowship prior to end of the study. Page 5, line 111, now reads, "The bone maturation was assessed with a left hand radiograph (BA), interpreted by one of two blinded investigators (AB, KK) according to the Greulich and Pyle method (11). There were no differences in readings between investigators on 20 independently read images."

Reviewer: 2

"1. It would be helpful if the authors included the number of girls in their clinic eligible for the study, the number who consented and the number who dropped out before completion. I was surprised that "Growth was considered complete when 3 consecutive heights were all within 0.5 cm (HV ≤ 0.5 cm/yr)". Wouldn't one measurement with such a low GV have sufficed to establish final height, and did they lose any patients because they dropped out before the 3rd visit showing no growth?"

Authors' response: This is an excellent point. Three heights were used to be sure we captured end of height growth. Even though we are very careful with our stadiometer height measurements, we know that variability is possible and we wanted a study ensuring adult height data. Page 5, line 116, was edited as follows, "All girls participating in the study had a BA x-ray obtained every 6 months. Growth was considered complete when 3 consecutive heights were all within 0.5 cm (HV ≤ 0.5 cm/yr), to ensure no further growth from measurement error." We do not have record of number of girls to whom the study was offered, but we added the following sentence about about those who dropped out on page 4, line 83, "Twenty-one girls agreed to participate.

One girl dropped out prior to end of growth. One girl did not return for confirmatory end of growth height measure, but was included as last two height measures were within 0.7 cm and bone age was 15 years. We suspect that follow up was so consistent because these girls were treated in our clinic by one provider for many years and were thankful for continued care."

"2. I would like to see a better discussion of the cost-implications of their findings. For many girls with CPP, the main rationale for treatment is not to improve on PAH, which is often normal in the girls who are tall at the time of diagnosis, but to mitigate the stress of early menses. I have tended to stop treatment around age 10 in such girls if I feel they could handle menses by age 11. Continuing treatment until age 11-12.5, as was standard in this cohort, should in my view, be considered mainly in those girls with a PAH which is subnormal (e.g. <155 cm), even if the PAH is below the MPH when parents are tall. An extra year or 2 of GnRH therapy can cost quite a bit and may not be needed routinely."

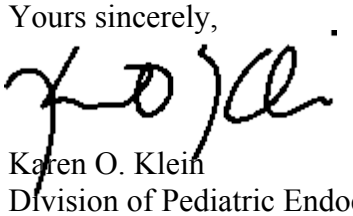
Authors' response: The cost-implications are not the focus of this study or discussion, so the editors can decide whether to add the following to the discussion or not. Other than cost-implications, the reviewer's question raises the discussion of whether longer treatment duration affects the results, so the following was added to page 12, line 259, "The girls in this study were all treated until almost 11 yo or more in order to maximize height benefit as well has have their physical findings of puberty coincide with their peers. When the primary goal of treatment is prevention of early menarche, treatment is often discontinued between 10 – 11 yo, anticipating onset of menarche on average 18 months post treatment. While not the focus of this study, the cost-implications of continuing treatment should always be kept in mind (reference added: Kaplowitz PB, Backeljauw PF, Allen DB. Toward more targeted and cost-effective gonadotropin-releasing hormone analog treatment in girls with central precocious puberty. Horm Res Paediatr 2018;90(1):1-7). Aside from cost-implications, however, the range of onset of menarche is 3 months to 2.5 years post treatment, similar to other published studies. Therefore, the older age at discontinuation in this cohort is unlikely to have affected the results of time to menarche or growth post treatment. It is always clinically relevant to inform families that a 10 yo girl who stops treatment may have menarche by 10 years 3 months, which would still be quite early after years of treatment to delay menarche."

"3. It would be nice if we could advise parents as to how long to menarche after cessation of therapy and yet this and other studies show that the interval is between 3 and 20 months. Do the authors have any theories as to why this is so variable?"

Authors' response: We continue to look for predictors of this variability, and add for the first time, some data on the relationship with bone age. We added the following sentence to page 13, line 291, "Since time to menarche post treatment is multifactorial and not yet able to be predicted, this study adds some guidelines and suggests continuation of treatment beyond a bone age of 12.5 years in younger girls, where earlier menarche continues to be a concern."

We thank you for your consideration of this manuscript and hope it is not acceptable for publication.

Yours sincerely,



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No Pubertal Growth Spurt, Rapid Bone Maturation, and Menarche Post GnRHa Treatment in Girls with Precocious Puberty

Running Title:

Growth and Menses in CPP

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Disclosure Summary

Karen Klein is a consultant for AbbVie Pharm, Arbor Pharm, and Tolmar Pharm. Audrey Briscoe and Katherine Chen have nothing to disclose

Abstract

Objective To study total growth, rate of bone maturation and menarche after discontinuation of Gonadotropin releasing hormone agonist (GnRHa) treatment for central precocious puberty (CPP).

Methods Twenty girls with CPP on treatment with GnRHa were followed from discontinuation of treatment to final height (FH). Height, height velocity (HV), and bone age were measured every 6 months. Age at menarche was collected.

Results Once treatment is discontinued, rate of bone maturation (bone age [BA]/chronological [CA]) accelerated from 0.7 ± 0.3 at end of treatment to 1.2 ± 0.8 post treatment, similar to BA/CA prior to treatment. BA at treatment discontinuation ranged from 11 – 14 years. On average, treatment was stopped when CA was within 9 months of BA.

All girls continued to grow from end of treatment to menarche averaging an increase of 4.7 ± 3.7 cm, with HV 3.2 ± 2.0 cm/y. Post-menarche they grew an additional 4.6 ± 2.1 cm, with HV 2.4 ± 1.9 cm/y. Acceleration of HV was not seen post treatment. The younger the BA at initiation or completion of treatment, the longer time to menarche. No one had menarche prior to a BA of 12.5 y.

Conclusion A pubertal growth spurt does not usually occur after treatment with GnRHa in girls with CPP. Rate of bone maturation accelerates post treatment. These factors are important in assessing optimal height outcome and decisions regarding cessation of treatment. This study will help clinicians give patients and families better estimates of growth and onset of menarche post treatment.

Introduction

Gonadotropin releasing hormone agonist (GnRHa) treatment is standard of care for central precocious puberty (CPP) (1-4). The treatment slows or halts pubertal progression, slows the rate of bone maturation, suppresses gonadotropins, and usually decreases height velocity (HV) to normal pre-pubertal levels. Predicted adult height (PAH) continues to improve while on treatment, and then slowly decreases post treatment as rapid bone maturation resumes.

The decision regarding when to stop treatment needs to be individualized (5) and is based on multiple factors. These include the child reaching an appropriate age for mid-puberty to resume and a PAH reasonable for genetic potential, with caution that PAH may decrease once treatment is discontinued (6). After discontinuation of GnRHa, the pubertal growth spurt typically does not resume, however growth will continue at a slower velocity until cessation of growth. This emphasizes the importance of not stopping treatment prematurely, as the remaining growth may be minimal. There are limited studies of growth and HV after discontinuation of GnRHa, especially as related to onset of menses (7). We studied rate of bone maturation and growth after GnRHa treatment was stopped. We hypothesized that HV will not accelerate after treatment is discontinued, and rate of bone maturation will accelerate. These results are helpful to parents anticipating pubertal development, growth and menses post treatment, and provide important information for the physician when deciding how long to continue GnRHa treatment based on anticipated growth and menarche post treatment.

Materials and Methods

Study population

All girls in our pediatric endocrinology clinic (Rady Children's Hospital, San Diego, CA) who were treated with the GnRHa Lupron Depot Ped (8,9) for CPP were offered participation in this study once ready to discontinue treatment. Recruitment took place between 2009 – 2012, and study follow-up visits were completed by 2016 once all girls reached final height. Determination of treatment cessation was based on physician judgement and parent request. Twenty-one girls agreed to participate. One girl dropped out prior to end of growth. One girl did not return for confirmatory end of growth height measure, but was included as last two height measures were within 0.7 cm and bone age was 15 years. We suspect that follow up was so consistent because these girls were treated in our clinic by one provider for many years and were thankful for continued care. All girls and their parents signed an assent and consent approved by our institutional review board. All authors complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Girls were included if chronological age (CA) at onset of pubertal symptoms was less than 8 years old, breast development was consistent with at least pubertal stage 2 at diagnosis, bone age (BA) was more than 2 SD above the mean for CA or PAH was at least 2 inches (5.08 cm) below midparental height (MPH). Pubertal stage was defined according to a modification of Tanner's description (10) to include breast palpation. Two patients were included without definitive data on onset of breast development. Patient #8 is included even though age at onset of CPP is listed at 8 y 4 m. This was by parent report of noticing breasts, however, since no physician saw her prior to that time and breast stage was already fully stage 3 with BA 3 years advanced, we suspect pubertal onset was likely prior to age 8 years. Patient #1 presented with menarche at 8 y

10 m, She had stage 4 breast and a report that breast had been present since birth. Her BA was 3 years advanced, so likely onset of CPP was less than 8 years old.

CPP was defined as a peak LH level above 5 IU/L during an aqueous leuprolide stimulation test. All girls had idiopathic central precocious puberty. Girls were excluded if they had any other condition interfering with growth, such as skeletal dysplasia, cerebral palsy, or a chronic illness requiring treatment that may have impacted their growth potential. Stable patients with intermittent asthma or patients on topical acne medication were included.

Study design

Girls underwent a general clinical examination and pubertal staging every 6 months until final height (FH) was reached. At each visit, height (using a Holtain stadiometer) and weight were recorded. The bone maturation was assessed with a left hand radiograph (BA), interpreted by one of two ~~a single~~ blinded investigators (AB, KK) according to the Greulich and Pyle method (11). There were no differences in readings between investigators on 20 independently read images. PAH was calculated at each visit according to Bailey-Pineau tables (11). All girls participating in the study had a BA x-ray obtained every 6 months. Growth was considered complete when 3 consecutive heights were all within 0.5 cm (HV ≤ 0.5 cm/yr), to ensure no further growth from measurement error. Girls were asked to record all menstrual cycles in detail in a calendar, with duration, severity, and timing noted.

Statistical analysis

All data is presented as mean ± SD. Linear regression analysis was used for correlations. MPH was calculated with parental report of height at the first visit; MPH=[maternal height

(cm)+paternal height(cm)-13]/2. MPH was available for 19/20 subjects. Height velocity was calculated based on the data as cm per year change (cm/y).

Rate of change of BA was calculated as the change in BA in years divided by time interval to yield a change in units of year per chronological year (y/y).

Results

Twenty girls remained in the study until FH was reached. Baseline characteristics are shown in Table 1, 2 and 3. GnRHa treatment was stopped at an average age of 11.8 ± 0.6 years (range 10.6 – 13.2 years). Girls were treated for an average duration of 4.5 ±2.4 years (range 1.5-10.4 years). The average reported age at onset of puberty was 5.8 ± 2.1 years (range 1.0 - 8.4 years). One girl was included in the study who had her onset of puberty estimated by parents at 8.4 years, however she had rapid progression of puberty, her BA was 2 years advanced, and her PAH was below her MPH.

Bone maturation

BA at discontinuation of treatment ranged from 11 – 14 years (average 12.5 ± 0.8 years). The BA at the onset and end of treatment both had a negative correlation with time to menarche post treatment ($0<0.01$). Girls with a younger BA at onset or end of treatment developed menarche later than girls with an older BA at either time point during treatment ($p<0.01$)(Figure 1). BA at end of treatment also had a negative correlation with the amount of growth accrued post

treatment, such that the younger the BA at end of treatment, the greater overall growth post-treatment ($p<0.01$)(Figure 2). No one had menarche prior to a bone age of 12.5 y.

The rate of change of BA advancement averaged 1.3 ± 0.15 prior to treatment, and decreased during treatment to 0.7 ± 0.3 years per chronological year (y/y). Post treatment, rate of BA change accelerated to 1.2 ± 0.8 y/y (range 0 – 3.0y/y). (Table 2, Figure 3).

On average, treatment was stopped when CA was within 9 months of BA. Three girls had a BA > 2 year beyond CA at end of treatment. They also had the 3 oldest BA at the end of treatment, but 2 girls surpassed their MPH (1 did not have MPH available).

Growth

Average HV on treatment was 4.8 ± 1.2 cm/y (range 2.9 - 7.6). All girls continued to grow from end of treatment to menarche with an average of 4.7 ± 3.7cm, with an average height velocity of 3.2 ± 2.0 cm/y. On average, they grew an additional 4.6 ± 2.1 cm from menarche until FH, with a HV of 2.4 ± 1.9 cm/y during that time. (Fig 4) Only 2/20 girls, had an increased height velocity (>6 cm/yr) after treatment was discontinued, similar to a pubertal height velocity. Growth after discontinuation of treatment ranged from 4.6 – 12.4 cm in girls who stopped GnRHa with a BA≥ 13 years, and 7.9 – 16.7 cm in girls who stopped GnRHa with a BA< 13 years.

Menarche

After treatment was discontinued, the average age at menarche was 13.2 ± 0.9 years, however ranged from 11.3 – 14.6 years. The average time from end of treatment to menarche was 15.2 ± 7.4 months, with a range of 3 – 30 months. The younger the BA was at initiation or completion of treatment, predicted a longer time to menarche. Menses occurred at regular intervals (defined

as cycles 21 – 35 days long) in some girls immediately post treatment, although other girls continued to have irregular menses up to 3 years post treatment. No predictions of time to regular menstrual cycles were found. When data was analyzed to compare girls with onset of CPP $\leq$6 yo compared to those > 6 yo, the only significant difference, other than the obvious age and BA, was age at onset of menarche. The younger girls had menarche on average one year later than the older girls, but at a similar BA.

Adult final height

FH occurred an average of 2 years post menarche, with a range of 0 to 3.7 years. FH was within 3 inches of MPH in 18/19 (1 child without MPH data) girls. Average difference between MPH and FH (MPH-FH) was 0.5 ± 6.0 cm. MPH was reached or surpassed by 11/19 (58%) girls treated with GnRHa for CPP. One girl attained a FH 14 cm below MPH, however her parents were tall (MPH =170 cm), pubertal onset was quite early at 5.5 years and her BA was 3.75 years advanced at start of treatment. Another girl attained a FH quite below her MPH, however she also had 2 tall parents (MPH = 176.5 cm) and onset of puberty at age 1.5 years old, although was able to reach a FH of 168 cm (Table 3).

Discussion

Time to menarche is a great concern for many children and their families. The range of onset of menarche post GnRHa treatment is consistent across studies (12-15) from 3 months to 3 years. The present study confirms that range but shows for the first time the relationship between BA and menarche. We found that the younger the BA was prior to treatment or the younger the BA was at the end of treatment, predicted a longer time to menarche. No girls had menarche prior to

BA of 12.5 years. When girls were compared by onset of CPP ≤ 6 years versus > 6 yo, the younger girls had menarche on average one year later than the older girls, but at a similar BA. This supports the importance of BA in predicting menarche. There were no other differences between these 2 groups, supporting the robustness of this population with truly rapidly progressing CPP, rather than a mixed population of early normal puberty and CPP. This is one of the strengths of the population, since many publications likely include some early normal puberty when girls close to 8 years old are studied.

Rate of BA advancement prior to treatment in girls with CPP is accelerated. On GnRHa treatment, rate of advancement slows to less than 1 year per year when treatment is optimal, and therefore leads to increases in growth potential and FH (20). Once treatment is stopped, we suspected that rate of BA advancement accelerates, and this is the first study with data to support that pathophysiology. Prior to GnRHa treatment rate of BA advancement in this population on average was 1.3 years per chronological year (5). This decreases to an average of 0.7 years per year on treatment. Post treatment, rate of BA advancement accelerates back to pretreatment rapid maturation averaging 1.2 years per year. This is expected since whatever process causes rapid bone maturation in CPP, is not cured by GnRHa treatment, but rather slowed down during treatment. Lazar et al (21) reported data from 115 girls post-GnRHa treatment. They describe greater height gain post treatment in girls who had onset of puberty prior to 6 years old compared to older girls. Final height in the older groups was less than PAH at end of treatment. They measured BA every 12 months and report that BA at end of treatment significantly contributed to prediction of height gain post treatment. However, they do not report change in BA post treatment. They raised the question of whether post-treatment growth would have been greater if

BA was closer to 11 at end of treatment. The present data support the opposite hypothesis, since rate of BA advance accelerates post treatment, stopping treatment at an older BA may lead to greater final height, achieved by more growth on treatment rather than more growth post-treatment. Shim et al also looked at growth post-GnRHa treatment in 85 boys but compared only PAH at end of treatment to final height, and did not delineate BA or growth rate changes over the years post treatment (22).

On average, treatment was stopped when CA was close to BA. Adequate pubertal suppression with GnRHa theoretically corrects the discrepancy between BA and CA seen prior to treatment. The girls in this study who did not reach a CA within 2 years of BA prior to end of treatment, were treated until an older BA than the rest of the girls, which was likely necessary to achieve improved FH outcomes. This supports the likely the importance of duration of treatment for optimal height outcome. Of course, we cannot know what height the girls would have achieved if treatment had been discontinued earlier, but we have shown the rapid acceleration of rate of bone maturation post treatment so it would be unlikely they would have reached the same FH. Previous studies showed that earlier onset of treatment, less BA advancement, and less delay from onset of CPP to onset of treatment have taller height outcomes as compared to MPH (6,16-19). In the present study, girls with less BA advancement had greater growth post treatment. This is the first study to quantify growth and growth rate between end of treatment and menarche and between menarche and FH. This is very important for clinicians and families when making decisions about the timing for cessation of GnRHa treatment. Those decisions cannot be based on expectations for resumption of a pubertal growth spurt post treatment. Only 2 girls in this population had increased HV to > 6 cm/y post treatment. All others grew at ≤ 5 cm/y after

GnRHa was stopped. An interrupted pubertal growth spurt does not resume post GnRHa treatment for CPP.

The lack of rapid pubertal growth post treatment is likely related to growth plate senescence. Our understanding of growth plate senescence includes the effect of estrogen to first stimulate growth plate chondrogenesis and therefore linear growth, while simultaneously decreasing the number of resting zone chondrocytes, accelerating the rate of chondrocyte proliferation leading to earlier proliferative exhaustion with eventual growth plate fusion (23,24). The early exposure of estrogen in precocious puberty causes early growth plate senescence. Perhaps GnRHa treatment is usually timed to interrupt this process at the point when estrogen has already diminished enough chondrocytes, such that as bone aging continues during treatment and no further increase is possible post treatment. The older the BA was at the beginning of treatment, the less growth is accrued post treatment. We can interrupt the process of CPP causing rapid maturation of BA, but the process of senescence, although slowed during treatment, is not stopped, so we cannot regain all the growth potential that was lost. This is further support of continuation of GnRHa longer in those girls with late onset of treatment who have short height potential compared to MPH.

It cannot be determined from this study whether growth would have been different if treatment was stopped earlier in the girls with more advanced BA. However, in all girls HV was good throughout treatment, there was similar amount of growth post treatment, and height outcomes compared to MPH were similar across all girls. This suggests that the individual treatment decisions of when to stop GnRHa treatment led to the best expected outcomes.

The girls in this study were all treated until almost 11 yo or more in order to maximize height benefit as well has have their physical findings of puberty coincide with their peers. When the

primary goal of treatment is prevention of early menarche, treatment is often discontinued between 10 – 11 yo, anticipating onset of menarche on average 18 months post treatment. While not the focus of this study, the cost-implications of continuing treatment should always be kept in mind (25). Aside from cost-implications, however, the range of onset of menarche is 3 months to 2.5 years post treatment, similar to other published studies. Therefore, the older age at discontinuation in this cohort is unlikely to have affected the results of time to menarche or growth post treatment. It is always clinically relevant to inform families that a 10 yo girl who stops treatment may have menarche by 10 years 3 months, which would still be quite early after years of treatment to delay menarche."

The major strengths of this study include following girls to FH with measures of growth, bone maturation and menses every 6 months for as long as 5 – 6 years post GnRHa treatment. This study also has girls who continued GnRHa until 13 – 14 years with good growth during the treatment interval, as well as post treatment. We found that 18/19 had FH within 3 inches of MPH, with more than half of the girls reaching or surpassing MPH. The wide range of age at onset of puberty allowed assessment of age as a factor contributing to timing of menarche.

The major limitations include the small number of participants, although this does show a real-world example from one center. All girls were offered participation, but not all girls wanted to continue to be followed every 6 months once treatment was discontinued, so there may have been some bias in those who continued coming. For example, those who did not continue follow up may have been obviously growing less and therefore not interested in continued bone age to assess potential growth. It is unlikely that those who preferred not to continue were those growing more rapidly, but that cannot be determined. Another limitation is that bone age at

cessation of treatment was not randomized, so physician decisions to stop treatment could bias outcomes. However, it would not be ethical to randomize treatment cessation if physician assessment indicated possible benefit to continued treatment.

In conclusion, the present study quantitates growth after treatment with GnRHa in girls with CPP. In most girls, there is no resumption of pubertal growth spurt post GnRHa treatment. Time to onset of menarche post-GnRHa treatment is proportionate to BA at the start as well as the end of treatment, with no girls in the present study having menarche prior to BA of 12.5 years. Since time to menarche post treatment is multifactorial and not yet able to be predicted, this study adds some guidelines and suggests continuation of treatment beyond a bone age of 12.5 years in younger girls, where earlier menarche continues to be a concern.

Rate of bone maturation accelerates post treatment. These factors are important in assessing potential height outcome and the decision regarding timing for cessation of treatment. This study will help clinicians give patients and families better estimates of growth and onset of menarche post treatment.

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Table 1. Patient age and time to menarche

Patient ID	Ethnicity	CA at onset CPP	CA at start Rx	CA at stop Rx	Duration of Rx (y)	CA at Menarche	Time post Rx to Menarche (months)	Time menarche to FH (y)
20	White	1.5	1.5	11.2	9.7	13.5	28.0	0.7
9	Hispanic	5.0	8.8	12.8	4.0	13.6	11.0	1.2
18	Hispanic	7.5	8.0	11.0	3.0	13.5	30.0	0.0
6	White	7.5	7.5	11.3	3.8	12.3	12.0	2.2
12	White	5.6	6.6	11.6	5.0	13.3	22.0	3.2
19	Asian	6.6	7.8	11.8	4.0	13.2	16.4	1.6
21	Hispanic	1.0	1.6	12.0	10.4	14.2	26.4	1.1
5	White	7.0	8.2	12.1	3.9	13.8	20.0	3.2
15	White	5.9	7.3	13.3	6.0	14.6	16.2	1.7
3	White	7.8	9.3	11.8	2.5	12.6	10.0	3.7
10	White	2.4	3.8	12.5	8.7	14.2	19.0	2.8
4	White	6.0	8.9	11.5	2.6	13.9	5.0	2.3
16	White	5.5	6.3	11.7	5.4	13.0	16.0	0.0
2	White	7.3	8.5	11.9	3.4	12.3	8.0	2.6
7	Hispanic	7.0	7.8	12.0	4.2	13.2	14.0	2.3
11	Hispanic	6.0	8.5	12.6	4.1	13.6	11.0	2.0
17	Hispanic	5.0	8.6	12.6	4.0	13.8	13.8	2.5
1	Hispanic	*	9.2	10.7	1.5	11.3	7.0	2.7
13	White	7.4	8.8	11.3	2.6	11.6	3.0	1.2
8	Asian	8.4	9.3	11.5	2.2	12.8	16.0	2.2
Average		5.8	7.3	11.8	4.5	13.2	15.2	2.0
Minimum		1.0	1.5	10.7	1.5	11.3	3.0	0.0
Maximum		8.4	9.3	13.3	10.4	14.6	30.0	3.7
SD		2.1	2.3	0.6	2.4	0.9	7.4	1.0

* menarche at 8.8, but breast onset not known
BA = bone age, Rx = treatment, FH = final height, CA - chronological age, CPP = central precocious puberty

Table 2. Bone age data during and after treatment with GnRHa

Patient ID	BA start Rx (y)	BA/CA start Rx	BA end of Rx (y)	BA at menarche (y)	BA at FH (y)	BA/CA during Rx	BA/CA end Rx to menarche	BA/CA menarche to FH
20	**	**	11.0	15.0	15.0	**	1.7	0.0
9	8.8	1.0	11.5	13.5	16.0	0.7	2.2	2.2
18	10.0	1.3	12.0	16.5	16.8	0.7	1.8	0.5
6	**	**	12.0	15.0	15.0	**	3.0	0.0
12	6.8	1.0	12.0	12.5	16.5	1.0	0.3	1.3
19	7.8	1.0	12.0	13.8	15.5	1.1	1.3	1.1
21	2.0	1.3	12.0	15.0	15.5	1.0	1.4	0.4
5	***	***	12.0	13.5	17.0	***	0.9	1.1
15	10.0	1.4	12.0	14.0	16.3	0.3	1.5	1.4
3	11.0	1.2	12.5	13.8	***	0.6	1.5	***
10	4.6	1.2	12.5	14.0	16.5	0.9	0.9	0.9
4	12.0	1.3	13.0	16.0	17.0	0.4	1.23	0.4
16	10.0	1.6	13.0	***	***	0.6	***	***
2	11.0	1.3	13.0	13.0	16.0	0.6	0.0	1.2
7	11.0	1.4	13.0	14.3	15.0	0.5	1.1	0.3
11	11.0	1.3	13.0	15.0	15.5	0.5	2.2	0.3
17	10.0	1.2	13.0	13.0	15.8	0.8	0.0	1.1
1	11.5	1.3	13.5	13.5	16.0	1.4	0.0	0.9
13	12.0	1.4	13.6	13.8	17.0	0.6	0.8	2.6
8	11.5	1.2	14.0	15.0	17.0	1.1	0.8	0.9
Average	9.5	1.3	12.5	14.2	16.1	0.7	1.2	0.9
Minimum	2.0	1.0	11.0	12.5	15.0	0.3	0.0	0.0
Maximum	12.0	1.6	14.0	16.5	17.0	1.4	3.0	2.6
SD	2.8	0.2	0.8	1.0	0.7	0.3	0.81	0.7

** came to us on treatment without records

*** missing data

BA = bone age, Rx = treatment, FH = final height, CA - chronological age

Table 3. Growth changes during and after treatment with GnRHa

Patient ID	Ht start of Rx (cm)	Ht end Rx (cm)	Growth end Rx to menarche (cm total)	HV end Rx to menarche (cm/y)	Ht at menarche (cm)	FH (cm)	CA at FH (y)	Growth menses to FH (cm total)	HV menarche to FH (cm/y)	MPH (cm)	MPH - FH (cm)
20	**	154.9	7.4	3.2	162.3	168.0	14.2	5.7	8.5	176.4	8.4
9	134.0	152.7	2.6	2.8	155.3	161.0	14.8	5.7	5.0	156.1	-4.9
18	141.7	162.5	8.0	3.2	170.5	170.5	14.0	0.0	0.0	168.5	-2.0
6	**	155.6	2.6	2.6	158.2	165.2	14.5	7.0	3.2	157.3	-7.9
12	114.5	140.3	10.6	5.8	150.9	155.5	16.4	4.6	1.5	155.1	-0.4
19	122.8	152.7	10.3	7.5	163.0	164.7	14.8	1.7	1.0	161.1	-3.6
21	80.5	152.7	9.3	4.2	162.0	165.5	15.3	3.5	3.1	171.3	5.8
5	125.0	141.4	7.9	4.7	149.3	153.3	17.0	4.0	1.3	161.1	7.8
15	119.7	146.6	5.5	4.1	152.1	154.5	16.3	2.4	1.5	162.4	7.9
3	147.5	163.4	3.2	3.8	166.6	172.0	16.3	5.4	1.4	171.3	-0.7
10	106.5	160.0	10.5	6.6	170.5	176.7	17.0	6.2	2.2	168.8	-7.9
4	142.3	153.5	0.0	0.0	153.5	160.5	16.2	7.0	3.1	152.3	-8.3
16	120.7	147.6	3.0	1.3	150.6	155.7	15.8	5.1	0.0	170.0	14.3
2	136.9	162.9	2.1	3.1	165.0	169.5	14.9	4.5	1.7	165.0	-4.6
7	130.5	142.7	3.6	3.1	146.3	148.5	15.5	2.2	1.0	156.1	7.6
11	127.4	148.7	0.6	0.7	149.3	153.3	15.6	4.0	2.0	158.6	5.3
17	124.3	140.2	3.6	3.1	143.8	152.6	16.3	8.8	3.5	153.5	0.9
1	135.0	142.3	1.8	3.1	144.1	151.0	13.9	6.9	2.6	***	***
13	140.8	151.7	0.0	0.0	151.7	156.3	12.8	4.6	3.7	152.9	-3.4
8	147.5	155.8	1.4	1.0	157.2	160.4	15.0	3.2	1.5	156.1	-4.3
Average	127.6	151.4	4.7	3.2	156.1	160.7	15.3	4.6	2.4	161.8	0.5
Minimum	80.5	140.2	0.0	0.0	143.8	148.5	12.8	0.0	0.0	152.3	-8.3
Maximum	147.5	163.4	10.6	7.5	170.5	176.7	17.0	8.8	8.5	176.4	14.3
SD	16.4	7.6	3.7	2.0	8.3	7.9	1.1	2.1	1.9	7.4	6.7

** came to us on treatment without records

*** missing data

HV = height velocity, Rx = treatment, FH = final height, CA - chronological age, Ht – height, MPH = mid-parental height

384

385

386

387 **Figure Legends**

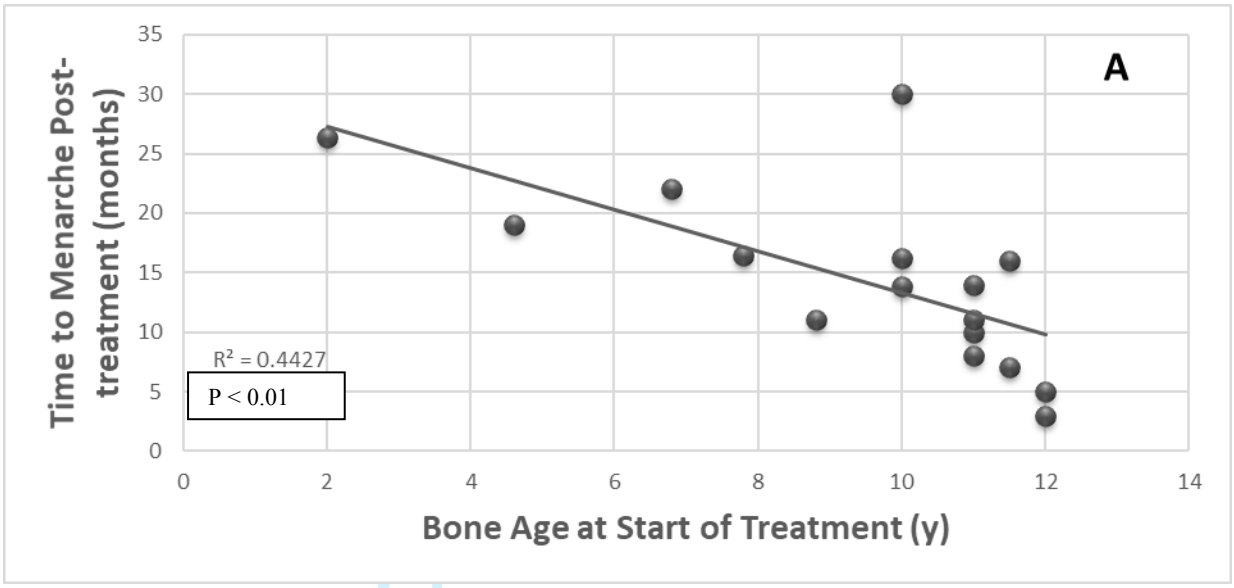
Figure 1: Time to Menarche post GnRHa treatment by bone age at start of treatment (A) and by bone age at end of treatment (B)

Figure 2. Total Growth post GnRHa treatment by BA at end of Treatment

Figure 3. BA/CA ratio from Treatment to Final Height

Figure 4. Height velocity from Treatment to Final Height – Individual patients shown. Solid bold line is average.

Figure 1:


A
Time to Menarche Post-treatment (months)
Bone Age at Start of Treatment (y)
R² = 0.4427
P < 0.01
0
5
10
15
20
25
30
35
0
2
4
6
8
10
12
14

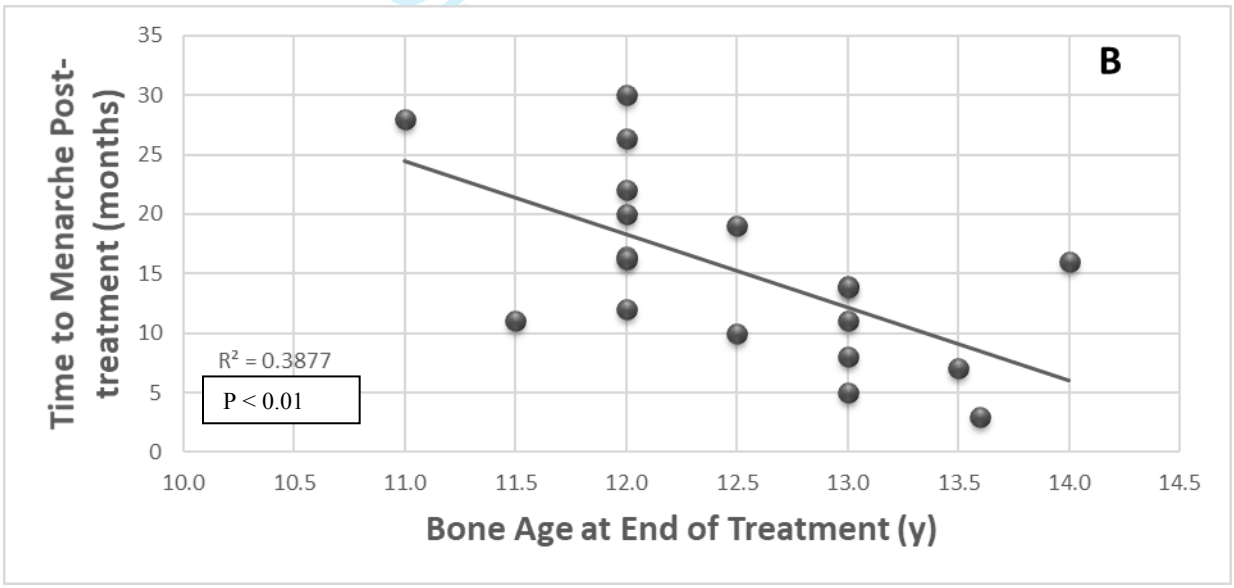

B
Time to Menarche Post-treatment (months)
Bone Age at End of Treatment (y)
R² = 0.3877
P < 0.01
0
5
10
15
20
25
30
35
10.0
10.5
11.0
11.5
12.0
12.5
13.0
13.5
14.0
14.5

Figure 2.

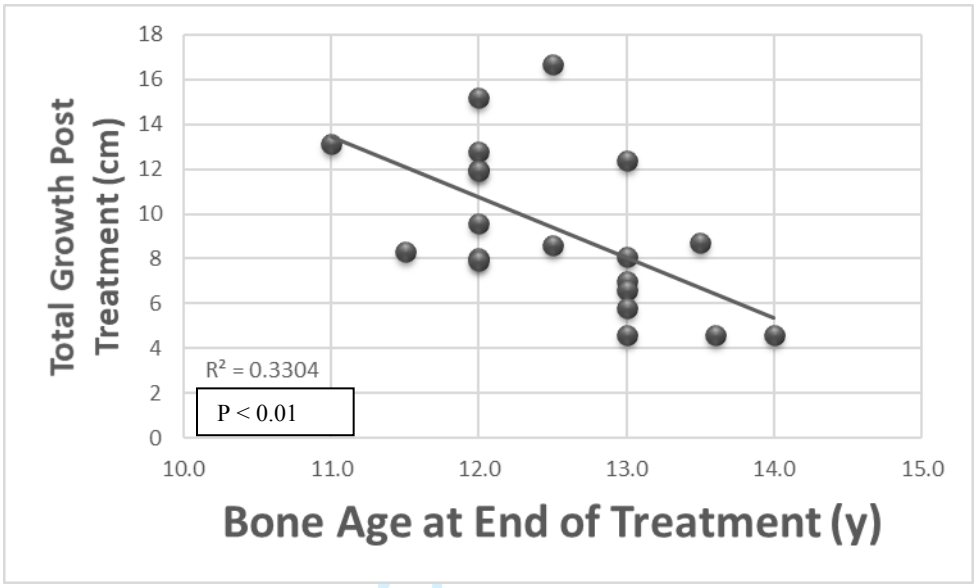


Figure 3.

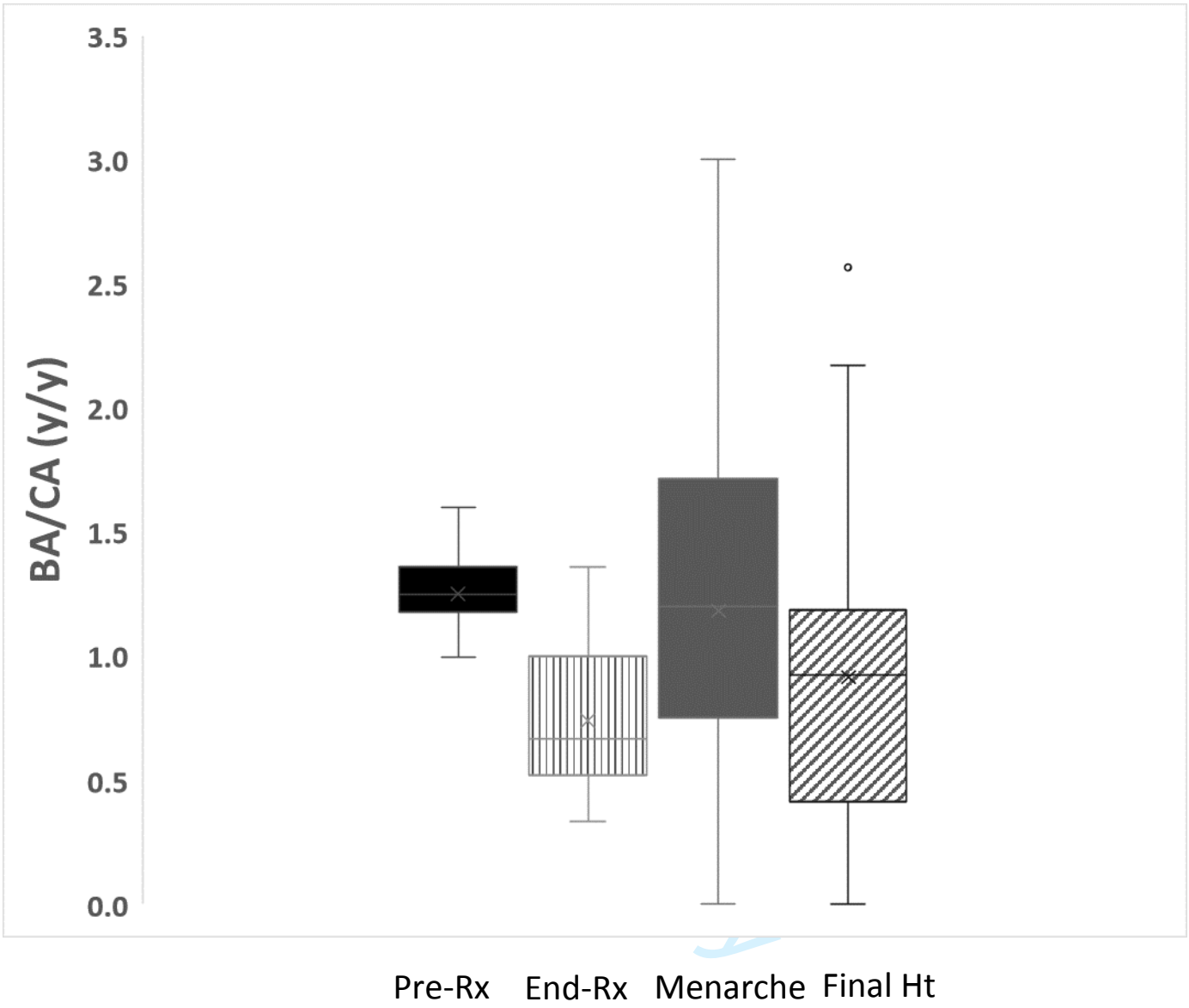
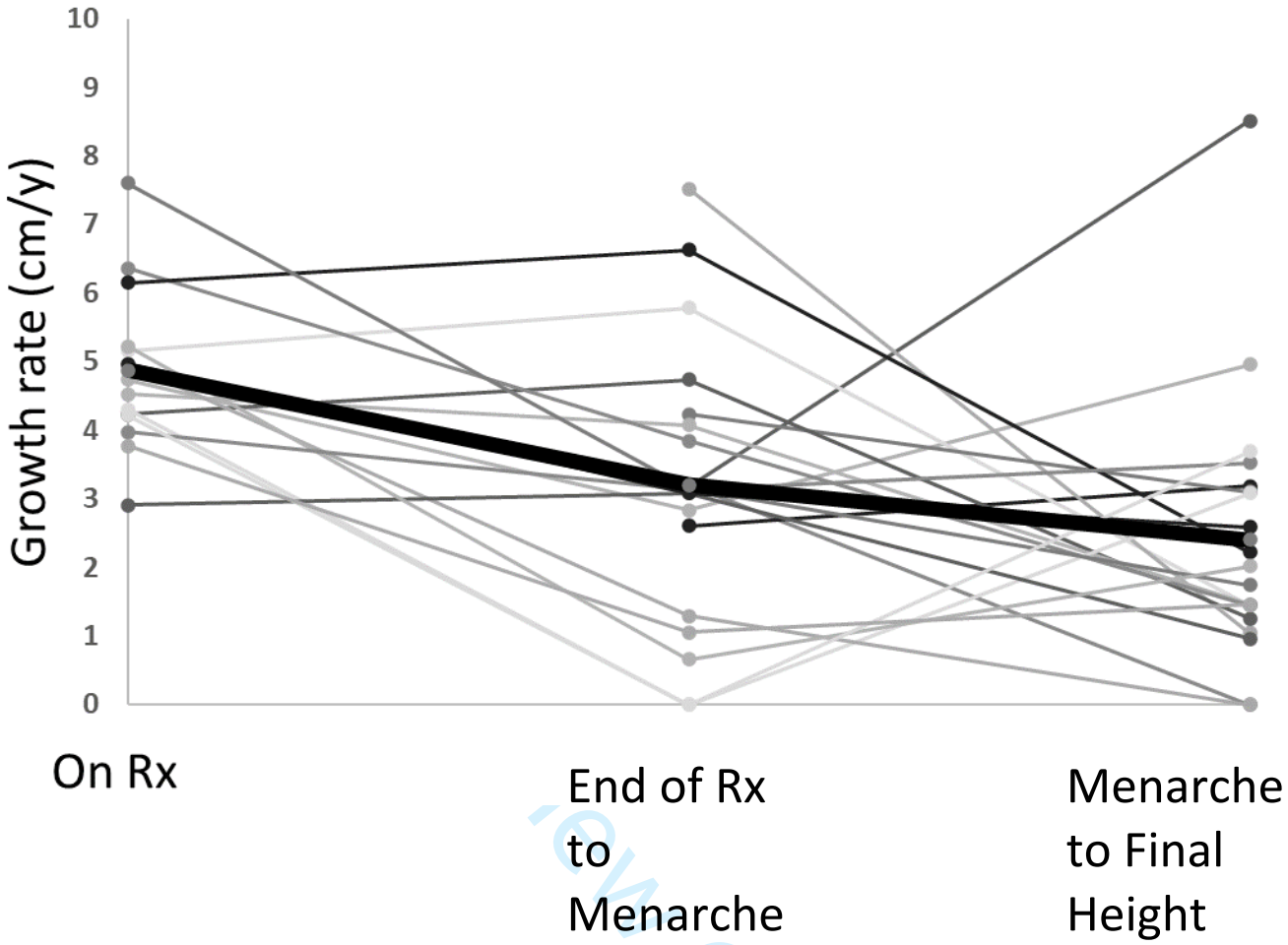


Figure 4.

459


Growth rate (cm/y)
10
9
8
7
6
5
4
3
2
1
0
On Rx
End of Rx to Menarche
Menarche to Final Height

8/29/22

No Pubertal Growth Spurt, Rapid Bone Maturation, and Menarche Post GnRHa Treatment in Girls with Precocious Puberty

Running Title:

Growth and Menses in CPP

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Keywords: precocious puberty, Leuprolide, LH, growth, menses, menarche

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Disclosure Summary

Karen Klein is a consultant for AbbVie Pharm, Arbor Pharm, and Tolmar Pharm. Audrey Briscoe and Katherine Chen have nothing to disclose

Abstract

Objective To study total growth, rate of bone maturation and menarche after discontinuation of Gonadotropin releasing hormone agonist (GnRHa) treatment for central precocious puberty (CPP).

Methods Twenty girls with CPP on treatment with GnRHa were followed from discontinuation of treatment to final height (FH). Height, height velocity (HV), and bone age were measured every 6 months. Age at menarche was collected.

Results Once treatment is discontinued, rate of bone maturation (bone age [BA]/chronological [CA]) accelerated from 0.7 ± 0.3 at end of treatment to 1.2 ± 0.8 post treatment, similar to BA/CA prior to treatment. BA at treatment discontinuation ranged from 11 – 14 years. On average, treatment was stopped when CA was within 9 months of BA.

All girls continued to grow from end of treatment to menarche averaging an increase of 4.7 ± 3.7 cm, with HV 3.2 ± 2.0 cm/y. Post-menarche they grew an additional 4.6 ± 2.1 cm, with HV 2.4 ± 1.9 cm/y. Acceleration of HV was not seen post treatment. The younger the BA at initiation or completion of treatment, the longer time to menarche. No one had menarche prior to a BA of 12.5 y.

Conclusion A pubertal growth spurt does not usually occur after treatment with GnRHa in girls with CPP. Rate of bone maturation accelerates post treatment. These factors are important in assessing optimal height outcome and decisions regarding cessation of treatment. This study will help clinicians give patients and families better estimates of growth and onset of menarche post treatment.

Introduction

Gonadotropin releasing hormone agonist (GnRHa) treatment is standard of care for central precocious puberty (CPP) (1-4). The treatment slows or halts pubertal progression, slows the rate of bone maturation, suppresses gonadotropins, and usually decreases height velocity (HV) to normal pre-pubertal levels. Predicted adult height (PAH) continues to improve while on treatment, and then slowly decreases post treatment as rapid bone maturation resumes.

The decision regarding when to stop treatment needs to be individualized (5) and is based on multiple factors. These include the child reaching an appropriate age for mid-puberty to resume and a PAH reasonable for genetic potential, with caution that PAH may decrease once treatment is discontinued (6). After discontinuation of GnRHa, the pubertal growth spurt typically does not resume, however growth will continue at a slower velocity until cessation of growth. This emphasizes the importance of not stopping treatment prematurely, as the remaining growth may be minimal. There are limited studies of growth and HV after discontinuation of GnRHa, especially as related to onset of menses (7). We studied rate of bone maturation and growth after GnRHa treatment was stopped. We hypothesized that HV will not accelerate after treatment is discontinued, and rate of bone maturation will accelerate. These results are helpful to parents anticipating pubertal development, growth and menses post treatment, and provide important information for the physician when deciding how long to continue GnRHa treatment based on anticipated growth and menarche post treatment.

Materials and Methods

Study population

All girls in our pediatric endocrinology clinic (Rady Children's Hospital, San Diego, CA) who were treated with the GnRHa Lupron Depot Ped (8,9) for CPP were offered participation in this study once ready to discontinue treatment. Recruitment took place between 2009 – 2012, and study follow-up visits were completed by 2016 once all girls reached final height. Determination of treatment cessation was based on physician judgement and parent request. Twenty-one girls agreed to participate. One girl dropped out prior to end of growth. One girl did not return for confirmatory end of growth height measure, but was included as last two height measures were within 0.7 cm and bone age was 15 years. We suspect that follow up was so consistent because these girls were treated in our clinic by one provider for many years and were thankful for continued care. All girls and their parents signed an assent and consent approved by our institutional review board. All authors complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Girls were included if chronological age (CA) at onset of pubertal symptoms was less than 8 years old, breast development was consistent with at least pubertal stage 2 at diagnosis, bone age (BA) was more than 2 SD above the mean for CA or PAH was at least 2 inches (5.08 cm) below midparental height (MPH). Pubertal stage was defined according to a modification of Tanner's description (10) to include breast palpation. Two patients were included without definitive data on onset of breast development. Patient #8 is included even though age at onset of CPP is listed at 8 y 4 m. This was by parent report of noticing breasts, however, since no physician saw her prior to that time and breast stage was already fully stage 3 with BA 3 years advanced, we suspect pubertal onset was likely prior to age 8 years. Patient #1 presented with menarche at 8 y

10 m, She had stage 4 breast and a report that breast had been present since birth. Her BA was 3 years advanced, so likely onset of CPP was less than 8 years old.

CPP was defined as a peak LH level above 5 IU/L during an aqueous leuprolide stimulation test. All girls had idiopathic central precocious puberty. Girls were excluded if they had any other condition interfering with growth, such as skeletal dysplasia, cerebral palsy, or a chronic illness requiring treatment that may have impacted their growth potential. Stable patients with intermittent asthma or patients on topical acne medication were included.

Study design

Girls underwent a general clinical examination and pubertal staging every 6 months until final height (FH) was reached. At each visit, height (using a Holtain stadiometer) and weight were recorded. The bone maturation was assessed with a left hand radiograph (BA), interpreted by one of two blinded investigators (AB, KK) according to the Greulich and Pyle method (11). There were no differences in readings between investigators on 20 independently read images. PAH was calculated at each visit according to Bailey-Pineau tables (11). All girls participating in the study had a BA x-ray obtained every 6 months. Growth was considered complete when 3 consecutive heights were all within 0.5 cm (HV $\leq$ 0.5 cm/yr), to ensure no further growth from measurement error. Girls were asked to record all menstrual cycles in detail in a calendar, with duration, severity, and timing noted.

Statistical analysis

All data is presented as mean ± SD. Linear regression analysis was used for correlations. MPH was calculated with parental report of height at the first visit; MPH=[maternal height

(cm)+paternal height(cm)-13]/2. MPH was available for 19/20 subjects. Height velocity was calculated based on the data as cm per year change (cm/y).

Rate of change of BA was calculated as the change in BA in years divided by time interval to yield a change in units of year per chronological year (y/y).

Results

Twenty girls remained in the study until FH was reached. Baseline characteristics are shown in Table 1, 2 and 3. GnRHa treatment was stopped at an average age of 11.8 ± 0.6 years (range 10.6 – 13.2 years). Girls were treated for an average duration of 4.5 ±2.4 years (range 1.5-10.4 years). The average reported age at onset of puberty was 5.8 ± 2.1 years (range 1.0 - 8.4 years). One girl was included in the study who had her onset of puberty estimated by parents at 8.4 years, however she had rapid progression of puberty, her BA was 2 years advanced, and her PAH was below her MPH.

Bone maturation

BA at discontinuation of treatment ranged from 11 – 14 years (average 12.5 ± 0.8 years). The BA at the onset and end of treatment both had a negative correlation with time to menarche post treatment ($0<0.01$). Girls with a younger BA at onset or end of treatment developed menarche later than girls with an older BA at either time point during treatment ($p<0.01$)(Figure 1). BA at end of treatment also had a negative correlation with the amount of growth accrued post treatment, such that the younger the BA at end of treatment, the greater overall growth post-treatment ($p<0.01$)(Figure 2). No one had menarche prior to a bone age of 12.5 y.

The rate of change of BA advancement averaged 1.3 ± 0.15 prior to treatment, and decreased during treatment to 0.7 ± 0.3 years per chronological year (y/y). Post treatment, rate of BA change accelerated to 1.2 ± 0.8 y/y (range 0 – 3.0y/y). (Table 2, Figure 3).

On average, treatment was stopped when CA was within 9 months of BA. Three girls had a BA > 2 year beyond CA at end of treatment. They also had the 3 oldest BA at the end of treatment, but 2 girls surpassed their MPH (1 did not have MPH available).

Growth

Average HV on treatment was 4.8 ± 1.2 cm/y (range 2.9 - 7.6). All girls continued to grow from end of treatment to menarche with an average of 4.7 ± 3.7cm, with an average height velocity of 3.2 ± 2.0 cm/y. On average, they grew an additional 4.6 ± 2.1 cm from menarche until FH, with a HV of 2.4 ± 1.9 cm/y during that time. (Fig 4) Only 2/20 girls, had an increased height velocity (>6 cm/yr) after treatment was discontinued, similar to a pubertal height velocity. Growth after discontinuation of treatment ranged from 4.6 – 12.4 cm in girls who stopped GnRHa with a BA≥ 13 years, and 7.9 – 16.7 cm in girls who stopped GnRHa with a BA< 13 years.

Menarche

After treatment was discontinued, the average age at menarche was 13.2 ± 0.9 years, however ranged from 11.3 – 14.6 years. The average time from end of treatment to menarche was 15.2 ± 7.4 months, with a range of 3 – 30 months. The younger the BA was at initiation or completion of treatment, predicted a longer time to menarche. Menses occurred at regular intervals (defined as cycles 21 – 35 days long) in some girls immediately post treatment, although other girls continued to have irregular menses up to 3 years post treatment. No predictions of time to

regular menstrual cycles were found. When data was analyzed to compare girls with onset of CPP $\leq$ 6 yo compared to those > 6 yo, the only significant difference, other than the obvious age and BA, was age at onset of menarche. The younger girls had menarche on average one year later than the older girls, but at a similar BA.

Adult final height

FH occurred an average of 2 years post menarche, with a range of 0 to 3.7 years. FH was within 3 inches of MPH in 18/19 (1 child without MPH data) girls. Average difference between MPH and FH (MPH-FH) was 0.5 ± 6.0 cm. MPH was reached or surpassed by 11/19 (58%) girls treated with GnRHa for CPP. One girl attained a FH 14 cm below MPH, however her parents were tall (MPH =170 cm), pubertal onset was quite early at 5.5 years and her BA was 3.75 years advanced at start of treatment. Another girl attained a FH quite below her MPH, however she also had 2 tall parents (MPH = 176.5 cm) and onset of puberty at age 1.5 years old, although was able to reach a FH of 168 cm (Table 3).

Discussion

Time to menarche is a great concern for many children and their families. The range of onset of menarche post GnRHa treatment is consistent across studies (12-15) from 3 months to 3 years. The present study confirms that range but shows for the first time the relationship between BA and menarche. We found that the younger the BA was prior to treatment or the younger the BA was at the end of treatment, predicted a longer time to menarche. No girls had menarche prior to BA of 12.5 years. When girls were compared by onset of CPP $\leq$ 6 years versus > 6 yo, the younger girls had menarche on average one year later than the older girls, but at a similar BA.

This supports the importance of BA in predicting menarche. There were no other differences between these 2 groups, supporting the robustness of this population with truly rapidly progressing CPP, rather than a mixed population of early normal puberty and CPP. This is one of the strengths of the population, since many publications likely include some early normal puberty when girls close to 8 years old are studied.

Rate of BA advancement prior to treatment in girls with CPP is accelerated. On GnRHa treatment, rate of advancement slows to less than 1 year per year when treatment is optimal, and therefore leads to increases in growth potential and FH (20). Once treatment is stopped, we suspected that rate of BA advancement accelerates, and this is the first study with data to support that pathophysiology. Prior to GnRHa treatment rate of BA advancement in this population on average was 1.3 years per chronological year (5). This decreases to an average of 0.7 years per year on treatment. Post treatment, rate of BA advancement accelerates back to pretreatment rapid maturation averaging 1.2 years per year. This is expected since whatever process causes rapid bone maturation in CPP, is not cured by GnRHa treatment, but rather slowed down during treatment. Lazar et al (21) reported data from 115 girls post-GnRHa treatment. They describe greater height gain post treatment in girls who had onset of puberty prior to 6 years old compared to older girls. Final height in the older groups was less than PAH at end of treatment. They measured BA every 12 months and report that BA at end of treatment significantly contributed to prediction of height gain post treatment. However, they do not report change in BA post treatment. They raised the question of whether post-treatment growth would have been greater if BA was closer to 11 at end of treatment. The present data support the opposite hypothesis, since rate of BA advance accelerates post treatment, stopping treatment at an older BA may lead to

greater final height, achieved by more growth on treatment rather than more growth post-treatment. Shim et al also looked at growth post-GnRHa treatment in 85 boys but compared only PAH at end of treatment to final height, and did not delineate BA or growth rate changes over the years post treatment (22).

On average, treatment was stopped when CA was close to BA. Adequate pubertal suppression with GnRHa theoretically corrects the discrepancy between BA and CA seen prior to treatment. The girls in this study who did not reach a CA within 2 years of BA prior to end of treatment, were treated until an older BA than the rest of the girls, which was likely necessary to achieve improved FH outcomes. This supports the likely the importance of duration of treatment for optimal height outcome. Of course, we cannot know what height the girls would have achieved if treatment had been discontinued earlier, but we have shown the rapid acceleration of rate of bone maturation post treatment so it would be unlikely they would have reached the same FH. Previous studies showed that earlier onset of treatment, less BA advancement, and less delay from onset of CPP to onset of treatment have taller height outcomes as compared to MPH (6,16-19). In the present study, girls with less BA advancement had greater growth post treatment. This is the first study to quantify growth and growth rate between end of treatment and menarche and between menarche and FH. This is very important for clinicians and families when making decisions about the timing for cessation of GnRHa treatment. Those decisions cannot be based on expectations for resumption of a pubertal growth spurt post treatment. Only 2 girls in this population had increased HV to > 6 cm/y post treatment. All others grew at ≤ 5 cm/y after GnRHa was stopped. An interrupted pubertal growth spurt does not resume post GnRHa treatment for CPP.

The lack of rapid pubertal growth post treatment is likely related to growth plate senescence. Our understanding of growth plate senescence includes the effect of estrogen to first stimulate growth plate chondrogenesis and therefore linear growth, while simultaneously decreasing the number of resting zone chondrocytes, accelerating the rate of chondrocyte proliferation leading to earlier proliferative exhaustion with eventual growth plate fusion (23,24). The early exposure of estrogen in precocious puberty causes early growth plate senescence. Perhaps GnRHa treatment is usually timed to interrupt this process at the point when estrogen has already diminished enough chondrocytes, such that as bone aging continues during treatment and no further increase is possible post treatment. The older the BA was at the beginning of treatment, the less growth is accrued post treatment. We can interrupt the process of CPP causing rapid maturation of BA, but the process of senescence, although slowed during treatment, is not stopped, so we cannot regain all the growth potential that was lost. This is further support of continuation of GnRHa longer in those girls with late onset of treatment who have short height potential compared to MPH.

It cannot be determined from this study whether growth would have been different if treatment was stopped earlier in the girls with more advanced BA. However, in all girls HV was good throughout treatment, there was similar amount of growth post treatment, and height outcomes compared to MPH were similar across all girls. This suggests that the individual treatment decisions of when to stop GnRHa treatment led to the best expected outcomes.

The girls in this study were all treated until almost 11 yo or more in order to maximize height benefit as well has have their physical findings of puberty coincide with their peers. When the primary goal of treatment is prevention of early menarche, treatment is often discontinued between 10 – 11 yo, anticipating onset of menarche on average 18 months post treatment. While

not the focus of this study, the cost-implications of continuing treatment should always be kept in mind (25). Aside from cost-implications, however, the range of onset of menarche is 3 months to 2.5 years post treatment, similar to other published studies. Therefore, the older age at discontinuation in this cohort is unlikely to have affected the results of time to menarche or growth post treatment. It is always clinically relevant to inform families that a 10 yo girl who stops treatment may have menarche by 10 years 3 months, which would still be quite early after years of treatment to delay menarche."

The major strengths of this study include following girls to FH with measures of growth, bone maturation and menses every 6 months for as long as 5 – 6 years post GnRHa treatment. This study also has girls who continued GnRHa until 13 – 14 years with good growth during the treatment interval, as well as post treatment. We found that 18/19 had FH within 3 inches of MPH, with more than half of the girls reaching or surpassing MPH. The wide range of age at onset of puberty allowed assessment of age as a factor contributing to timing of menarche.

The major limitations include the small number of participants, although this does show a real-world example from one center. All girls were offered participation, but not all girls wanted to continue to be followed every 6 months once treatment was discontinued, so there may have been some bias in those who continued coming. For example, those who did not continue follow up may have been obviously growing less and therefore not interested in continued bone age to assess potential growth. It is unlikely that those who preferred not to continue were those growing more rapidly, but that cannot be determined. Another limitation is that bone age at cessation of treatment was not randomized, so physician decisions to stop treatment could bias

outcomes. However, it would not be ethical to randomize treatment cessation if physician assessment indicated possible benefit to continued treatment.

In conclusion, the present study quantitates growth after treatment with GnRHa in girls with CPP. In most girls, there is no resumption of pubertal growth spurt post GnRHa treatment. Time to onset of menarche post-GnRHa treatment is proportionate to BA at the start as well as the end of treatment, with no girls in the present study having menarche prior to BA of 12.5 years. Since time to menarche post treatment is multifactorial and not yet able to be predicted, this study adds some guidelines and suggests continuation of treatment beyond a bone age of 12.5 years in younger girls, where earlier menarche continues to be a concern. Rate of bone maturation accelerates post treatment. These factors are important in assessing potential height outcome and the decision regarding timing for cessation of treatment. This study will help clinicians give patients and families better estimates of growth and onset of menarche post treatment.

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Table 1. Patient age and time to menarche

Patient ID	Ethnicity	CA at onset CPP	CA at start Rx	CA at stop Rx	Duration of Rx (y)	CA at Menarche	Time post Rx to Menarche (months)	Time menarche to FH (y)
20	White	1.5	1.5	11.2	9.7	13.5	28.0	0.7
9	Hispanic	5.0	8.8	12.8	4.0	13.6	11.0	1.2
18	Hispanic	7.5	8.0	11.0	3.0	13.5	30.0	0.0
6	White	7.5	7.5	11.3	3.8	12.3	12.0	2.2
12	White	5.6	6.6	11.6	5.0	13.3	22.0	3.2
19	Asian	6.6	7.8	11.8	4.0	13.2	16.4	1.6
21	Hispanic	1.0	1.6	12.0	10.4	14.2	26.4	1.1
5	White	7.0	8.2	12.1	3.9	13.8	20.0	3.2
15	White	5.9	7.3	13.3	6.0	14.6	16.2	1.7
3	White	7.8	9.3	11.8	2.5	12.6	10.0	3.7
10	White	2.4	3.8	12.5	8.7	14.2	19.0	2.8
4	White	6.0	8.9	11.5	2.6	13.9	5.0	2.3
16	White	5.5	6.3	11.7	5.4	13.0	16.0	0.0
2	White	7.3	8.5	11.9	3.4	12.3	8.0	2.6
7	Hispanic	7.0	7.8	12.0	4.2	13.2	14.0	2.3
11	Hispanic	6.0	8.5	12.6	4.1	13.6	11.0	2.0
17	Hispanic	5.0	8.6	12.6	4.0	13.8	13.8	2.5
1	Hispanic	*	9.2	10.7	1.5	11.3	7.0	2.7
13	White	7.4	8.8	11.3	2.6	11.6	3.0	1.2
8	Asian	8.4	9.3	11.5	2.2	12.8	16.0	2.2
Average		5.8	7.3	11.8	4.5	13.2	15.2	2.0
Minimum		1.0	1.5	10.7	1.5	11.3	3.0	0.0
Maximum		8.4	9.3	13.3	10.4	14.6	30.0	3.7
SD		2.1	2.3	0.6	2.4	0.9	7.4	1.0

* menarche at 8.8, but breast onset not known
BA = bone age, Rx = treatment, FH = final height, CA - chronological age, CPP = central precocious puberty

Table 2. Bone age data during and after treatment with GnRHa

Patient ID	BA start Rx (y)	BA/CA start Rx	BA end of Rx (y)	BA at menarche (y)	BA at FH (y)	BA/CA during Rx	BA/CA end Rx to menarche	BA/CA menarche to FH
20	**	**	11.0	15.0	15.0	**	1.7	0.0
9	8.8	1.0	11.5	13.5	16.0	0.7	2.2	2.2
18	10.0	1.3	12.0	16.5	16.8	0.7	1.8	0.5
6	**	**	12.0	15.0	15.0	**	3.0	0.0
12	6.8	1.0	12.0	12.5	16.5	1.0	0.3	1.3
19	7.8	1.0	12.0	13.8	15.5	1.1	1.3	1.1
21	2.0	1.3	12.0	15.0	15.5	1.0	1.4	0.4
5	***	***	12.0	13.5	17.0	***	0.9	1.1
15	10.0	1.4	12.0	14.0	16.3	0.3	1.5	1.4
3	11.0	1.2	12.5	13.8	***	0.6	1.5	***
10	4.6	1.2	12.5	14.0	16.5	0.9	0.9	0.9
4	12.0	1.3	13.0	16.0	17.0	0.4	1.23	0.4
16	10.0	1.6	13.0	***	***	0.6	***	***
2	11.0	1.3	13.0	13.0	16.0	0.6	0.0	1.2
7	11.0	1.4	13.0	14.3	15.0	0.5	1.1	0.3
11	11.0	1.3	13.0	15.0	15.5	0.5	2.2	0.3
17	10.0	1.2	13.0	13.0	15.8	0.8	0.0	1.1
1	11.5	1.3	13.5	13.5	16.0	1.4	0.0	0.9
13	12.0	1.4	13.6	13.8	17.0	0.6	0.8	2.6
8	11.5	1.2	14.0	15.0	17.0	1.1	0.8	0.9
Average	9.5	1.3	12.5	14.2	16.1	0.7	1.2	0.9
Minimum	2.0	1.0	11.0	12.5	15.0	0.3	0.0	0.0
Maximum	12.0	1.6	14.0	16.5	17.0	1.4	3.0	2.6
SD	2.8	0.2	0.8	1.0	0.7	0.3	0.81	0.7

** came to us on treatment without records

*** missing data

BA = bone age, Rx = treatment, FH = final height, CA - chronological age

Table 3. Growth changes during and after treatment with GnRHa

Patient ID	Ht start of Rx (cm)	Ht end Rx (cm)	Growth end Rx to menarche (cm total)	HV end Rx to menarche (cm/y)	Ht at menarche (cm)	FH (cm)	CA at FH (y)	Growth menses to FH (cm total)	HV menarche to FH (cm/y)	MPH (cm)	MPH - FH (cm)
20	**	154.9	7.4	3.2	162.3	168.0	14.2	5.7	8.5	176.4	8.4
9	134.0	152.7	2.6	2.8	155.3	161.0	14.8	5.7	5.0	156.1	-4.9
18	141.7	162.5	8.0	3.2	170.5	170.5	14.0	0.0	0.0	168.5	-2.0
6	**	155.6	2.6	2.6	158.2	165.2	14.5	7.0	3.2	157.3	-7.9
12	114.5	140.3	10.6	5.8	150.9	155.5	16.4	4.6	1.5	155.1	-0.4
19	122.8	152.7	10.3	7.5	163.0	164.7	14.8	1.7	1.0	161.1	-3.6
21	80.5	152.7	9.3	4.2	162.0	165.5	15.3	3.5	3.1	171.3	5.8
5	125.0	141.4	7.9	4.7	149.3	153.3	17.0	4.0	1.3	161.1	7.8
15	119.7	146.6	5.5	4.1	152.1	154.5	16.3	2.4	1.5	162.4	7.9
3	147.5	163.4	3.2	3.8	166.6	172.0	16.3	5.4	1.4	171.3	-0.7
10	106.5	160.0	10.5	6.6	170.5	176.7	17.0	6.2	2.2	168.8	-7.9
4	142.3	153.5	0.0	0.0	153.5	160.5	16.2	7.0	3.1	152.3	-8.3
16	120.7	147.6	3.0	1.3	150.6	155.7	15.8	5.1	0.0	170.0	14.3
2	136.9	162.9	2.1	3.1	165.0	169.5	14.9	4.5	1.7	165.0	-4.6
7	130.5	142.7	3.6	3.1	146.3	148.5	15.5	2.2	1.0	156.1	7.6
11	127.4	148.7	0.6	0.7	149.3	153.3	15.6	4.0	2.0	158.6	5.3
17	124.3	140.2	3.6	3.1	143.8	152.6	16.3	8.8	3.5	153.5	0.9
1	135.0	142.3	1.8	3.1	144.1	151.0	13.9	6.9	2.6	***	***
13	140.8	151.7	0.0	0.0	151.7	156.3	12.8	4.6	3.7	152.9	-3.4
8	147.5	155.8	1.4	1.0	157.2	160.4	15.0	3.2	1.5	156.1	-4.3
Average	127.6	151.4	4.7	3.2	156.1	160.7	15.3	4.6	2.4	161.8	0.5
Minimum	80.5	140.2	0.0	0.0	143.8	148.5	12.8	0.0	0.0	152.3	-8.3
Maximum	147.5	163.4	10.6	7.5	170.5	176.7	17.0	8.8	8.5	176.4	14.3
SD	16.4	7.6	3.7	2.0	8.3	7.9	1.1	2.1	1.9	7.4	6.7

** came to us on treatment without records
*** missing data
HV = height velocity, Rx = treatment, FH = final height, CA - chronological age, Ht – height, MPH = mid-parental height

Figure Legends

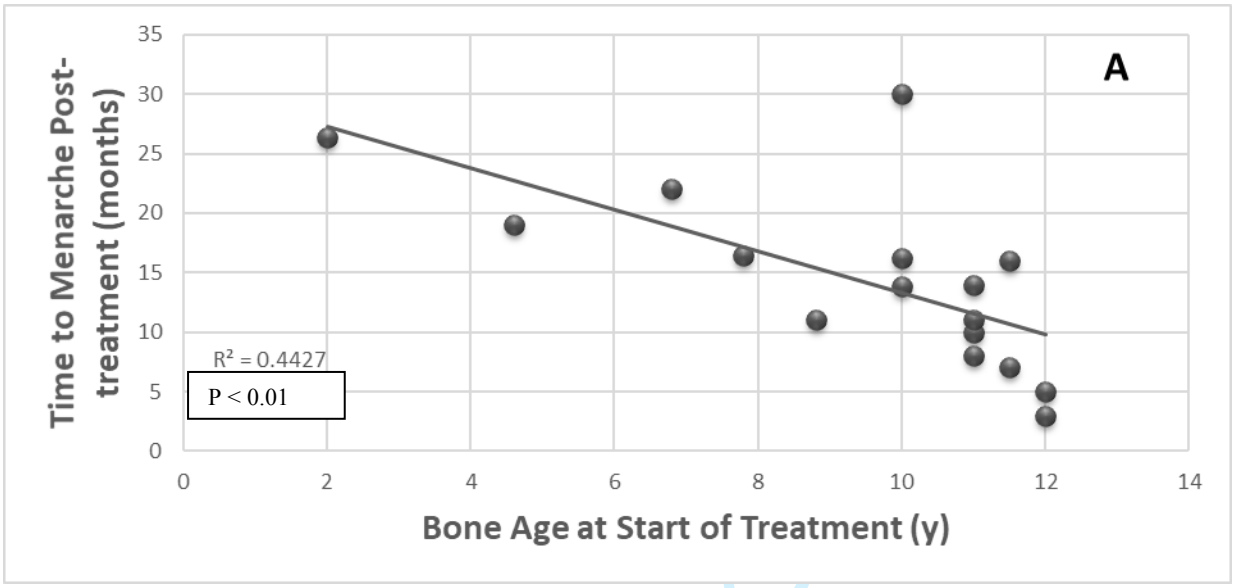
Figure 1: Time to Menarche post GnRHa treatment by bone age at start of treatment (A) and by bone age at end of treatment (B)

Figure 2. Total Growth post GnRHa treatment by BA at end of Treatment

Figure 3. BA/CA ratio from Treatment to Final Height

Figure 4. Height velocity from Treatment to Final Height – Individual patients shown. Solid bold line is average.

Figure 1:


A
Time to Menarche Post-treatment (months)
Bone Age at Start of Treatment (y)
R² = 0.4427
P < 0.01
0
5
10
15
20
25
30
35
0
2
4
6
8
10
12
14

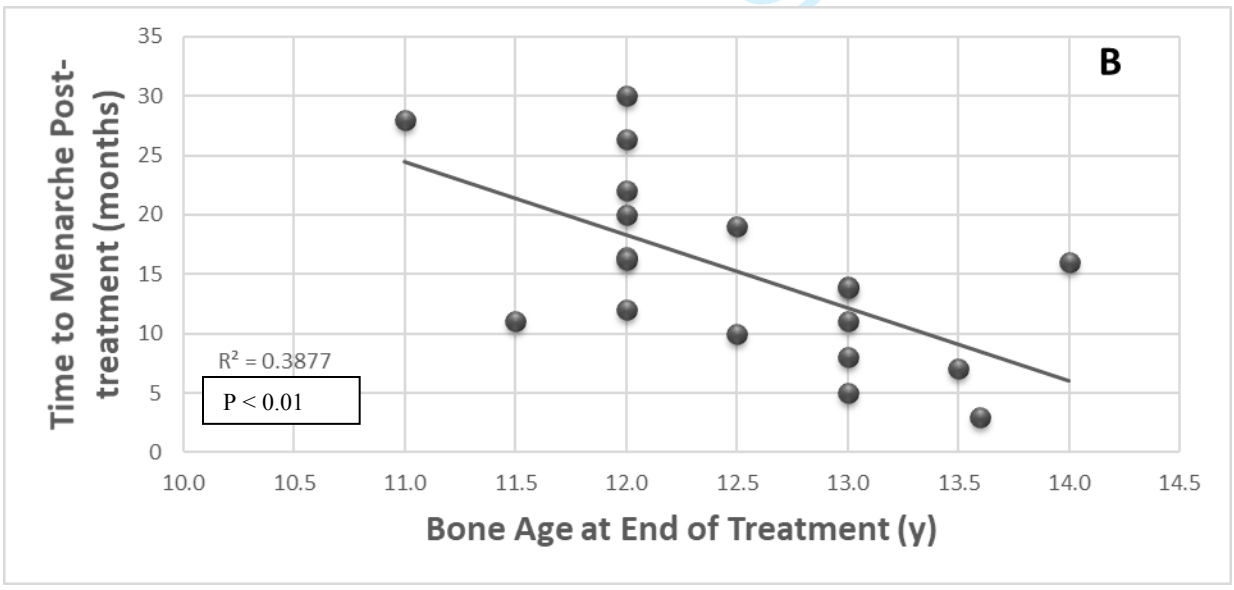

B
Time to Menarche Post-treatment (months)
Bone Age at End of Treatment (y)
R² = 0.3877
P < 0.01
0
5
10
15
20
25
30
35
10.0
10.5
11.0
11.5
12.0
12.5
13.0
13.5
14.0
14.5

Figure 2.

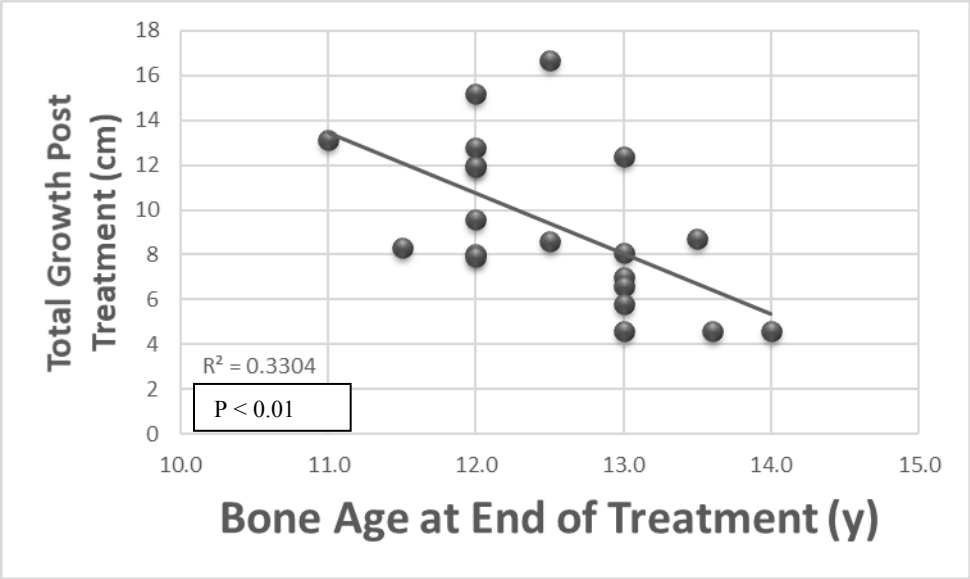


Figure 3.

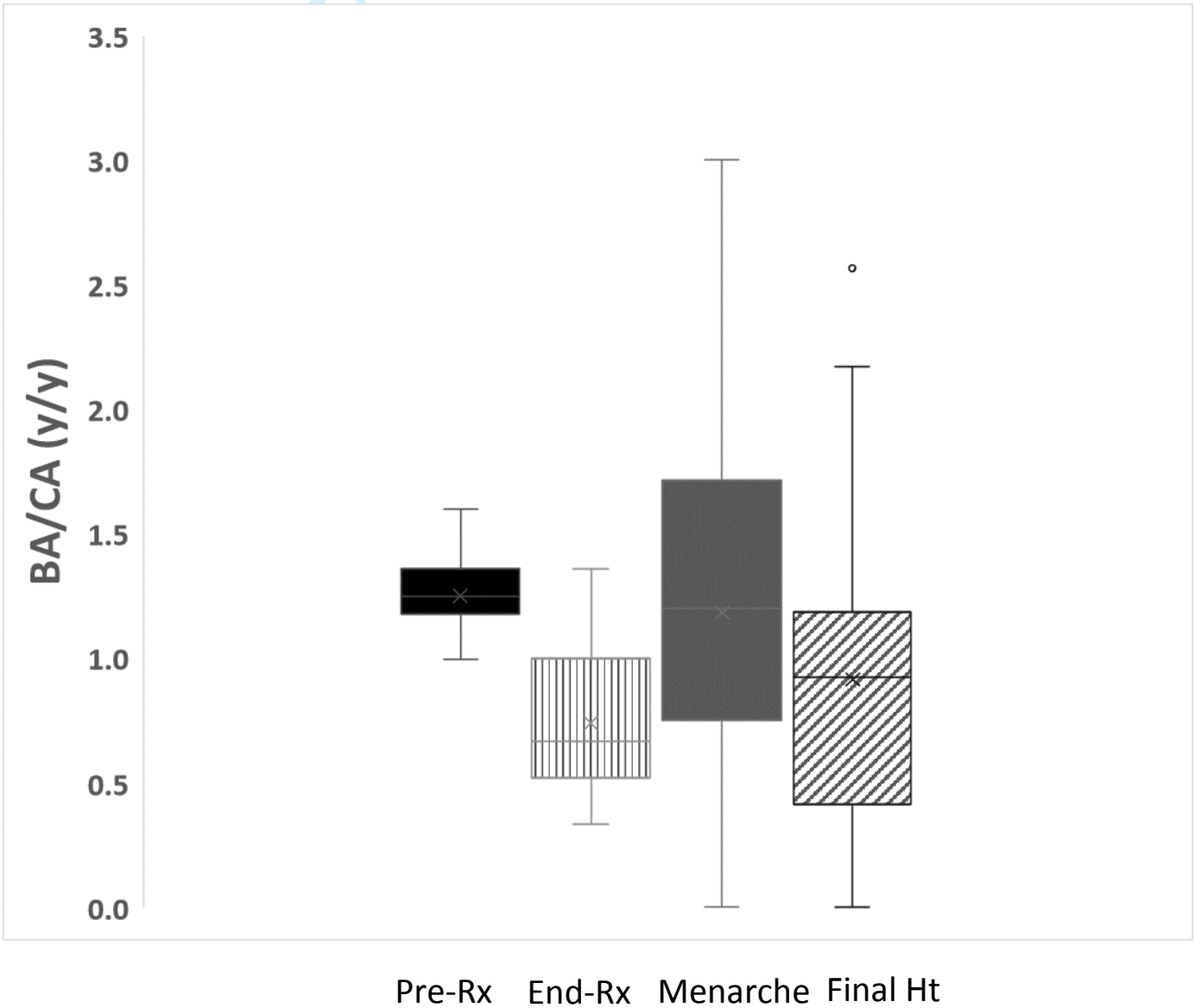
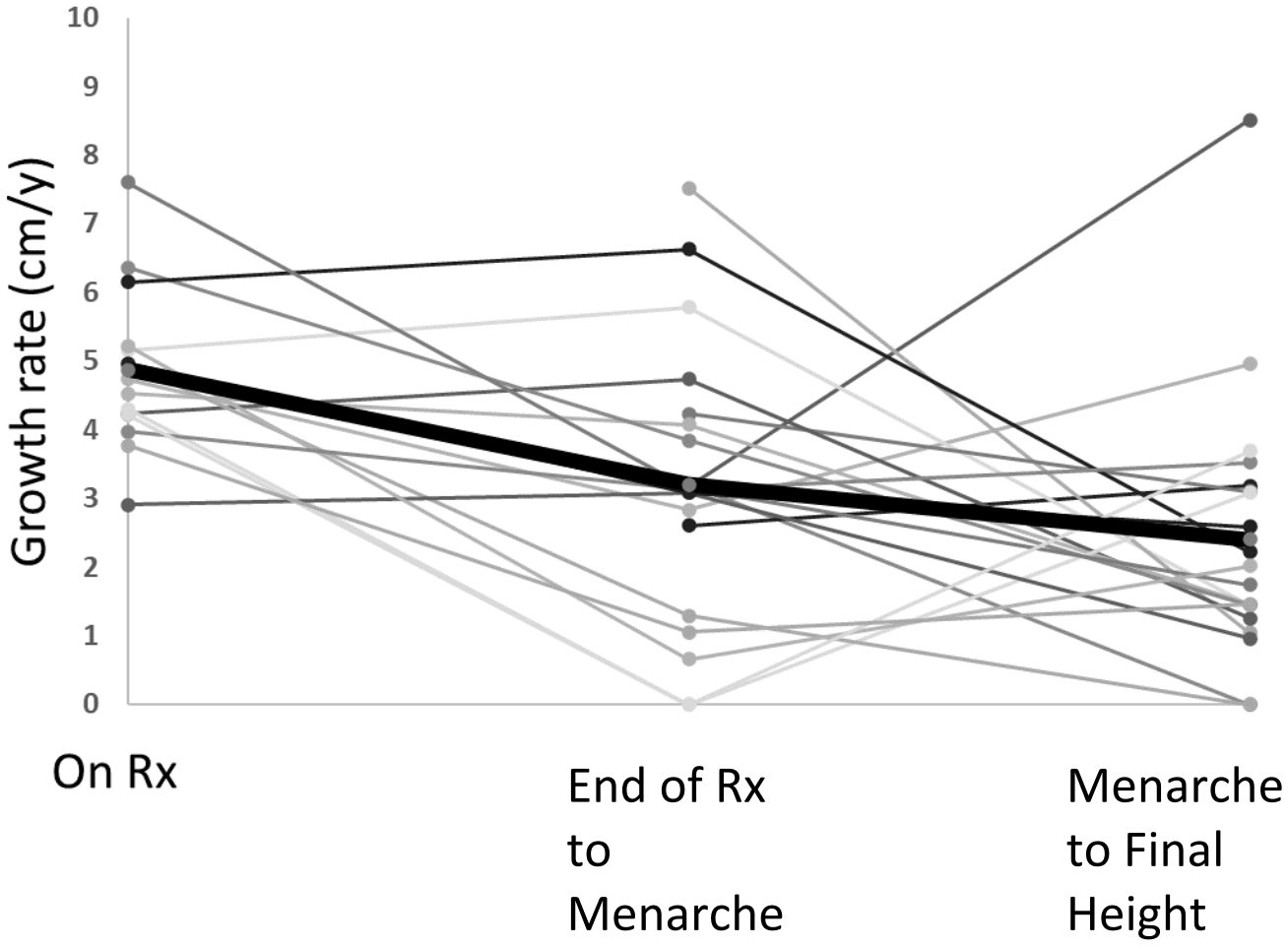


Figure 4.


Growth rate (cm/y)
10
9
8
7
6
5
4
3
2
1
0
On Rx
End of Rx to Menarche
Menarche to Final Height

Strictly adhere to the given format!
In case informed consent or ethical approval do not apply the statements should read: "Not applicable".

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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

Karen Klein is a consultant for AbbVie Pharm, Arbor Pharm, and Tolmar Pharm. Audrey Briscoe and Katherine Chen have nothing to disclose

Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

The local Institutional Review Board approved this study.