Evaluation and phenotypic characteristics of 293 Danish girls with tall stature: effects of oral administration of natural 17β-estradiol

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BACKGROUND: Reduction of adult height by sex steroid treatment was introduced decades ago in tall statured children, but controlled trials are lacking and treatment is controversial. In this study, we wanted to evaluate the phenotypic characteristics in girls referred due to tall stature and the effect of oral administration of 17β-estradiol on predicted adult height in girls.

METHODS: A single-centre retrospective observational study of 304 girls evaluated consecutively due to tall stature between 1993 and 2013. 207 patients diagnosed with constitutionally tall stature (CTS), 60 (29%) girls ended up being treated with 17β-estradiol with a duration of 1.7 y (1.2; 2.5) (median (25; 75 percentile)), and final height was available in 26 girls.

RESULTS: At baseline, 20% of girls with CTS had supranormal IGF-I, whereas reproductive hormones were within the normal range. Final adult height was reduced with 1.6 ± 2.1 cm in the girls treated with 17β-estradiol when compared to initial prediction. Chronological age, bone age, estradiol, and IGF-I at baseline or estrogen dose did not predict height reduction.

CONCLUSIONS: Serum IGF-I was elevated tall statured children, but did not predict the effect of treatment with 17β-estradiol, which caused a modest reduction in final adult height.

Tall stature in children is defined as a height greater than two standard deviations above the mean for a given sex, age, and population group. No international agreement has been made on the diagnostic classification of tall stature, but it is commonly divided into three subgroups: (i) normal variation of growth (constitutionally tall stature (CTS)), (ii) primary growth disorders (sex chromosome–related disorders and overgrowth syndromes), and (iii) secondary growth disorders (precocious puberty and growth hormone excess) (1). CTS is frequently characterized by the child having one or two tall parents and no abnormalities at physical examination (1). Extreme tall stature can be associated with psychosocial distress, frequent teasing, and hurtful remarks about their height (1,2). Therefore, reduction of adult height by estrogen treatment in females was introduced decades ago.

The decision whether height-reducing treatment is advisable for a child with tall stature depends on an overall assessment, and the justification for treatment frequently include psychosocial aspects. We acknowledge that tall stature has become more socially acceptable in recent decades, which may have reduced the demand for height-reducing treatment.

The estimation of predicted adult height (PAH) is an important tool in the assessment of whether height-reducing treatment is advisable in a tall-statured girl. To estimate a child's PAH, an X-ray of the left hand and wrist is analyzed to determine bone age (BA). BA is a measurement of skeletal maturation and a tool used to predict a child's remaining growth potential (3). Height reduction by sex hormone therapy has been used frequently and for many decades in tall-statured girls. However, the treatment still remains controversial, and may be viewed by some as a medicalization of a normal physical variation (4). Furthermore, recent epidemiological studies revealed that height appeared to have minimal consequences for health-related quality of life (5). Most of the experience with sex hormone treatment for CTS has been obtained with extremely high doses of synthetic estrogens (ethinyl estradiol (EE2)), which results in a significant height reduction depending on BA at start of treatment (2,6–8).

High-dose EE2 treatment has been associated with severe short-term adverse events such as deep venous thrombosis (9) and coagulation defects (10). However, less is known about potential long-term side effects of estrogen treatment in CTS girls. Recent studies have indicated that there may be reduced fertility later in life after treatment with high doses of EE2 in adolescence (11,12), and that there may be a possible dose-relationship between oestrogen dose and increased infertility (12). Previous studies have also shown a change in coagulation parameters during treatment with high-dose EE2 (10). In addition, a recent study showed an increased risk for melanoma in treated tall stature girls (13).

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EE2 treatment of extreme tall stature has become increasingly debated and controversial. In Sweden, percutaneous epiphysiodesis operation is suggested as an alternative to EE2 treatment (14). In our center, we introduced an alternative clinical practise 20 y ago, when we introduced early treatment with oral administration of natural estrogens (17β-estradiol (E2)). This treatment principle is based on the assumption that 17β-estradiol will be more physiological by increasing estradiol to higher but not overt pharmacological serum levels. This results in less suppression of gonadotropins compared to EE2, and increased serum estradiol levels are assumed to initiate or ensure a more rapid progression of puberty (and epiphyseal closure).

In the present study, we evaluated our retrospective 20-y single center experience with evaluation of 304 tall statured girls, and the possible clinical and biochemical effects of oral administration of E2.

RESULTS

Diagnostic Classification

A total of 304 patients were identified from our registry, of whom 11 (4%) were excluded from the study due to missing data or reclassification resulting in 293 girls for evaluation. 28 (9%) of these girls were diagnosed with a well-known overgrowth syndrome (Marfan, TripleX, Sotos, and Beckwidt-Wiedemann syndrome), as shown in **Figure 1**. Growth curves for patients with Marfan syndrome, TripleX, Sotos, and Beckwith-Wiedemann syndrome are shown in **Supplementary Figure S1** online. Thus, a total of 265 (87%) girls were further evaluated and 207 (78%) of these girls fulfilled the auxological criteria of CTS at the time of referral whereas 58 (22%) patients did not (**Figure 1**). The 58 patients were subdivided into three of the following subgroups: (i) Height SD > 2 or PAH > 2 SD during follow up (n = 26, 45%), (ii) Tall Normal Variation: Height SD 1–2 during follow up (n = 28, 48%), and (iii) Height SD < 1 during follow up (n = 4, 7%) (**Figure 1**). The 207 girls fulfilling the auxological criteria for CTS at the time of referral were further analyzed.

Baseline Characteristics

Of the 207 patients diagnosed with CTS, 60 (29%) girls ended up being treated with oral E2, 3 (1%) girls with both oral E2 and surgical epiphysiodesis, and 1 girl (<1%) with surgical epiphysiodesis alone. The remaining 143 (69%) girls did not receive treatment for CTS. The four girls who received surgical epiphysiodesis were not included in the subsequent analyses.

Table 1 presents the clinical, biochemical, and auxological results from the first clinical evaluation. Height and PAH at first evaluation are shown according to chronological age, with or without subsequent oral E2 treatment (**Figure 2**). The girls who later received treatment had a significantly taller height at referral compered to the girls who never received treatment, but there were no differences in height SD or PAH between the

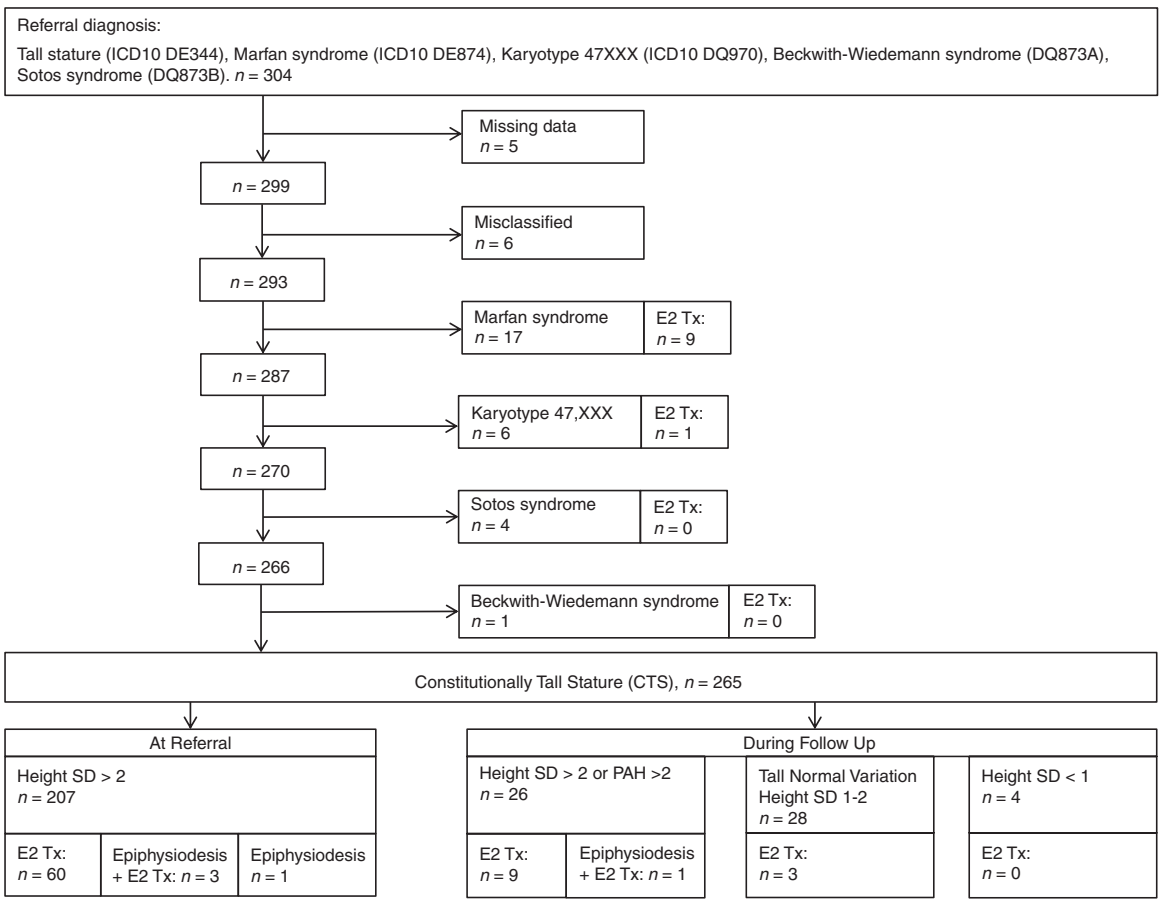

Figure 1. Reclassification of 304 girls referred with tall stature and overgrowth syndromes.

Table 1. Auxological data, clinical data, and reproductive hormone levels in 203 tall stature girls at time of referral; here of 60 who later received treatment with 17β-estradiol, and 143 who never received any treatment

	All ($n = 203$)				Untreated ($n = 143$)				Treated ($n = 60$)				
	n	Median (range)	25p	75p	*n*	Median (range)	25p	75p	*N*	Median (range)	25p	75p	*P*
Chronological age (year)	203	11.58 (1.09–16.82)	7.95	13.36	143	10.67 (1.09–16.2)	7.81	13.12	60	12.28 (3.27–16.82)	8.34	13.65	<0.05
Bone age (year)	130	11.79 (1.1–17.79)	8.9	13.31	98	11.46 (1.1–17.79)	8.86	13.5	32	12.15 (3.0–13.65)	8.9	13.0	0.56
CA-BA (year)	130	−0.21 (−2.95–3.86)	−1	0.59	98	−0.41 (−2.95–3.1)	−1.12	0.23	32	0.26 (−2.15–3.86)	−0.66	1.33	<0.05
Tanner Breast Stage 1/2/3/4/5	179	69/11/24/44/31			127	54/6/15/32/20			52	15/5/9/12/11			
Tanner Pubic Stage 1/2/3/4/5	158	70/10/24/27/27			112	53/6/15/22/16			46	17/4/9/5/11			
Menarche, yes/no	193	43/150			137	32/105			56	11/45			
Height (cm)	203	168 (83–192.9)	145.6	178.1	143	163.4 (83–190.3)	143.3	176.8	60	174.1 (110.5–192.9)	152.4	179.1	<0.01
Height (SDS)	203	2.68 (2.01–4.93)	2.38	3.22	143	2.66 (2.01–4.1)	2.38	3.19	60	2.74 (2.07–4.93)	2.38	3.35	0.42
Target height (cm)	198	176.7 (159.5–193.1)	173.3	179.6	138	176.2 (159.5–193.1)	172.7	179.5	60	177.1 (166.2–188.9)	174.5	180.2	<0.05
Target height (SDS)	198	1.14 (−1.59–3.72)	0.6	1.59	138	1.06 (−1.59–3.72)	0.5	1.57	60	1.19 (−0.53–3.05)	0.78	1.69	<0.05
Predicted adult height (cm)	122	182.1 (171.5–191.3)	180.0	184.5	92	182 (171.5–191.3)	179.6	184.4	30	183.7 (175.5–187.2)	181.1	184.9	0.17
Predicted adult height (SDS)	122	1.99 (0.3–3.44)	1.65	2.37	92	1.98 (0.3–3.44)	1.59	2.36	30	2.23 (0.93–2.8)	1.83	2.43	0.17
PAH-TH (cm)	119	6.34 (−8.68–21.77)	2.81	9.49	89	6.24 (8.68–21.77)	2.93	9.46	30	6.67 (−6.93–16.67)	2.62	10.18	0.91
PAH-TH (SDS)	119	1 (−1.37–3.44)	0.44	1.5	89	0.99 (−1.37–3.44)	0.46	1.49	30	1.06 (−1.09–2.63)	0.41	1.6	0.91
BMI (kg/m^2)	203	18.5 (13.5–39.8)	16.9	20.2	143	19 (13.5–39.8)	17.5	20.6	60	17.9 (14.5–27.8)	16.3	19.4	<0.01
BMI (SDS)	203	0.7 (−2.33–6.7)	−0.33	1.65	143	0.89 (−2.32–6.7)	−0.09	1.84	60	0.07 (−2.33.3.43)	−0.9	1.05	<0.01
Mother's height (cm)	200	175.2 (152.7–193.7)	171	179.5	140	175.0 (152.7–193.7)	170	178.5	60	175.9 (163.8–188)	173	181	<0.05
Mother's height (SDS)	200	0.89 (−2.66–3.82)	0.23	1.57	140	0.86 (−2.66–3.82)	0.07	1.42	60	1.01 (−0.9–2.92)	0.55	1.81	<0.05
Father's height (cm)	198	190.0 (169.4–205.5)	185.2	195	138	190 (172.3–205.5)	185	194.6	60	190 (169.4–205)	186.9	196.3	0.33
Father's height (SDS)	198	1.37 (−1.83–3.77)	0.63	2.14	138	1.37 (−1.38–3.77)	0.59	2.08	60	1.37 (−1.83–3.69)	0.89	2.34	0.33
LH (IU/l)	169	1.66 (<0.05–44.1)	<0.05	3.83	116	1.36 (<0.05–44.1)	<0.05	3.73	53	2.21 (<0.05–12)	<0.05	4.01	0.48
FSH (IU/l)	169	3.63 (<0.05–12.6)	1.41	5.42	116	2.91 (0.18–12.6)	1.38	5.49	53	4.41 (<0.05–8.67)	1.53	5.28	0.49
Estradiol (pmol/l)	176	62.5 (<18.1–727)	<18.1	141.5	121	54 (<18.1–727)	<18.1	133	55	85 (<18.1–55.3)	25	164	0.09
Testosterone (nmol/l)	133	<0.35 (<0.35–2.72)	<0.35	0.85	96	<0.35 (<0.35–2.72)	<0.35	0.79	37	<0.35 (<0.35–2.43)	<0.35	0.97	0.45
Serum sex hormone-binding globulin (nmol/l)	169	68 (12–212)	55	92.5	119	65 (16–212)	53	94	50	73.5 (12–172)	61	91.5	0.26
Inhibin B 1993–2010 (pg/ml)	103	48 (<20–169)	<20	88	67	38 (<20–169)	<20	77	36	68 (<20–139)	<20	91.3	0.22
Inhibin B 2010–2013 (pg/ml)	54	36.5 (<3–295)	14.3	84.3	43	37 (<3–295)	17	84	11	34 (4–136)	10	117	0.68
IGF-I 1993–2008 (ng/ml)	87	345 (103–830)	236	490	50	335.5 (117–747)	225.8	469.8	37	375 (103–830)	258	509.5	0.49
IGF-I 2008–2013 (ng/ml)	88	438 (116–1,076)	258.8	554.8	68	438 (116–1,076)	239.3	573.8	20	406.5 (191–719)	320.3	517.8	0.85
IGFBP-3 1993–2008 (ng/ml)	85	4,013 (2,221–6,023)	3,487.5	4,582.5	48	3,986.5 (2,221–6,023)	3,456.3	4,636.3	37	4029 (2522–5577)	3,534	4,560.5	0.75
IGFBP-3 2008–2013 (ng/ml)	89	5,130 (2,530–7,420)	4,410	5,820	69	5,130 (2,530–7,420)	4,425	5,875	20	5140 (3730–6650)	4,235	5,637.5	0.94

BA, bone age; CA, chronological age; PAH, predicted adult height; SDS, SD score.

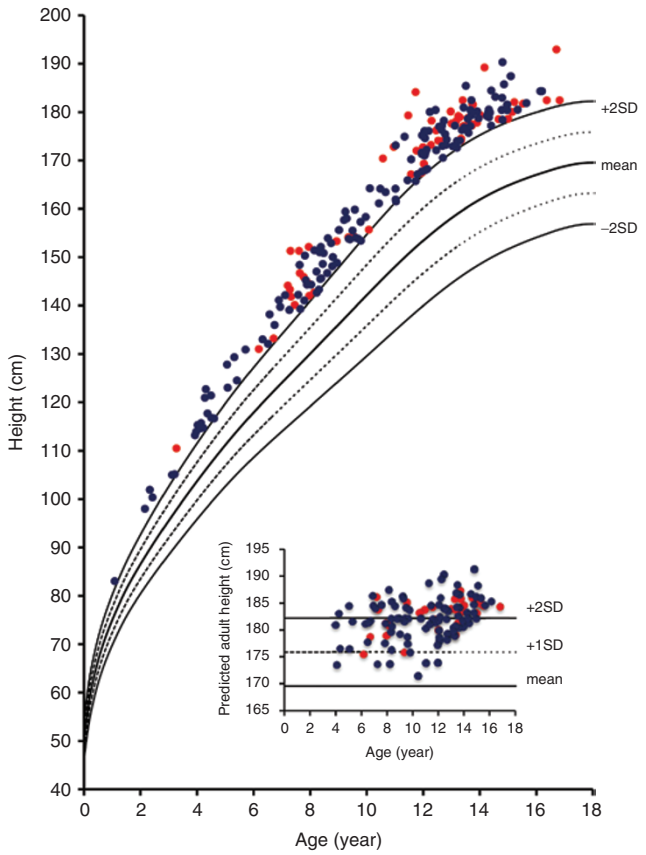

Figure 2. Height (cm) and predicted adult height (cm) (inserted figure) according to age at referral in girls diagnosed with tall stature. Red dots represent girls who later received treatment with 17β-estradiol, and blue dots represent girls who never received treatment. Black reference lines are derived from Danish references (29).

two groups (Table 1). Weight and BMI at referral are illustrated according to chronological age and age-related national references (**Supplementary Figure S2** online). The girls who never received treatment had a significantly higher BMI compered to the group who later received treatment. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, testosterone, serum sex hormone-binding globulin (SHBG), and inhibin B levels at baseline (Figure 3) did not differ between the two groups (Table 1). The reproductive hormone levels at first evaluation were within the normal range, except for SHBG values, which tended to cluster in the lower part of the normal range. IGF-I levels were supranormal (>2 SD) in 33 (20%) of the patients. IGF-I values were more frequently elevated in 2008–2013 (n = 25, 29%) compared to 1993–2008 (n = 8, 10%) (**Supplementary Figure S3** online). There was a significant difference in IGF-I (SDS) between 2008–2013 and 1993–2008, 1.14 SDS ± 1.21 SDS vs. 0.29 SDS ± 1.0 SDS respectively (P < 0.01). IGFBP-3 levels were supranormal (>2) in 19 of the patients, 2008–2013 (n = 17, 19%) and 1991–2008 (n = 2, 2%).

Treatment With 17β-Estradiol in Girls With CTS

One girl (2%) of 60 treated girls was excluded from further analysis due to missing start date of treatment. The average treatment duration was 1.7 y (1.2; 2.5). The start dose of E2 was <2 mg E2 (n = 14, 24%), 2 mg E2 (n = 34, 58%), and 4 mg (n = 10, 17%), respectively, depending on an individual evaluation of each girl. The dosage of oral E2 was not specified for one (2%) of the 59 girls.

In Figure 4, height, height SD, and PAH are shown before, during, and after treatment in 59 treated CTS girls. PAH at baseline (± 0.3 y) and final height (FH) were available in 26 (44%) of the girls. FH was defined as, growth velocity <2 cm/y or cessation of growth for more than 6 mo. For these 26 girls, chronological age and BA at treatment start was 13.37 (10.37–16.83) years and 12.2 (10.5–15.5) years, respectively. PAH at baseline decreased from 185.7 ± 3.9 cm to a FH of 184.1 ± 4.8 cm (P < 0.001). That corresponds to an average height reduction of −1.6 ± 2.1 cm. There was no significant decrease in PAH after 1 y of oral E2 treatment (n = 32) (P = 0.99). There was no significant difference between FH in treated (183.8 ± 4.3 cm) (n = 35) compared to untreated (181.5 ± 4.6 cm) (n = 16) girls (P = 0.09).

The 26 girls with PAH at baseline and FH were divided into the following two groups: height reduction (n = 18, 69%) and no height reduction (n = 8, 31%). There were no statistical differences at start of treatment between the groups with respect to chronological age, BA, PAH, target height, BMI, or serum estradiol. The height SD at baseline was significantly higher in the girls with no or negative effect compared to the group who achieved height reduction (P < 0.05). Baseline BA tended to be negatively associated with height reduction (r = −0.34, P = 0.08). There was no statistical difference in E2 dosage during treatment between the groups. Figure 5 shows individual estradiol dose, serum estradiol, and IGF-I (SDS) before and during treatment in the 26 treated girls according to whether or not height reduction was achieved. IGF-I (SDS) tended to be higher at baseline in girls who achieved height reduction compared to girls who had no or negative effect, 0.7 (−1.01–2.57) vs. −0.49 (−1.39–0.24) (P = 0.068).

Six of the 59 girls reported one or more of the following problems during treatment: hyperpigmentation of nipple, stretch marks located to the breasts, heavy menstrual bleeding, nausea, joint, and muscle pain. No serous side effects were reported.

DISCUSSION

In this large cohort study of 304 girls, we re-evaluated the diagnostic criteria of CTS and found that 207 tall girls fulfilled the auxological criteria for CTS, height > 2 SD at referral. Serum IGF-I levels were supranormal in 20% of the CTS subjects at baseline, whereas reproductive hormone levels were within the normal range for their age. Oral administration of natural E2 reduced final height by an average of 1.6 cm compared to initial prediction.

Our results suggest that oral E2 treatment in CTS girls reduced final height marginally, which is in line with the only previous study on oral E2 in CTS (15), which report an average height reduction of 2.4 cm (15). Estrogens as height reducing treatment is well known, but most previous experience are made

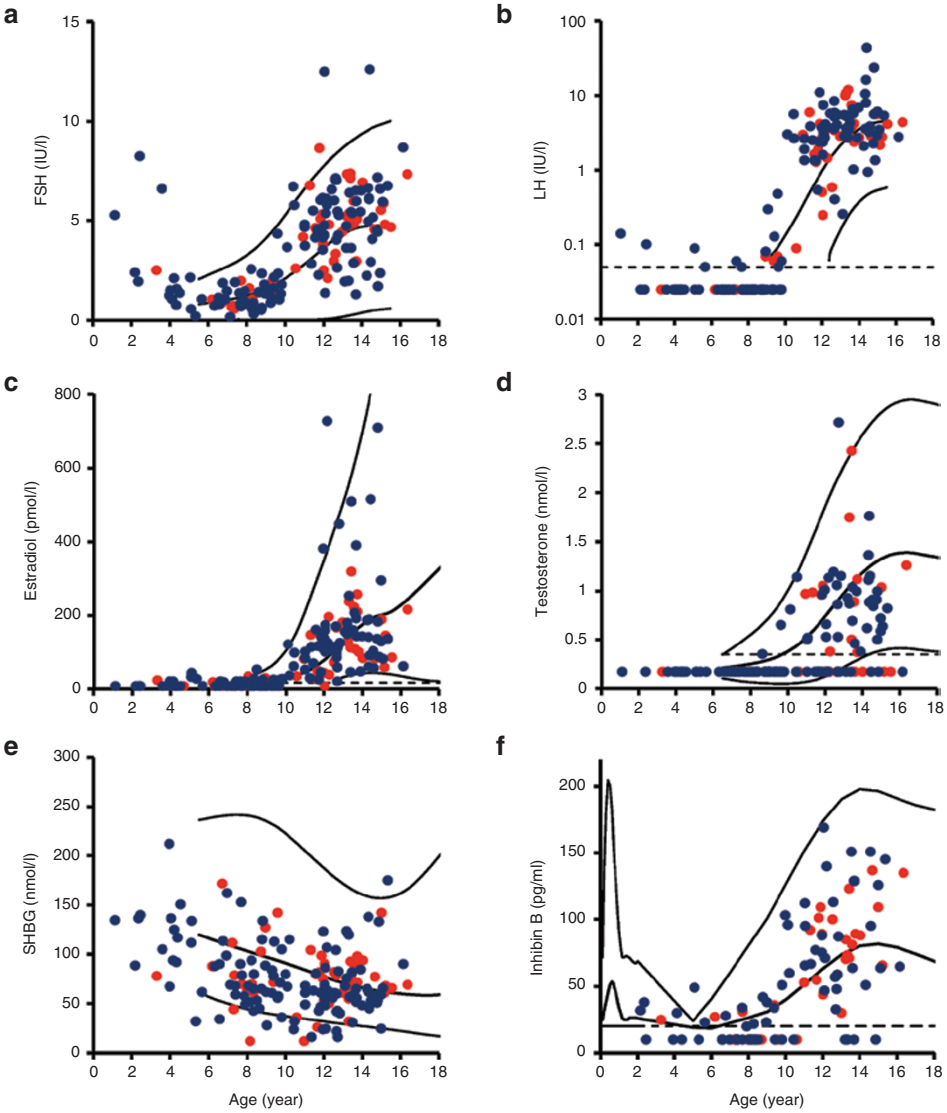

Figure 3. Levels of FSH (**a**), LH (**b**), estradiol (**c**), testosterone (**d**), serum sex hormone-binding globulin (**e**), and Inhibin B (**f**) according to age at time of referral in 203 girls with tall stature; here of 60 who later received treatment with 17β-estradiol (red dots), and 143 who never received any treatment (blue dots). Black reference lines are estimated from Danish cross-sectional data of healthy girls. The black dotted line represents the detection limit of the assay.

with high-dose synthetic EE2 or conjugated estrogens. Height reduction due to treatment with EE2 or conjugated estrogens varies among different studies, and height reductions of 1.4–7.4 cm have been reported (2,6–8,16–18). Variation in the height reduction achieved with EE2 or conjugated estrogens treatment is probably due to methodological differences. This, differences in BA measurement method, PAH method, BA and chronological age at start of treatment, EE2 doses used and differences in treatment duration may all influence response (2,6–8,16–18).

In our study, 18 of the 26 girls followed until final height, showed a reduction in gained final height after treatment with E2. When comparing the girls who achieved height reduction with the group who did not, E2 dose, estradiol, and BA at start of treatment did not predict height reduction. IGF-I (SDS) tended to be higher at baseline in girls who achieved height reduction compared to girls who had no or negative effect, but the difference was not statistically significant. However, no definite conclusions can be drawn considering the small subgroups. Furthermore, we do not have the final adult height of all 59 treated girls as some have not yet reached final height or was discharged from the outpatient clinic before they reached final adult height. This is clearly a limitation to the conclusions drawn from our study.

In our study, treatment with E2 was initiated at a rather advanced BA, which may partly explain the moderate results observed. Previous studies have shown an association between height reduction and BA prior to start of EE2. The greatest effect on height reduction was observed when EE2 treatment was initiated at a younger BA. In accordance Joss *et al.* (8) observed a larger height reduction following EE2 in girls with BA < 12 y, compared to girls with more advanced BA.

Estimation of PAH is one factor that is taken into account when deciding whether or not height-reducing treatment is

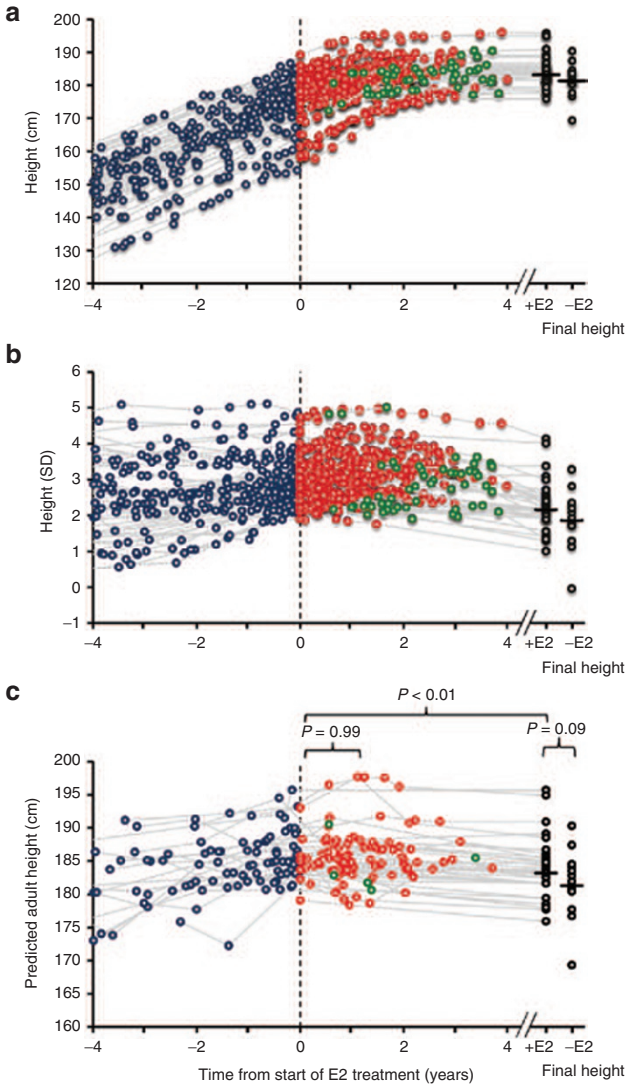

Figure 4. Height (cm) (**a**), height (SD) (**b**), and predicted adult height (cm) (**c**) in 59 girls diagnosed with tall stature, before, during and after oral E2 treatment. Blue dots represent measurement prior to treatment, red dots during treatment, and green dots after treatment. Black dots represent final height in treated (n = 35) and untreated (n = 16) girls.

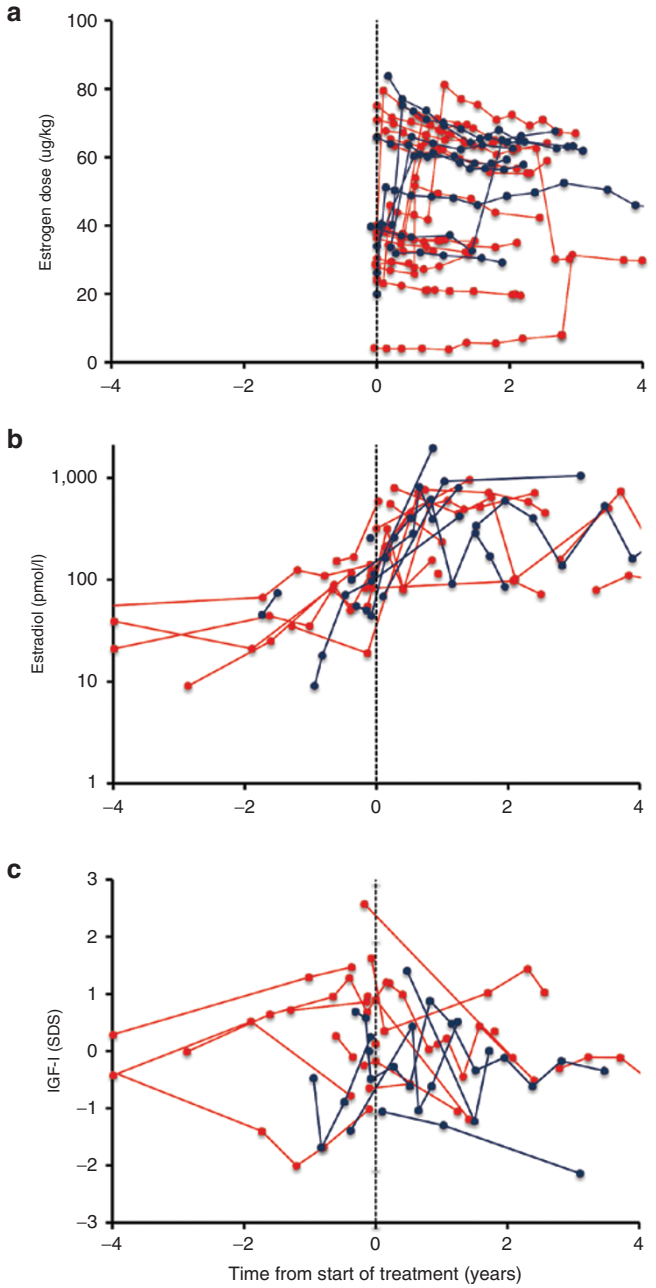

Figure 5. Estrogen dose (**a**), serum estradiol concentration (pmol/l) (**b**), and IGF-I (SDS) (**c**) before, during and after oral E2 treatment of 26 girls with constitutionally tall stature. Red lines (n = 18) represent girls who achieved a reduction of final height compared to those who did not (blue lines, n = 8).

advisable for a child with tall stature. Furthermore, FH compered with PAH before start of height-reducing treatment is a frequently used method to evaluate the effect of treatment. The risk of error in height-prediction methods is well known, and studies have shown both underestimation and overestimation of final adult height depending on the method used (16,19). Individual variations in height prediction have also been shown to occur. Joss *et al.* (19) state that it is more informative to indicate a range of possible adult heights. However, the methods of height prediction were found to be clinically acceptable in tall stature girls (16). Furthermore, manually determined BA is subject to both inter- and intraobserver variability.

In our study, we chose to calculate adult height prediction using the newly developed BoneXpert method (3), which depends on the BA assessment. In our present study, BA was calculated according to the methods of GP (20) using manual readings between 1993–2008, and by automated bone age estimation from 2008–2013 (21). Thodberg *et al.* (22) showed that the manual (GP) rating is better than the manual Tanner-Whitehouse 3 method (23), and the automated BA rating is as good as the manual BA rating. Furthermore, automatic BA rating eliminates the risk of inter- and intraobserver variability. Unfortunately, we were not able to retrieve all radiographs from the period of 1993–2008 to be re-evaluated with BoneXpert. However, we felt it unlikely that the use of different methods should introduce any systematic bias in our study.

The treatment with E2 was generally well tolerated. No serious side effects were reported during the study period. Six of the 59 girls reported one or more of the following problems during treatment: hyperpigmentation of nipple, stretch marks located to the breasts, heavy menstrual bleeding, nausea, joint, and muscle pain. However, it is important to note that this was a retrospective clinical study reflecting real life and therefore no specific registration of adverse events as in a research clinical trial was made. The reports of potential adverse effects of treatment were collected from patient records. Furthermore, our follow-up was to short too evaluate any long-term side effects.

Our findings, obtained at the first clinical visits, have contributed in describing the phenotypic characteristics of tall statured girls. In addition, we found that LH, FSH, estradiol, testosterone, IGF-I, IGFBP-3, and inhibin B were normal, whereas SHBG tended to be low. Importantly, we found significantly increased IGF-I levels among girls with CTS which is in line with previous studies (24,25). In the present study, we used two different IGF-I assays; in-house radioimmunoassay (RIA) between 1993–2008 (26) and commercially available immunoassay (Immulite) between 2008–2013 (27). We cannot exclude that the more frequent supranormal IGF-I values between 2008–2013 compared to 1993–2008 is due to differences in the two assays.

Considering the recent indications of association between high-dose EE2 treatment in adolescence and possible long-term side-effects as reduced fertility (11,12), altered coagulation parameters (10), and increase risk of melanoma (13), alternative treatments for CTS must be investigated. In our study, treatment with oral administration of E2 in CTS girls led to a 1.6 ± 2.1 cm reduction in final height and the short-term side effects were few and mild. However, it is important to keep in mind the lack of long-term follow up and shortage of randomized trials, which is necessary in order to state the efficacy and safety of treatment with E2 in CTS girls. There are alternatives to pharmacological treatment for CTS. Benyi *et al.* (14) reported a height reduction, compared to prediction, of 4.1 cm in 12 girls subjected to bilateral percutaneous epiphysiodesis. No side effects except postoperative pain were reported. However, bilateral percutaneous epiphysiodesis is, by many, considered to be an invasive procedure in an otherwise healthy child.

Our study took place in a single centre, which implies that the auxological and biochemical methods have been similar during the whole study period. Only one person conducted the review of patient record files to obtain medical history. This contributes to the consistency of the data collection.

Since this is an observational study, and not a randomized trial, there is a risk of bias. The girls were treated individually to initiate or ensure rapid progression of puberty (and epiphyseal closure). The E2 dose used, age at treatment start and treatment duration did vary among the girls. The clinical examinations were carried out by different physicians, which make subjective evaluations, as Tanner staging, at risk of interobserver variability. The blood samples for estradiol measurement were not standardized according to menstruation cycle in postmenarcheal girls.

In conclusion, this large observational study of 293 tall statured girls has contributed to the description of this patient population by presenting auxological, clinical, and biochemical data. We have shown a modest height reduction with oral E2 in our observational study, and were not able to identify predictors of beneficial effects. Thus, treatment of CTS with oral E2 cannot be recommended in general, but should be reserved for selected cases. It remains to be seen if early intervention will improve final height reduction, and further controlled studies are needed.

METHODS

Patients

The patient population consisted of girls who were referred with tall stature or overgrowth syndromes (ICD10 34.4, and ICD10 DE874, DQ970, DQ873A, DQ873B) to the Department of Growth and Reproduction at Rigshospitalet in Copenhagen, Denmark, during a 20-y period (between 1993 and 2013). A total of 304 patients were identified, and the diagnoses were re-evaluated as part of the present study.

Clinical Data and Medical History

Medical history was obtained using a structured review of patient record files. Clinical data on pubertal development were obtained from each patient visit. Puberty was evaluated by inspection and palpation of the breasts and pubic hair according to Marshall and Tanner (28). A wall-mounted stadiometer (Holtain, Crymych, UK) was used to measure standing height to the nearest 0.1 cm. The girls were weighed on a digital electronic scale (Seca delta, model 707; Seca, Hamburg, Germany) with a precision of 0.1 kg while wearing light clothing and no shoes. BMI was calculated as weight (kg) divided by height (m^2). The Danish growth references published by Tinggaard *et al.* (29) were used in this study. BA was calculated according to the methods of Greulich and Pyle (GP) (20) using manual readings between 1993–2008 (n = 368), and automated BA estimation from 2008–2013 (n = 378) (21). Target height was calculated as the sum of the mother's and father's heights (cm) minus 13 cm, divided by 2. Adult height prediction was calculated using BoneXpert (21).

17β-Estradiol Treatment

The decision to initiate treatment with E2 was determined individually, and made by the physician together with the patient and her parents, depending on height SDS, age, PAH, and target height. Oral E2 was administered in increasing doses depending on age and pubertal stage. The starting doses ranged between 0.2 to 4 mg E2. The speed of dose increment depended on the individual girl (age, BA, maturity, pubertal stage, and PAH). The treatment consisted of 17β-estradiol supplemented with norethisteronacetat after 1–2 y of E2, or after the first menstrual bleeding. Oral E2 treatment was administered in the form of Trisekvens (Novo Nordisk Scandinavia AB, Copenhagen, Denmark), Trisekvens Forte (Novo Nordisk Scandinavia AB), and/or Femanest (Sandoz A/S, Copenhagen, Denmark). Clinical and biochemical values were recorded at baseline and at each year thereafter. The treatment was generally terminated when the X-ray showed closed epiphyseal lines at final or near-final (<2 cm/y) height.

Laboratory Analysis

Blood samples were drawn from the antecubital vein between 8 AM and 1 PM in the nonfasting state. Blood samples were clotted and centrifuged, and serum was stored at −20 °C until hormone analyses were performed. Serum FSH and LH were measured by time-resolved immunofluorometric assays (Delfia, Wallac, Turku, Finland). The detection limits (dL) for FSH and LH were 0.06 and 0.05 IU/l, respectively. Intra- and interassay coefficients of variation (CV) were < 5% in both gonadotropin assays. Testosterone was measured by RIA (Coat-a-count, Diagnostic Products Corporation, Los Angeles, CA) with

a dL of 0.23 nmol/l and intra- and interassay CV both < 10%. The assay was validated against LC-MS/MS methodology in 757 serum samples. SHBG was measured by time-resolved immunofluorometric assays (Delfia, Wallac, Turku, Finland) with a dL of 0.20 nmol/l, and intra- and interassay CVs of 5.8 and 6.4%, respectively. Serum insulin-like growth factor I (IGF-I) was measured using a highly sensitive in-house RIA as previously described by Juul *et al.* (26). The intra- and interassay CV were 3.9 and 8.7%, respectively. From 2008, IGF-I levels were determined by conventional immunoassays (IMMULITE 2000 IGF-1; Siemens Healthcare Diagnostics, Los Angeles, CA) on automated IMMULITE 2000 (Siemens). The CV were less than 4 and 9%, respectively (27). Serum insulin-like growth factor-binding protein 3 (IGFBP-3) was measured by RIA, as previously described by Blum *et al.* (30). The intraassay CV was 2.4% and the interassay CV was 10.7% (31). Between 1993 and 2013, serum inhibin B was measured using one of two double antibody enzyme immunometric assays (Inhibin B DSL or Oxford Bio-Innovation Inhibin B), both with a dL of 20 pg/ml and intra- and interassay CV < 16%. Estradiol was measured by RIA (Pantex, Santa Monica, CA; before 1998 distributed by Immuno Diagnostic Systems, Bolton, UK) with a dL of 18 pmol/l, and intra- and interassay CVs < 8% and <13%, respectively. Assays for IGF-I changed during the 20-y study period, and we compared the two assays rigorously and ensured that they yielded similar results before changing the assay. All assays were accredited by the Danish Accreditation Fund (DANAK (http://english.danak.dk/)).

Statistical Analysis

Data are displayed as the median with interquartile range (25th; 75th percentiles) and/or the range (min – max). Results of treatment effect and IGF-I (SD) are displayed as the mean ± SD. Hormone values below the dL of the assay were assigned a value corresponding to the dL/2. The Mann-Whitney *U*-test and student's *t*-test was used to determine significance when comparing clinical, auxological, and laboratory data between groups, and student's *t*-test was used when comparing the effect of treatment within the group of girls treated with oral E2. All statistical analyses were performed using SPSS software (IBM Corporation, Armonk, NY, version 22).

Ethical Considerations

Clinical data and blood samples were collected as part of the patient's routine clinical follow-up, and all parents gave informed consent. This retrospective study based on patient record files was approved by our institution and the Danish Data Protection Agency (RH-2015–218, I-Suite no: 04161) and Danish Health Authorities (3-3013-1333/1/). The study was registered in www.Clinicaltrials.gov (#NCT02638922).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

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