Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Does height and IGF-I determine pubertal timing in girls?

Emmie N. Upners[1,2], Alexander S. Busch[1,2], Kristian Almstrup[1,2], Jørgen H. Petersen[1,2], Maria Assens[1,2], Katharina M. Main[1,2], Rikke B. Jensen[1,2] and Anders Juul[1,2]

BACKGROUND: Pubertal timing is closely linked to growth regulated by the growth hormone/insulin-like factor (GH/IGF) axis that includes IGF-regulating factors such as pregnancy-associated plasma protein-A/A2 (PAPP-A/PAPP-A2) and stanniocalcin 2 (STC2). We investigated the association between height, IGF-I concentration, and *PAPPA*, *PAPPA2*, and *STC2* genotypes on the timing of female pubertal milestones.

METHODS: Height, IGF-I, and genotypes were analyzed in 1382 Danish girls from the general population, 67 patients with tall stature (height ≥2 SD), and 124 patients with short stature (height ≤−2 SD). The main outcomes were breast stage and menarche.

RESULTS: Thelarche occurred significantly earlier in patients with tall stature (mean age 9.37 years [95% confidence interval (CI) 8.87–9.87]) and later in patients with short stature (11.07 years [95% CI 10.7–11.43]) compared with girls within the normal range (9.96 years [95% CI 9.85–10.07]) ($p = 0.02$ and $p < 0.01$, respectively). Girls with higher IGF-I levels experienced thelarche and menarche earlier compared with the rest of the cohort ($p < 0.01$). Genotypes were not associated with age at thelarche nor menarche, but the *PAPPA2* minor allele carriers were shorter compared with major allele carriers, $p = 0.03$.

CONCLUSIONS: Height and IGF-I, but not *PAPP-A*, *PAPP-A2*, and *STC2* genotypes, were negatively associated with age at thelarche and menarche.

Pediatric Research (2021) 90:176–183; https://doi.org/10.1038/s41390-020-01215-6

IMPACT:

- Girls with tall and short stature enter puberty earlier and later compared with girls with normal height.
- Girls with higher insulin-growth factor-I in childhood enter puberty earlier.
- Pubertal timing is influenced by longitudinal growth and IGF-I levels earlier in childhood.
- Childhood growth and the levels of IGF-I in childhood may be biomarkers of pubertal timing.

INTRODUCTION

Pubertal timing varies considerably among girls. The regulation of pubertal timing is multifactorial and involves genetic, nutritional, and environmental factors.[1] Activation of the gonadotropin-releasing hormone (GnRH) pulse generator initiates the pubertal transition. Throughout this transition, growth of estrogen-responsive tissue occurs, ultimately leading to breast development (thelarche) and menarche in girls. The GnRH surge is initiated by complex interactions of neural and endocrine signals. However, the mechanism is not well understood.

Pubertal transition is closely linked to linear growth suggesting common pathways between reproductive development and the growth hormone/insulin-like factor-I (GH/IGF-I) axis. Patients with dysfunction of the GH/IGF-I axis, for example, patients with GH-insensitivity syndrome, present with delayed puberty, short stature, and small genitalia.[2] Moreover, there is mounting preclinical evidence that IGF-I is involved in pubertal development. For example, mice lacking IGF-I receptors on GnRH neurons showed delayed pubertal onset.[3] In addition, IGF-I administered into the ventricles of the brain in mice led to earlier onset of puberty, which highlights the potential role of IGF-I in pubertal development.[4]

IGF-I circulates in high concentrations; nevertheless, the majority of IGF-I is bound to specific IGF-binding proteins, leaving only a small fraction to be free and biologically active. Furthermore, pregnancy-associated plasma protein-A (PAPP-A),[5] pregnancy-associated plasma protein-A2 (PAPP-A2),[6] and stanniocalcin 2 (STC2)[7] regulate the binding of IGF-binding proteins to IGF-I and thereby contribute to IGF-I bioavailability. In a large genome-wide association study (GWAS), single-nucleotide polymorphisms (SNPs) in the IGF-I gene were not associated with adult height, whereas variants in genes coding for modifiers of IGF bioavailability, that is, encoding PAPP-A, PAPP-A2, and STC2, were strongly associated with adult height.[8] In addition, mutations in the PAPP-A2 gene caused short stature.[9] Thus, the regulation of IGF-I and its actions are important for the regulation of human growth. However, it remains unknown if this in turn influences the timing of puberty. To our knowledge, pubertal timing in girls with short and tall stature has not been evaluated previously. We hypothesize that height influences timing of puberty directly or via IGF-I and its regulators.

Therefore, we investigated the impact of height, IGF-I, and genetic variants in *PAPP-A*, *PAPP-A2*, and *STC2* on pubertal onset

[1]Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen, Denmark and [2]International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen, Denmark

Correspondence: Emmie N. Upners (emmie.nicolina.upners.02@regionh.dk)

Received: 27 March 2020 Revised: 8 July 2020 Accepted: 23 September 2020
Published online: 3 November 2020

and menarche in a large cohort of girls with tall stature and short stature, and normal height. In addition, we explored a possible relationship between height and pubertal timing and genotypes of SNP of *PAPP-A*, *PAPP-A2*, and *STC2*.

SUBJECTS AND METHODS

Study population

The cohort of 1573 girls was recruited from two population-based cohorts of Danish children and two patient cohorts.

Girls from the general population

(I) *The COPENHAGEN Puberty Study*: 1097 girls, aged 6–20 years, were recruited in 2006–2008 from 10 schools in Copenhagen (overall participation rate 35%).[10,11] In total, 108 of these girls participated in a follow-up with repeated pubertal examinations every 6 months. Girls without available DNA were excluded from the present study ($n = 223$). A total of 785 and 89 girls, from the cross-sectional and longitudinal cohort respectively, were included in the study.

(II) *The Copenhagen Mother–Child Cohort*: 1210 girls born in 1997 and 2002 were examined at several timepoints during infancy and childhood.[12,13] Between 2006 and 2013, 672 of the girls attended one to five annual examinations and 317 of these girls were again examined once between 2015 and 2016. A total of 508 girls with available DNA were included in the present study.

Patients. The patient populations included girls with short and tall stature referred to the Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark, a tertiary pediatric endocrinology unit. The included girls fulfilled the criteria for tall stature (height standard deviation score (HSDS) $\geq +2$, $n = 170$) and short stature (HSDS ≤ -2, $n = 218$) and were referred to the department during the study periods of 2003–2013 and 1992–2015, respectively. A total of 66 girls with tall stature and 124 with short stature had available DNA and were included in the present study. The girls were examined at several visits at the outpatient clinic (mean number of visits 2.97 (2.05 SD) and 4.18 (5.73 SD) in girls with short and tall stature, respectively).

Study groups

We divided the girls into five groups depending on their height: (A) patients evaluated because of short stature, (B) short girls from the population-based cohorts (HSDS ≤ -2), (C) girls from the population-based cohorts ($-2 <$ HSDS $< +2$), (D) tall girls from the population-based cohorts with HSDS $\geq +2$, and (E) patients evaluated because of tall stature. The groups were divided based on their calculated height SDS at first examination in girls recruited from the COPENHAGEN Puberty Study, at first examination in mid-childhood in girls recruited from the Copenhagen Mother–Child Cohort, and at the first clinical visit in patients with short and tall stature. Out of 124 girls in group A, 21 girls were born small for gestational age (SGA) and 2 girls were diagnosed with Silver–Russell syndrome. In group A, seven girls were treated with GH (five due to SGA, one due to Silver–Russell syndrome, and one due to extremely short stature). None of the girls had partial or complete GH deficiency.

Clinical examination

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, Germany) with a precision of 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). The anthropometric data were expressed as standard deviation scores (SDS) to enable us to compare children of different ages. The Danish growth references published by Tinggaard et al.[14] were used as reference for height and BMI. Puberty was evaluated by inspection and palpation of the breasts according to Marshall and Tanner. Pubertal onset (thelarche) was defined as having breast stage 2. Age at menarche was collected through information obtained at an outpatient clinic visit for the patients or through a questionnaire for the healthy girls. Bone age (BA) was measured according to the methods of Greulich and Pyle.

Genotyping

Peripheral blood (EDTA-preserved) was used for isolation of genomic DNA (QuickGene-810 Nucleic Life Science Products, Tokyo, Japan) and quantified on a NanoDrop ND-1000 spectrophotometer (Saveen Werner, Limhamn, Sweden). KASP™ genotyping assays were designed by LGC genomics, United Kingdom, toward the following sequences: rs751543 (*PAPPA*): GAGCAGACTC [Y]GGCTACTTCT; rs889014 (*STC2*): ARYTATTAACT[Y]TCAAYTACT AGA; and rs1325598 (*PAPPA2*): CATAAATGAAKAAM[R]TAATTTTTCC AGC, and genotyped in all 1573 girls. The analysis was performed in the laboratory of the Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark. In a few cases, genotyping was impossible due to poor DNA quality. Consequently, *PAPPA*, *PAPPA2*, and *STC2* was genotyped in 1556, 1562, and 1561 girls, respectively.

Hormone analyses

Blood samples were drawn from the antecubital vein, clotted and centrifuged, and stored at −20 °C. Serum IGF-I concentrations were measured in 874 girls from the COPENHAGEN Puberty Study using IMMULITE 2000 conventional immunoassay (Siemens Healthcare Diagnostics, Los Angeles, CA, USA) with an intra-assay coefficient of variation (CV) of 2.1%, inter-assay CV of 10.1%, and limit of detection (LOD) of 20 µg/l. IGF-I SDS was calculated from our reference data.[15] IGF-I was measured as part of the routine workup in patients with tall and short stature. During the study period, the department used three different assays for IGF-I. A radioimmunoassay was used until 2008,[16] with an inter-assay CV of 14.1%, intra-assay CV of 10.3%, and a LOD of 20 µg/l. IGF-I SDS was calculated from our reference data.[16] IMMULITE 2000 was used between 2008 and 2013. From 2013, IGF-I was determined by an iSYS assay (Immunodiagnostic Systems, UK). The CVs for inter- and intra-assay were 7.2% and 2.1% and the LOD was 10 µg/l. We calculated age- and sex-adjusted IGF-I SDS to compare measurements in children of different ages and with different assays.

Statistical analyses

We performed probit analyses (proc lifereg; SAS Institute, Cary, USA), integrating left-, right-, and interval-censored data to estimate the mean age [95% confidence interval (CI)] at thelarche and menarche. Longitudinal measurements were used as interval-censored data (interval-censored data, $n = 402$ for thelarche, $n = 568$ for menarche). If a girl had examinations before and examinations after entering puberty, the interval contained the exact age of thelarche. Some girls had already entered puberty when the examination took place, in which case the current age was the upper bound for the age at thelarche (left-censored data). Some had not entered puberty before the examinations and the current age was the lower bound for age at thelarche (right-censored data). The five height groups were used to evaluate the effects of height (Table 1). Furthermore, to evaluate the effects of height and IGF-I levels in the entire population and in the population-based cohorts (groups B–D), we used height SDS quintiles (Q1–Q5) and IGF-I SDS (Q1–Q5). If a girl had longitudinal measurements, we used the height SDS and IGF-I SDS closest to 8 years of age. Girls with an age >15 years were excluded from the probit analyses ($n = 133$). Two subanalyses were performed, including only girls who were prepubertal at study entry

Table 1. Basic characteristics of the patient and population-based cohorts divided into groups.

	Group A patients (HSDS ≤ −2) $n = 124$	Group B population (HSDS ≤ −2) $n = 21$	Group C population (−2 < HSDS < +2) $n = 1326$	Group D population (HSDS ≥ +2) $n = 35$	Group E patients (HSDS ≥ +2) $n = 67$
Age (years)	5.65 (3.72; 11.29)	11.58 (10.48; 12.24)	10.97 (9.36; 12.33)	12.12 (10.48; 13.31)	10.57 (7.40; 13.19)
Height (cm)	103.0 (91.9; 129.9)	135.5 (127.0; 139.7)	146.9 (136.8; 157.6)	170.8 (161.7; 173.9)	164.1 (144.3; 177.2)
Height (SDS)	−2.70 (−3.02; −2.33)	−2.27 (−2.37; −2.09)	−0.08 (−0.65; 0.60)	2.32 (2.15; 2.51)	2.79 (2.45; 3.41)
BMI (kg/m^2)	15.4 (14.5; 16.6)	16.7 (15.5; 18.8)	17.3 (15.8; 19.4)	19.0 (17.8; 20.6)	18.4 (16.9; 20.2)
BMI (SDS)	−0.52 (−1.31; 0.14)	−0.43 (−0.99; 0.66)	0.06 (−0.64; 0.74)	0.65 (−0.13; 1.04)	0.55 (−0.17; 1.35)
IGF-I (SDS)	−0.97 (−1.76; 0.08)	−1.04 (−1.56; −0.86)	−0.02 (−0.61; 0.67)	0.38 (−0.43; 1.07)	1.03 (0.23; 2.01)

Median (25th; 75th percentile).
Auxological data and IGF-I in girls with short, normal, and tall stature, respectively.
SDS SD score, *HSDS* height SD score, *BMI* body mass index.

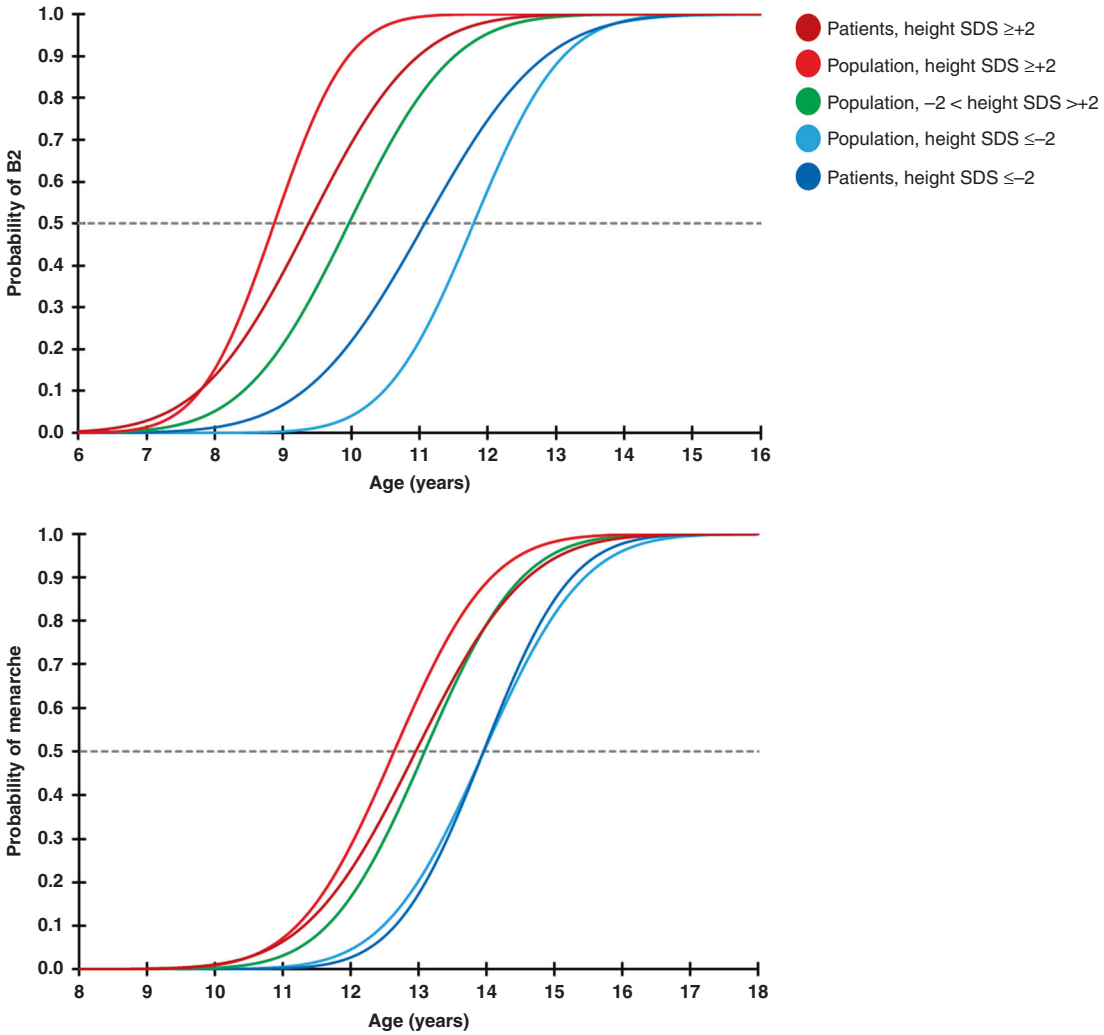

Fig. 1 Pubertal milestones in girls with short, normal or tall stature. Probability of having Tanner breast stage 2 or more (B2+) (**a**) or menarche (**b**) according to age. Patients with short stature (height SDS (HSDS) ≤ −2) are shown as dark blue lines, girls from the population-based cohorts (HSDS ≤ −2) as light blue lines, girls from the population-based cohorts (−2 < HSDS < +2) as green lines, girls from the population-based cohorts (HSDS ≥ +2) as light red lines, and patients with tall stature (HSDS ≥ +2) as dark red lines.

(<breast stage 2) with an available IGF-I measurement ($n = 377$) or height SDS ($n = 558$), respectively, to evaluate the effect of prepubertal levels of IGF-I and height SDS on age at thelarche and menarche. We performed all probit analyses unadjusted and adjusted by BMI SDS. BMI SDS was included as a covariate (continuous variable) in the model.

The puberty score was calculated as individual mean breast-stage SDS in the girls with multiple puberty evaluations. We used

Table 2. Age at thelarche and menarche in the height groups of the population-based and patient cohorts.

Unadjusted	Thelarche (years)	*n*	*p* Value	Menarche (years)	*n*	*p* Value
(A) Patients (HSDS ≤ −2)	11.07 (10.7–11.43)	85	<0.01	13.96 (13.48–14.44)	27	<0.01
(B) Population, (HSDS ≤ −2)	11.79 (10.99–12.60)	21	<0.01	13.95 (13.17–14.73)	19	0.03
(C) Population, (-2 < HSDS < +2)	9.96 (9.85–10.07)	1315	Ref.	13.09 (13.00–13.18)	1282	Ref.
(D) Population, (HSDS ≥ +2)	8.88 (8.00–9.77)	35	0.02	12.64 (12.19–13.09)	35	0.05
(E) Patients (HSDS ≥ +2)	9.37 (8.87–87)	63	0.02	12.93 (12.59–13.28)	61	0.39
Adjusted for BMI SDS	**Thelarche (years)**	***n***	***p* Value**	**Menarche (years)**	***n***	***p* Value**
(A) Patients (HSDS ≤ −2)	10.93 (10.57–11.30)	85	<0.01	13.77 (13.30–14.23)	27	<0.01
(B) Population (HSDS ≤ −2)	11.66 (10.87–12.44)	21	<0.01	14.01 (13.24–14.77)	19	0.02
(C) Population (−2 < HSDS < +2)	9.96 (9.86–10.07)	1315	Ref.	13.10 (13.01–13.19)	1282	Ref.
(D) Population (HSDS ≥ +2)	9.14 (8.23–10.06)	35	0.08	12.80 (12.36–13.23)	35	0.18
(E) Patients (HSDS ≥ +2)	9.57 (9.06–10.07)	63	0.13	13.07 (12.73–13.41)	61	0.87

Mean age (95% confidence interval).
Age at thelarche (defined as breast stage 2) and age at menarche, unadjusted and adjusted for BMI SDS, in patients with short stature (height SDS (HSDS) ≤ −2), girls from the population-based cohorts (HSDS ≤ −2), girls from the population-based cohorts (−2 < HSDS < +2), girls from the population-based cohorts (HSDS ≥ +2) and patients with tall stature (HSDS ≥ +2), respectively.
HSDS height SD score, *BMI* body mass index.

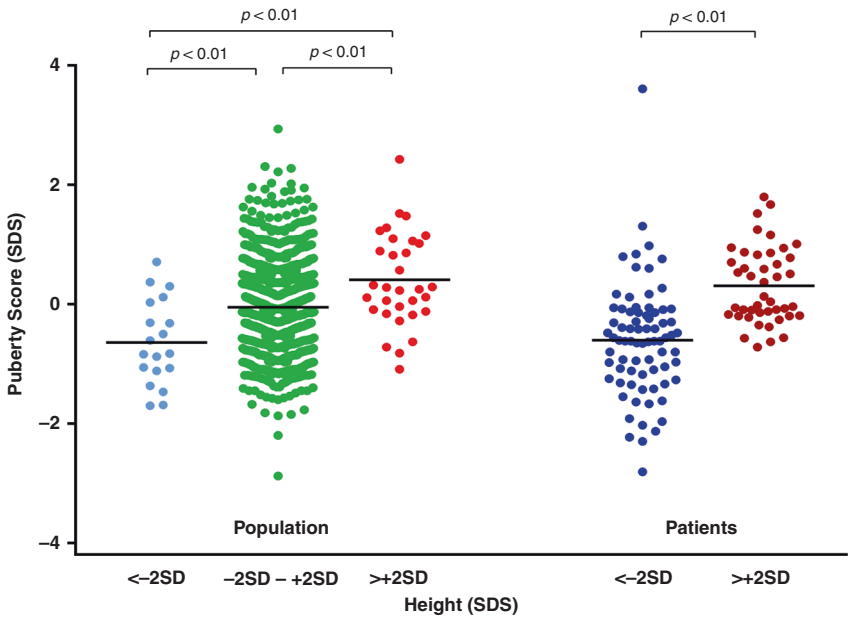

Fig. 2 Individual puberty timing (puberty scores) in girls with tall, normal or short stature. Average puberty score (mean breast stage SDS) for each girl are shown according to stature. Patients with short stature (height SDS (HSDS) ≤ −2) are shown as dark blue dots, girls from the population-based cohorts (HSDS ≤ −2) as light blue dots, girls from the population-based cohorts (−2 < HSDS < +2) as green dots, girls from the population-based cohorts (HSDS ≥ +2) as light red dots, and patients with tall stature (HSDS ≥ +2) as dark red dots. Horizontal bars mark the groups' mean breast stage.

previously described Danish breast-stage nomogram to display progression through puberty.[17] The patients with short stature treated with GH were excluded ($n = 6$) from the analysis.

For the genotype analysis, all girls were included with only one auxological and biochemical measurement, at first examination in girls recruited from the COPENHAGEN Puberty Study, at first examination in mid-childhood in girls recruited from the Copenhagen Mother–Child Cohort, and at the first clinical visit in patients with short and tall stature. We performed a Fisher's exact test and χ^2 test to investigate the genotype distribution between the height groups. The association between genotype and phenotype (height SDS and IGF-I SDS) was tested by an analysis of variance and analysis of covariance. The effect size (β) was calculated for the effect of each genetic variant.

All statistical analyses were generated using the SAS version 9.4 and IBM SPSS Statistics 22. $P < 0.5$ was considered statistically significant.

Ethical considerations

All girls or their parents who participated in the population-based studies gave informed consent. The COPENHAGEN Puberty Study (ClinicalTrials.gov ID: NCT01411527) and the Copenhagen

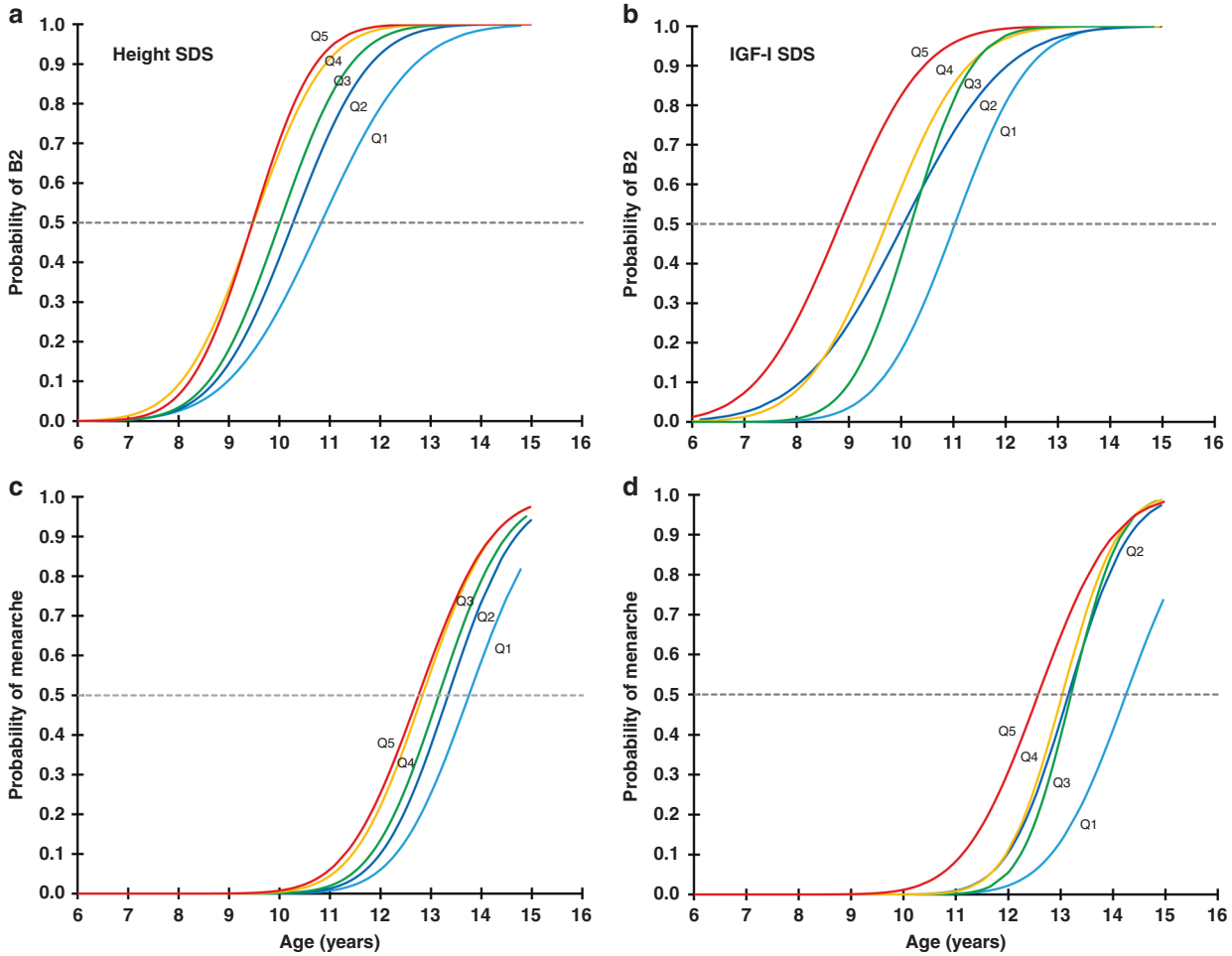

Fig. 3 Pubertal milestones in girls stratified by height SDS and IGF-I SDS. Probability of having Tanner breast stage 2 or more (B2+) (**a**) or menarche (**c**) according to age in girls with short, normal, and tall stature stratified by height SDS quintiles and the predicted probability of having Tanner breast stage 2 or more (B2+) (**b**) or menarche (**d**) according to age in girls stratified by IGF-I SDS quintiles. Quintile (Q) 1 is shown as light blue lines, Q2 as dark blue lines, Q3 as green lines, Q4 as yellow lines and Q5 as red lines.

Mother–Child Cohort were approved by the Ethical Committee at the Capital Region of Denmark (H-KF-282214 and KF 01 030/97, H-1-2009-074) and the Danish Data Protection Agency (2015-41-4494 and 2003-41-2996). All patient samples were taken as part of the clinical follow-up. Use of data from patients with short or tall stature was approved by the Danish Patient Safety Authority (nos. 3-3013-2022/1 and 3-3013-1333/1/) and the Danish Data Protection Agency (RH-2016-177-04732 and RH-2015-218-04161). The genetic analyses of the patient cohorts were approved by the Ethical Committee at the Capital Region of Denmark (H-18013128).

RESULTS

Height and puberty

Clinical characteristics for all girls divided into five height groups are presented in Table 1. Thelarche was significantly earlier in patients with tall stature (9.37 years [95% CI 8.87–9.87], $p < 0.01$) (group E) and later in patients with short stature (11.07 years [95% CI 10.7–11.43], $p = 0.02$) (group A), compared with girls with normal height (9.96 years [95% CI 9.85–10.07]) (group C) (Fig. 1 and Table 2). This was confirmed within the population-based cohort. The tallest girls (group D) had earlier thelarche and the shortest girls (group B) later thelarche compared with girls with normal height (group C), $p < 0.01$ and 0.02, respectively. Adjusting for BMI did not substantially change the estimates for the short girls (groups A and B), but the difference between tall girls (groups D and E) and girls with normal height became statistically insignificant. Menarche was significantly later in the short girls (groups A and B) compared with girls with normal height (group C). Adjusting for BMI did not change the results (Table 2). There was no significant difference in age of menarche between tall girls (groups D and E) and girls with normal height (group C).

The puberty nomogram (Supplementary Fig. S1) transforms a distinct age at a specific pubertal stage to a continuous puberty score and illustrates the individual progression through puberty.

The puberty scores, calculated as the average breast-stage score of an individual evaluated longitudinally (mean breast SDS), were higher in tall girls (group D: mean SDS 0.41 (0.78 SD)) compared with girls with normal height (group C: −0.05 (0.70 SD)), and lower in short girls (group B: −0.64 (0.71 SD)) from the population cohort ($p < 0.01$ and $p < 0.01$, respectively). Patients with tall stature had a higher puberty score compared with patients with short stature (group E: mean SDS 0.31 (0.64 SD) vs. group A: −0.60 (0.94 SD)), $p < 0.01$ (Fig. 2).

In addition, we combined the cohorts and divided the entire group into quintiles (Q) depending on their height SDS: Q1 −3.78 to −1.08 SDS, Q2 −1.08 to −0.34 SDS, Q3 −0.34 to 0.16 SDS, Q4 0.17–0.98 SDS, and Q5 0.98–4.96 SDS. Thelarche and menarche were significantly later in girls within the lowest height quintile (Q1) and earlier in girls within the two highest quintiles (Q4–Q5) compared with the middle quintile (Q3) (all p values below

Table 3. Age at thelarche and menarche according to (A) prepubertal height SDS and (B) prepubertal IGF-I SDS.

(A) Prepubertal height SDS

Unadjusted	HSDS[a]	Age at HSDS (years)[a]	Thelarche (years)[b]	*n*	*p* Value	Menarche (years)[b]	*n*	*p* Value
HSDS < −1 SDS	−1.77 (−2.47; −1.32)	8.65 (6.54; 10.30)	11.54 (11.30–11.78)	174	<0.001	14.03 (13.70–14.37)	118	0.04
HSDS −1 ≥ SDS ≤ 1	−0.16 (−0.53; 0.23)	8.65 (7.53; 9.67)	10.94 (10.74–11.14)	305	Ref.	13.59 (13.30–13.87)	300	Ref.
HSDS > 1 SDS	1.54 (1.28; 2.67)	8.35 (7.15; 9.24)	10.12 (9.76–10.48)	76	<0.001	12.55 (12.08–13.02)	71	<0.001
Adjusted for BMI SDS	**HSDS[a]**	**Age at HSDS (years)[a]**	**Thelarche (years)[b]**	***n***	***p* Value**	**Menarche (years)[b]**	***n***	***p* Value**
HSDS < −1 SDS	−1.77 (−2.47; −1.32)	8.65 (6.54; 10.30)	11.48 (11.23−11.73)	174	<0.001	13.96 (13.61−14.31)	118	0.07
HSDS −1 ≥ SDS ≤1	−0.16 (-0.53; 0.23)	8.65 (7.53; 9.67)	10.93 (10.73−11.12)	305	Ref.	13.56 (13.28−13.85)	300	Ref.
HSDS > 1 SDS	1.54 (1.28; 2.67)	8.35 (7.15; 9.24)	10.18 (9.81−10.54)	76	<0.001	12.65(12.15−13.14)	71	<0.01

(B) Prepubertal IGF-I SDS

Unadjusted	IGF-I SDS[a]	Age at IGF-I (years)[a]	Thelarche (years)[b]	*n*	*p* Value	Menarche (years)[b]	*n*	*p* Value
IGF-I < −1 SDS	−1.52 (−1.95; −1.20)	9.27 (7.63; 10.66)	11.87 (11.47–12.27)	74	<0.01	14.12 (13.51–14.73)	58	0.09
IGF-I ≥ SDS ≤ 1	−0.09 (−0.47; 0.40)	8.45 (7.33; 9.43)	11.13 (10.85–11.40)	256	Ref.	13.51 (13.15–13.88)	240	Ref.
IGF-I > 1 SDS	1.31 (1.14; 1.69)	7.56 (6.97; 8.60)	10.02 (9.46–10.58)	45	<0.001	12.23 (11.52–12.94)	44	0.001
Adjusted for BMI SDS	**IGF-I SDS[a]**	**Age at IGF-I (years)[a]**	**Thelarche (years)[b]**	***n***	***p* Value**	**Menarche (years)[b]**	***n***	***p* Value**
IGF-I < −1 SDS	−1.52 (−1.95; −1.20)	9.27 (7.63–10.66)	11.80 (11.40–12.20)	74	<0.01	13.99 (13.39–14.58)	58	0.17
IGF-I ≥ SDS ≤ 1	−0.09 (−0.47; 0.40)	8.45 (7.33; 9.43)	11.11 (10.84–11.38)	256	Ref.	13.51 (13.16–13.87)	240	Ref.
IGF-I > 1 SDS	1.31 (1.14; 1.69)	7.56 (6.97; 8.60)	10.13 (9.55–10.71)	45	<0.01	12.42 (11.70–13.15)	44	<0.01

Age at thelarche (defined as breast stage 2) and age at menarche, unadjusted and adjusted for BMI SDS, in girls with prepubertal height SDS (A) and IGF-I SDS (B) ≤−1 SDS, −1 < SDS < +1 and SDS > +1, respectively.
HSDS height standard deviation score, *IGF-I* insulin-like growth factor-I, *SDS* standard deviation score, *BMI* body mass index.
[a]Median (25th; 75th percentile).
[b]Mean age (95% confidence interval).

$p < 0.05$) (Fig. 3 and Supplemental Table S1A). Exclusion of the patients with short and tall stature (groups A and E) and dividing the group of girls from the population-based cohorts (groups B–D) into height quintiles confirmed the findings of later thelarche and menarche in those with lower height (Supplemental Table S2A). A similar relation between height SDS and age at thelarche was revealed in a subanalysis exclusively including height SDS of prepubertal girls (Table 3A).

BA only available in the patient cohorts (groups A and E) was slightly advanced in patients with tall stature (group E: 0.27 years (1.04 SD)) corresponding to 3 months that is considered age-appropriate. In contrast, patients with short stature had a delayed BA (group A: −1.45 years (1.04 SD)).

IGF-I and puberty

IGF-I concentrations were available in 956 girls from the population-based and patient cohorts. The girls were divided into quintiles depending on their IGF-I SDS: Q1 −3.05 to −0.87 SDS, Q2 −0.86 to −0.25 SDS, Q3 −0.25 to 0.24 SDS, Q4 0.24–0.90 SDS, and Q5 0.91–4.68 SDS. Age at thelarche and menarche was later in girls within the lowest IGF-I SDS quintile (Q1) and earlier in girls within the highest quintile (Q5) compared with the reference group (Q3) (all *p* values below <0.001) (Fig. 3 and Supplementary Table S1B). Exclusion of the patients with short and tall stature (groups A and E) and dividing the group of girls from the population-based cohorts (groups B–D) into quintiles showed the same results, later thelarche and menarche in those with lower IGF-I (Supplementary Table S2B). Including only prepubertal girls divided into groups depending on prepubertal IGF-I SDS revealed the same pattern with an inverse relationship between IGF-I and age at thelarche (Table 3B).

Genotype distribution

The girls had the following allele distributions: *PAPPA* rs751543 A > G AA 48.4% ($n = 753$), AG 41.4% ($n = 644$), GG 10.2% ($n = 159$), *PAPPA2* rs1325598 C > T CC 32.2% ($n = 502$), CT 49.7% ($n = 776$), TT 18.1% ($n = 283$) and *STC2* rs889014 C > T CC 43.1% ($n = 674$), CT 44.9% ($n = 702$), and TT 12.0% ($n = 186$). The distribution of genotypes in the five height groups (groups A–E) is shown in Fig. 4 and Supplementary Table S3. There was a significant difference between the genotype distribution for *PAPPA2* rs1325598 between the girls from the population-based cohorts according to their height (groups B–D) ($p = 0.02$) (Fig. 4). No significant differences were found between the frequency distributions for *PAPPA* rs751543 and *STC2* rs889014 across groups.

In the entire cohort, girls homozygous for the minor allele (*PAPPA2* rs1325598 TT) were −0.27 (−0.47; −0.08) SDS shorter and carriers of the heterozygote genotype (*PAPPA2* rs1325598 CT) were −0.09 (−0.25; 0.06) SDS shorter than carriers of the genotype CC ($p = 0.03$). This association remained significant after adjusting for IGF-I SDS ($p = 0.02$). There was no association between height SDS and *PAPPA* rs751543 or *STC2* rs889014. No differences in IGF-I SDS between the three genotypes (data not shown) were found. None of the genotypes were associated with thelarche or menarche (data not shown).

DISCUSSION

In this large study of 1573 girls, we investigated the relation between statural height and distinct female pubertal milestones and found a negative association between height and pubertal onset. Girls with tall stature and an age-appropriate BA had breast

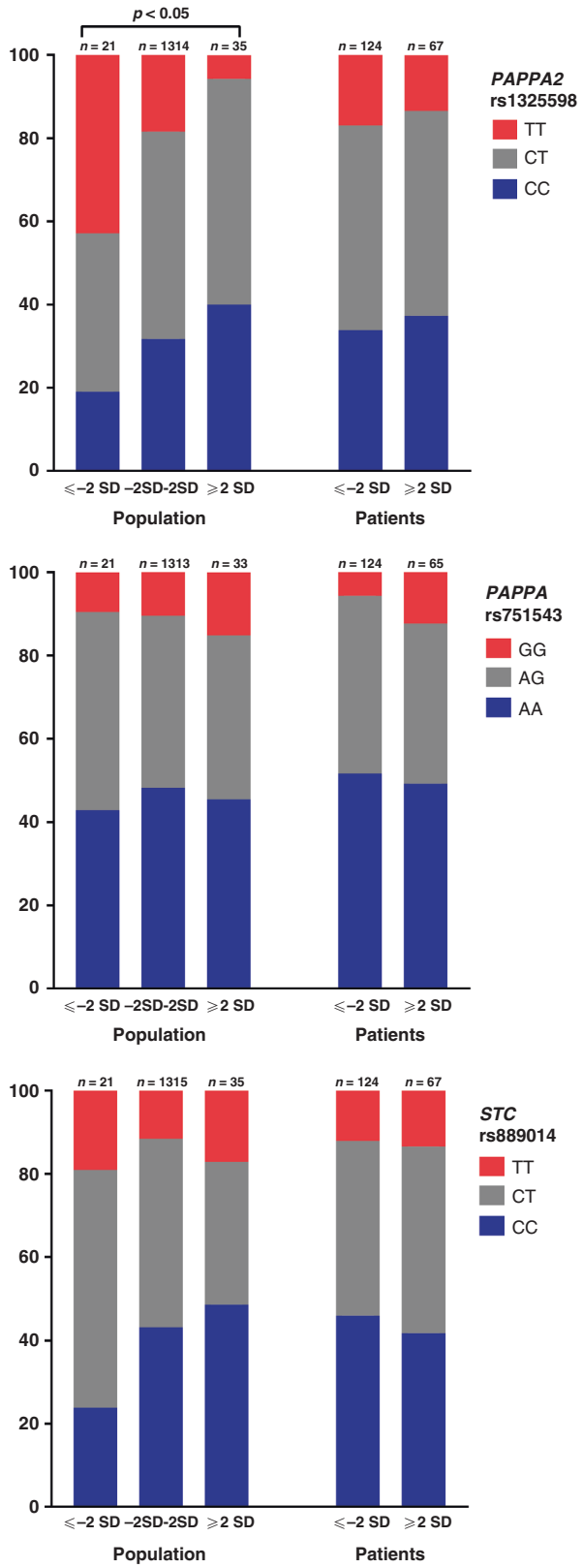

Fig. 4 Distribution of genotypes. Distribution of genotypes *PAPP-A2* (rs1325598), *PAPP-A* (rs751543), and *STC-2* (rs889014) in girls from the population-based and the patient cohorts. Population-based and patient groups were compared using Fisher's exact test and χ^2 test, respectively.

development at an earlier age than girls with normal or short stature. In the entire cohort, we found that girls with higher IGF-I had earlier thelarche and menarche. This was not only true for patients with short or tall stature, but also seen in the population-based cohorts. Height, but neither thelarche nor menarche, was associated with *PAPPA2* genotypes.

It is well known that IGF-I levels increase linearly in childhood with a significant rise during puberty. Our data suggest an influence of IGF-I and height on pubertal timing, and we therefore propose that the GH/IGF-I axis could serve as a link between growth and sexual maturation. To support this hypothesis, patients with mutations that impair the GH/IGF-I axis are short and present with pubertal delay.[2,18] In animal studies, intracerebroventricular-administered IGF-I in prepubertal rodents leads to earlier pubertal onset,[3] and knockout of IGF-I receptors on GnRH neurons in mice resulted in delayed pubertal onset,[4] suggesting a central regulatory role of GH/IGF-I-axis on pubertal timing. Furthermore, sexual maturation is delayed in female GH receptor knockout mice and can be advanced by administration of IGF-I.[19] In children with short stature, treatment with GH or recombinant IGF-I may advance the timing of puberty, although the results are diverging.[20–22] Our findings of earlier menarche in girls with higher IGF-I concentrations are in line with a previous Australian study concluding that menarche occurred earlier in children with high levels of IGF-I.[23] In **a**dditionally, a previous study demonstrated that girls with central precocious puberty had higher IGF-I concentrations compared with both age- and BA-matched healthy girls.[15] In our study, girls with tall stature had an earlier breast development than those with normal or short stature. Although it would be evident to speculate that they had earlier puberty and thereby increased height, the fact that girls with tall stature had an age-appropriate BA suggests that their increased height was genetically determined, and not driven by earlier maturation per se. In addition, in a subanalysis, prepubertal IGF-I levels were inversely associated with age at thelarche. However, our study is an observational study and therefore cannot provide evidence of a causal relation between IGF-I, height, and puberty, but can describe the associations. Consequently, there is a potential risk of reverse causality between height, IGF-I, and pubertal timing

We found a higher mean puberty score in girls with tall stature compared with girls with short stature. It can be speculated that this difference is not only influenced by the difference in pubertal onset, but that a faster tempo during pubertal development may also play a role.

Higher BMI in childhood[24] has previously been associated with earlier age at menarche, and possible mechanisms explaining the relation have been proposed previously. Overweight and obesity are followed by a compensatory hyperinsulinism because of insulin resistance and this may lead to decreased sex-hormone-binding globulin and thereby increased availability of sex hormones.[21] Furthermore, an increase in aromatase activity can increase the conversation of androgens to estrogens in peripheral fat tissue.[25] However, high IGF-I at the age of 8 years was independently associated with earlier age at menarche even after adjusting for body size (BMI and height).[26] In the current study, we adjusted for BMI that did not alter the association between short stature and later thelarche, whereas the association between tall stature and earlier thelarche became statistically insignificant. BMI is commonly used as a marker of body composition to evaluate overweight and obesity. The fact that girls with tall stature, in the current study, had higher BMI, is probable due to increased fat mass. However, a previous study shows that a higher BMI SDS may not necessarily reflect higher adiposity, but also changes in stature in early-maturing children.[27] Girls with premature adrenarche are characterized by having increased androgen levels, height above

average, high BMI, and pubarche.[28] High androgens could potentially lead to earlier gonadotropin-independent thelarche due to increased conversion of androgen to estrogen by aromatase. However, girls with premature adrenarche usually present with advanced BA[28] and appropriate height for BA, and in our cohort, girls with tall stature had a nearly age-appropriate BA.

New discoveries within the GH/IGF-I axis have increased our understanding of the complex regulation of linear growth. PAPP-A and PAPP-A2 cleave IGF-I from its binding protein and thereby increase the IGF-I bioavailability.[5,6] In addition, STC2 inhibits the activity of PAPP-A and PAPP-A2[7] and thereby longitudinal growth. We studied three common genetic variants in *PAPP-A2*, *PAPP-A*, and *STC2*, which were strongly associated with adult height in a previous study,[8] and found that girls homozygous for the minor allele of *PAPP-A2* were shorter compared with girls homozygous for the major allele. We did not find any associations to thelarche, menarche, or IGF-I with any of the three SNPs we tested. In GWAS, the study population often exceeds 100,000 participants, which enables these studies to detect associations between height and SNPs with very low frequency and small effect size. Our sample size could be too small to detect an association between height and pubertal development. However, we could not find genetic support for our hypothesis of a relation between height and puberty.

In conclusion, we demonstrated that height and IGF-I were negatively associated with age at thelarche. Girls with tall and short stature had an earlier and later breast development, respectively. Furthermore, we demonstrated that height in childhood is affected by a genetic variation in *PAPP-A2*, but there was no association with *PAPP-A*, *PAPP-A2*, and *STC2* genotypes and pubertal milestones.

ACKNOWLEDGEMENTS

We thank the participating children and their families. We also thank the personnel in the molecular laboratory. This work was supported by the Candys Foundation (grant no. 2017-224). A.J. received funding from The Danish Research Council and The Capital Region of Denmark Research Fund for Health Research.

AUTHOR CONTRIBUTIONS

E.N.U. contributed substantially to the conception and design, acquisition of data, or analysis and interpretation of data, drafted the initial paper, tables, and figures, and approved the final manuscript as submitted. A.S.B., K.A., and J.H.P. contributed substantially to the analysis and interpretation of data, revised the paper critically for important intellectual content, and approved the final version as submitted. K.M.M. and M.A. contributed substantially to the acquisition of data, revised the paper critically for important intellectual content, and approved the final version as submitted. A.J. and R.B.J. contributed substantially to the conception and design, acquisition of data or analysis, and interpretation of data, drafted the initial paper, and approved the final manuscript as submitted.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-020-01215-6) contains supplementary material, which is available to authorized users.

Competing interests: A.J. has received an unrestricted research grant from Novo Nordisk (North European Study on GH treatment in short SGA children) and lecture fees from Novo Nordisk, Ferring, Ipsen, Sandoz, and Pfizer International. K.M.M. and R.B.J. have received lecture fees from Novo Nordisk. The other authors have nothing to declare.

Informed consent: Informed consent was obtained from all girls or their parents who participated in the population-based studies. Use of data from patients with short or tall stature was approved by the Danish Patient Safety Authority.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Parent, A.-S. et al. The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr. Rev.* **24**, 668–693 (2003).
2. Messina, M. F. et al. Long-term auxological and pubertal outcome of patients with hereditary insulin-like growth factor-I deficiency (Laron and growth hormone-gene deletion syndrome) treated with recombinant human insulin-like growth factor-I. *J. Endocrinol. Invest.* **34**, 292–295 (2011).
3. Hiney, J. K., Srivastava, V., Nyberg, C. L., Ojeda, S. R. & Dees, W. L. Insulin-like growth factor I of peripheral origin acts centrally to accelerate the initiation of female puberty. *Endocrinology* **137**, 3717–3728 (1996).
4. Divall, S. A. et al. Divergent roles of growth factors in the GnRH regulation of puberty in mice. *J. Clin. Invest.* **120**, 2900–2909 (2010).
5. Lawrence, J. B. et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc. Natl. Acad. Sci. USA* **16**, 3149–3153 (1999).
6. Overgaard, M. T. et al. Pregnancy-associated plasma protein-A2 (PAPP-A2), a novel insulin-like growth factor-binding protein-5 proteinase. *J. Biol. Chem.* **15**, 21849–21853 (2001).
7. Jepsen, M. R. et al. Stanniocalcin-2 inhibits mammalian growth by proteolytic inhibition of the insulin-like growth factor axis. *J. Biol. Chem.* **6**, 3430–3439 (2015).
8. Lango Allen, H. et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* **14**, 832–838 (2010).
9. Dauber, A. et al. Mutations in pregnancy-associated plasma protein A2 cause short stature due to low IGF-I availability. *EMBO Mol. Med.* **8**, 363–374 (2016).
10. Aksglaede, L., Sørensen, K., Petersen, J. H., Skakkebaek, N. E. & Juul, A. Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics* **123**, 932–939 (2009).
11. Sørensen, K., Aksglaede, L., Petersen, J. H. & Juul, A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. *J. Clin. Endocrinol. Metab.* **95**, 263–270 (2010).
12. Boisen, K. A. et al. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* **17**, 1264–1269 (2004).
13. Chellakooty, M. et al. Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. *J. Clin. Endocrinol. Metab.* **88**, 3515–3520 (2003).
14. Tinggaard, J. et al. The 2014 Danish references from birth to 20 years for height, weight and body mass index. *Acta Paediatr.* **103**, 214–224 (2014).
15. Sørensen, K., Aksglaede, L., Petersen, J. H., Andersson, A.-M. & Juul, A. Serum IGF1 and insulin levels in girls with normal and precocious puberty. *Eur. J. Endocrinol. Eur. Fed. Endocr. Soc.* **166**, 903–910 (2012).
16. Juul, A. et al. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J. Clin. Endocrinol. Metab.* **78**, 744–752 (1994).
17. Lindhardt, M. J. et al. Pubertal progression and reproductive hormones in healthy girls with transient thelarche. *J. Clin. Endocrinol. Metab.* **1**, 1001–1008 (2017).
18. Kofoed, E. M. et al. Growth hormone insensitivity associated with a STAT5b mutation. *N. Engl. J. Med.* **18**, 1139–1147 (2003).
19. Danilovich, N., Wernsing, D., Coschigano, K. T., Kopchick, J. J. & Bartke, A. Deficits in female reproductive function in GH-R-KO mice; role of IGF-I. *Endocrinology* **140**, 2637–2640 (1999).
20. Crowe, B. J. et al. Effect of growth hormone dose on bone maturation and puberty in children with idiopathic short stature. *J. Clin. Endocrinol. Metab.* **91**, 169–175 (2006).
21. Midyett, L. K. et al. Recombinant insulin-like growth factor (IGF)-I treatment in short children with low IGF-I levels: first-year results from a randomized clinical trial. *J. Clin. Endocrinol. Metab.* **95**, 611–619 (2010).
22. Kamp, G. A. et al. High dose growth hormone treatment induces acceleration of skeletal maturation and an earlier onset of puberty in children with idiopathic short stature. *Arch. Dis. Child* **87**, 215–220 (2002).
23. Tam, C. S., de Zegher, F., Garnett, S. P., Baur, L. A. & Cowell, C. T. Opposing influences of prenatal and postnatal growth on the timing of menarche. *J. Clin. Endocrinol. Metab.* **91**, 4369–4373 (2006).
24. Biro, F. M. et al. Onset of breast development in a longitudinal cohort. *Pediatrics* **1**, 1019–1027 (2013).
25. Biro, F. M., Khoury, P. & Morrison, J. A. Influence of obesity on timing of puberty. *Int. J. Androl.* **29**, 272–277 (2006).
26. Thankamony, A. et al. Higher levels of IGF-I and adrenal androgens at age 8 years are associated with earlier age at menarche in girls. *J. Clin. Endocrinol. Metab.* **97**, 786–790 (2012).
27. Sørensen, K. & Juul, A. BMI percentile-for-age overestimates adiposity in early compared with late maturing pubertal children. *Eur. J. Endocrinol.* **173**, 227–235 (2015).
28. Paterson, W. F. et al. Exaggerated adrenarche in a cohort of Scottish children: clinical features and biochemistry. *Clin. Endocrinol.* **72**, 496–501 (2010).