RESEARCH

Deletion in the uridine diphosphate glucuronyltransferase 2B17 gene is associated with delayed pubarche in healthy boys

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Abstract

Objective: Only a few genetic loci are known to be associated with male pubertal events. The ability of excreting testosterone (T) and other steroids in the urine depends on sulfation and glucuronidation. One of several essential glucuronidases is encoded by the *UGT2B17* gene. In a preliminary report, we found that homozygous deletion of *UGT2B17* in boys was associated with lower urinary excretion of T. We hypothesized that boys with a lower glucuronidation capacity may have altered androgen action and excretion affecting pubarche, as this represents a T-dependent event.

Design, participants and measures: 668 healthy boys (cross-sectional) aged 6.1–21.9 years (COPENHAGEN puberty study conducted from 2005 to 2006) were included. 65 of the boys where followed longitudinally every 6 months. Participants were genotyped for *UGT2B17* copy number variation (CNV). Clinical pubertal staging including orchidometry, anthropometry and serum reproductive hormone levels.

Results: 59 of the 668 boys (8.8%) presented with a homozygous deletion of *UGT2B17* (del/del). These boys experienced pubarche at a mean age of 12.73 years (12.00–13.46) vs 12.40 years (12.11–12.68) in boys heterozygous for deletion of *UGT2B17* (del/ins) vs 12.06 years (11.79–12.33) in boys with the wild-type genotype (ins/ins) ($P = 0.029$, corrected for BMI z-score). The effect accounted for 0.34 years delay per allele (95% CI: 0.03–0.64). A comparable trend was observed for onset of testicular enlargement >3 mL but did not reach significance.

Conclusion: CNV of *UGT2B17* is a factor contributing to the timing of male pubarche.

Introduction

Testosterone (T) promotes maturation of the male reproductive organs, development of secondary sex characteristics and production of sperm by the testes. In puberty in boys, pulsatile gonadotropin stimulation upon reactivation of the hypothalamic–pituitary–gonadal axis results in testicular enlargement >3 mL and secretion of T produced by the testicular Leydig cells (gonadarche). Furthermore, gradually increasing secretion of androgens including T produced by the adrenal glands is occurring from mid-childhood (adrenarche) resulting in pubic hair development (pubarche). T exerts its effects by binding to the androgen receptor (AR). Activation of the AR by T binding may vary and depends on the free biologically active T concentration. Thus, the biological activity of circulating T depends on the concentration of total T, albumin and sex hormone-binding globulin (SHBG),

the transendothelial transport to target tissues, as well as the peripheral conversion rate of T into Estradiol (E2) and dihydrotestosterone (DHT). Finally, T concentration depends on the clearance rate, determined by, conversion of T to its metabolites, conjugation capacity, as well as urinary excretion. The ability of excreting T and other steroids in the urine depends on sulfation and glucuronidation of steroids to water-soluble conjugates. One of the several essential glucuronidases is uridine diphosphate glucuronyltransferase 2B17, encoded by the *UGT2B17* gene, and several studies observed a lower urinary excretion of T with unaltered or higher level in the blood in males with homozygous *UGT2B17* (1, 2, 3). In a pilot study, we observed a significantly reduction of the urinary T:epitestosterone (E) ratio in *UGT2B17* del/del boys, but without lower concentration of T in the blood (4). *In vitro* studies have suggested an inverse association between the level of UGT2B17 and the level of DHT (and other T metabolites) in local tissues (5).

We hypothesize that boys with lower glucuronidation of T due to a homozygous *UGT2B17* deletion have altered T clearance, which may consequently influence the age at which boys develop androgen-dependent pubic hair.

Materials and methods

Subjects

Healthy boys (668 boys from 6.1 to 21.9 years of age) participating in the cross-sectional COPENHAGEN Puberty Study were included in the study (6, 7). Of the 668 included boys, 65 were followed every six months for five years (2006–2010, median (range) 11 (2–15) visits per boy) in the longitudinal part of the study (6, 7). Other aspects of this study have previously been published (4, 8, 9, 10, 11, 12). The Copenhagen Puberty Study was approved by the local ethical committee (Videnskabsetiske Komité for Region Hovedstaden, # KF 01 282214 and # V200.1996/90). The study is registered in ClinicalTrials.gov (NCT01411527).

Consent has been obtained from each participant and parents after full explanation of the purpose and nature of all procedures used.

Clinical examination

Pubertal stages were evaluated by clinical examination according to Marshall and Tanner (13, 14). All evaluations of puberty in boys were performed by one of 3 male pediatricians. Testicular volume (TV) was measured by palpation to the nearest mL using Prader's orchidometer. In case of discrepancy between left and right side, the largest measurement was used for classification. Assessment of pubic hair staging was done by inspection. Onset of testicular enlargement was defined as development testicular volume >3 mL (TV > 3 mL). Pubarche was defined as pubic hair (PH) stage ≥ PH2. Pubertal onset was defined as TV > 3 mL or pubic hair stage > PH2.

Age at onset of pubic hair (PH2+) or testis volume >3 mL was assigned as the mean age between age at the first examination in stage 2 or >3 mL and the latest examination in stage 1 or ≤3 mL, respectively.

Genotyping

DNA was isolated from peripheral blood using a semi-automatic nucleic acid isolation method, either on a QuickGene-810 apparatus (Fujifilm, Life Science Products, Tokyo, Japan) or Maxwell-16 (Promega Biotech AB), and quantified on a NanoDrop ND-1000 spectrophotometer (Saveen Werner AB, Malmö, Sweden). The analysis of UGT2B17 copy number variation (CNV) was performed by quantitative PCR on the Mx3000P platform (Stratagene, Cedar Creek, TX, USA), using primers specific for UGT2B17 and for the internal normalization gene GAPDH, as previously described in details (4).

Hormone analyses

Blood samples were drawn between 8:00 h and 10:00 h. The samples were clotted, 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; PerkinElmer) with detection limits of 0.06 and 0.05 IU/L for FSH and LH, respectively. Intra- and inter-assay coefficients of variation (CV) were less than 5% in both gonadotropin assays. Estradiol levels were determined by RIA (Pantex, Santa Monica, CA, USA) with detection limit of 18 pmol/L and the intra- and inter-assays CV were 7.5% and 12.3% respectively. SHBG was determined buy a time-resolved immunofluorescence assay (Delfia; Wallac Oy, Turku, Finland) with a detection limit of 0.20 nmol/L. Intra- and inter-assays CV were 5.8% and 6.4% respectively.

DHEAS, adione (androstenedione) and T in serum were quantified using a validated TurboFlow-LC–MS/MS method (15). The Adione:DHEAS ratio was calculated as adione (nmol/L) divided with DHEAS (nmol/L). Relative standard deviations for the quality control samples at the low and high concentration levels, respectively in that

period were: DHEAS, 7.1% and 8.3%; adione, 11.0% and 11.1% and T, 10.0% and 5.7%. Limits of quantification (LOQ) were determined according to the International Conference on Harmonisation guidelines (ICH, 2005) and were DHEAS, 48 nmol/L; adione, 0.18 nmol/L and T, 0.10 nmol/L.

Statistical analysis

Data are resented as medians and 25 and 75 percentiles and as means and 95% confidence interval (CI). For hormone levels below the LOQ, the LOQ divided by the square root of 2 was used.

In order to estimate the mean age (95% CI) at pubertal onset, we performed probit analyses (SAS; proc lifereg). This allowed integration of left-, right- and interval-censored observations and pubic hair status (PH2+) or testis volume >3 mL, yes/no as a binomial response variable. Longitudinal data of boys entering puberty during follow-up was included in the probit analyses (interval censored data). If a boy entered puberty between two examinations, the date between these examinations was used as interval-censored data. If a boy had not entered puberty at his last examination (or only examination if seen once), his age at examination was used as right censored data. If a boy had entered puberty at his first examination, his age at examination was used as left censored data.

The cohort was standardized by applying WHO age-specific body mass index scores (BMI z-scores) (16). As longitudinal data were available in 65 boys, we calculated a mean of all individual BMI z-scores. Comparisons of hormone levels between groups were carried out using GLM Univariate using the SPSS software (version 19; SPSS). In the probit analyses, age at pubertal onset was adjusted for BMI z-score. Examinations above the age of 19 years ($n=18$) were not included into the analysis of

pubertal onset, as the WHO age-specific body mass index scores is not specified for this age.

A P value ≤0.05 was considered statistically significant.

Results

Approximately one out of ten boys presented with the homozygous deletion of the *UGT2B17* (del/del): 59 of the 668 boys in the cross-sectional study (8.8%) and 6 of the 65 boys in the longitudinal study (9.2%) (Table 1). Irrespective of UGT2B17 genotype, mean age at testicular enlargement >3 mL was 11.65 years (11.51–11.80, non-corrected) and 11.76 (11.62–11.90, corrected for BMI z-score) while mean age at pubarche was 12.16 (11.96–12.36; non-corrected) and 12.26 (12.06–12.46, corrected for BMI z-score) (Fig. 1).

Pubic hair developed at a later age in boys with the *UGT2B17* del/del genotype compared to the boys who were heterozygous and homozygous carriers of the *UGT2B17* gene (del/ins) and (ins/ins). The *UGT2B17* del/del boys experienced pubarche (PH2+) at a mean age of 12.73 years (12.00–13.46) compared to a mean age of 12.40 (12.11–12.68) in boys with the del/ins and a mean age of 12.06 (11.79–12.33) in boys with the ins/ins when corrected for BMI z-score ($P=0.029$). The effect accounted for 0.34 years delay per allele (95% CI: 0.03–0.64). The comparisons were also statistical significant without correction for zBMI ($P=0.043$; effect size: 0.32 years per allele; 95% CI: 0.00–0.65). Table 1 illustrates age at PH2+ in all three groups, with and without BMI z-score correction.

Although not statistically significant with and without BMI z-score correction (Table 1), we observed a later onset of testicular enlargement >3 mL in the boys with the homozygote deletion compared to the boys with the ins/del and the ins/ins genotype (12.09 years (11.54–12.64) vs 11.79 (11.58–11.99) and 11.69 years (11.50–11.89);

Table 1 Age, zBMI, age at pubertal onset PH2+ (pubarche) and TV (testicular volume) >3 mL stratified by *UGT2B17* genotype.

| UGT2B17 genotype	del/del ($n=59$)	del/ins ($n=309$)	ins/ins ($n=300$)	*P* value
Median age in yrs (25th and 75th percentile)	11.38 (8.56–14.83)	11.23 (9.16–14.05)	11.14 (9.27–13.62)	
Median zBMI (25th and 75th percentile)	0.20 (−0.63 to 0.98)	0.35 (−0.24 to 0.97)	0.23 (−0.41 to 0.91)	
Mean age at PH2+ in yrs (95% CI)				
No correction	12.65 (11.89–13.41)	12.28 (11.99–12.57)	11.97 (11.68–12.25)	*P*=0.043
Corrected for zBMI	12.73 (12.00–13.46)	12.40 (12.11–12.69)	12.06 (11.79–12.33)	*P*=0.029
Mean age at TV >3 mL in yrs (95% CI)				
No correction	12.08 (11.49–12.67)	11.65 (11.44–11.86)	11.60 (11.39–11.81)	*P*=0.253
Corrected for zBMI	12.09 (11.54–12.64)	11.79 (11.58–11.99)	11.69 (11.50–11.89)	*P*=0.204

n, number; yrs, years.
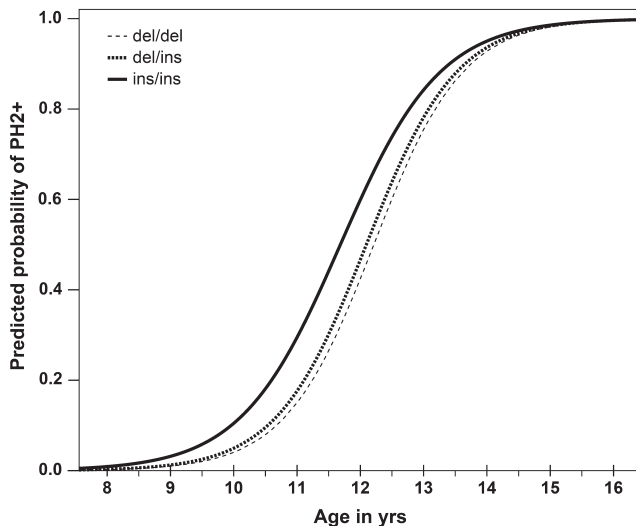
Figure 1
Predicted probability of age at PH2+ (onset of pubic hair Tanner stage 2) stratified by *UGT2B17* genotype.

$P = 0.204$; corrected for BMI *z*-score). Comparing the del/del as one group to the del/ins and ins/ins as another group did not result in a significant later age at TV > 3 mL.

The hormones FSH, E2, LH, T, DHEAS and adione increased with age and puberty, and SHBG decreased. No significant differences in hormone levels of FSH, E2, LH, T, SHBG, DHEAS or adione between groups corrected for age were observed (data not shown).

Although not statistically significant, we observed a higher level of DHEAS and a lower level of Adione. Analyzing the adione:DHEAS ratio corrected for age, we observed a significant lower level in the boys with the *UGT2B17* del/del ($P = 0.014$).

Discussion

This is the first demonstration of delayed pubarche in boys carrying a common deletion of the *UGT2B17* gene, which codes for an enzyme involved in urinary steroid excretion.

Knowledge is sparse regarding the genetic etiology of the timing of male pubertal events. However, recent research points to the fact that beside classical genes involved in central reactivation of the hypothalamic–pituitary–gonadal axis, also genes exerting an effect on peripheral target tissue are significantly associated with the occurrence of pubertal events (17, 18). We found deletion of *UGT2B17* to be significantly associated with pubarche but not with testicular enlargement > 3 mL.

The impact of UGT2B17 genotype on age at pubarche but not age at onset of testicular growth is in line with the assumption of an altered T action by UGT2B17 affecting pubarche, a testosterone-dependent event, but not testicular enlargement, which is a gonadotropin-dependent event (19). Further, our findings of an association of the *UGT2B17* deletion with delayed pubarche highlight a potential role of this CNV in ethnic differences in timing of pubertal events. As the deletion is relatively rare among Caucasians and very frequent among Asians (1, 2), it is intriguing that although in Chinese boys testicular enlargement > 3 mL occurs more than a year earlier compared to the Danish boys of our present study, pubarche occurs later in Chinese boys than in Danish boys (TV > 3: 10.55 vs 11.65 years; PH2+: 12.78 vs 12.16 years, respectively) (20). We speculate that the frequency of the *UGT2B17* deletion may contribute to ethnic differences in age at pubarche.

The observed prevalence of 10% of boys being homozygote for the *UGT2B17* deletion is in accordance with the prevalence in other studies of Caucasian boys (1, 4, 21).

In our pilot study, we found no difference in the serum concentration of T, but only in the urinary concentration of T (4). *In vitro* studies in prostatic cancer cells have revealed that both *UGT2B15* and *UGT2B17* are critical enzymes for the local inactivation of androgens (5). The circulating T is converted to DHT, a potent androgen receptor agonist, by 5α-reductase. As it is extremely important to modulate DHT levels locally, the substrates (DHT, 3α-DIOL and ADT) are regulated by steroidogenic enzymes and glucuronidation. Furthermore, it seems as an impaired clearance of T is regulated by a lower production of T to keep the serum level in a steady state. The lower production could be due to the conversion of precursors through other pathways than the adione to T pathway. As observed the boys with the *UGT2B17* del/del had a lower adione:DHEAS ratio, indicating that the conversion of DHEAS to adione could be impaired.

Although some studies have observed associations between the deletion polymorphism of *UGT2B17* with osteoporosis in males (2), prostate cancer (22), lower incidence of colorectal cancer in males (23), other studies observed no association with osteoporosis risk (24) or with risk of prostate cancer (25). A recent CNV study found that the UGT2B gene deletions are very common and may indicate that the effects are neutral or minor (26). The *UGTB15* substitutes for *UGT2B17* and other genes may have a compensatory role in the UGT activity.

Conclusion

UGT2B17 is involved in the timing of male pubarche. The boys with the homozygous deletion experienced delayed pubic hair development, although circulating T, DHEAS and androstenedione did not differ between genotype groups. *UGT2B17* may contribute to the differential timing of male pubertal events across ethnicities.

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