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Androgen biosynthesis during minipuberty favors the backdoor pathway over the classic pathway: insights into enzyme activities and steroid fluxes in healthy infants during the first year of life from the urinary steroid metabolome

Abbreviated title: Minipuberty uses the backdoor androgen pathway

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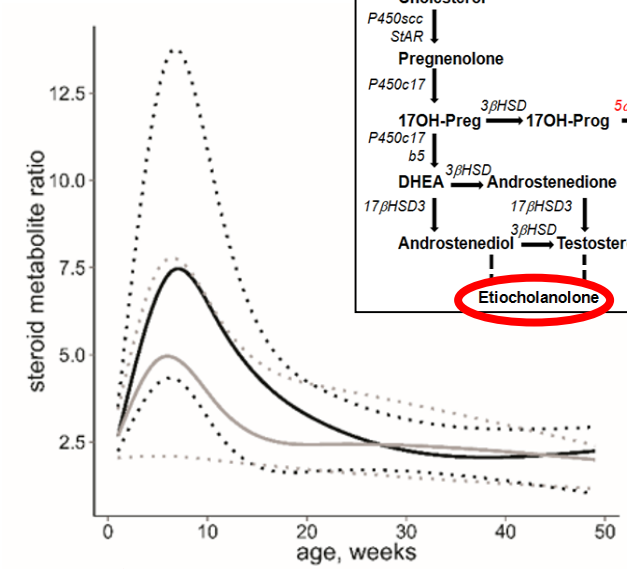
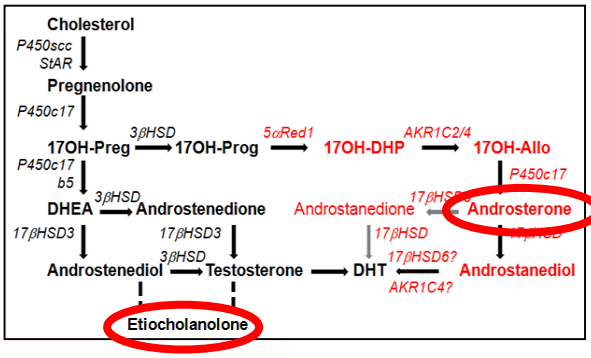
Androsterone/Etiocholanolone





Fetal-placental unit
Minipuberty
Fetal adrenal involution

Highlights

- Male androgen biosynthesis shows a significant peak at week 7 during minipuberty
- Androgens in minipuberty are (at least in part) produced through the backdoor pathway
- Steroid enzyme activities in the first year of life are all age-, some sex-specific
- Steroid enzyme ratios obtained from urine GC-MS are comparable between laboratories

Abstract

The steroid profile changes dramatically from prenatal to postnatal life. Recently, a novel backdoor pathway for androgen biosynthesis has been discovered. However, its role remains elusive. Therefore, we investigated androgen production from birth to one year of life with a focus on minipuberty and on production of androgens through the backdoor pathway. Additionally, we assessed the development of the specific steroid enzyme activities in early life. To do so, we collected urine specimens from diapers in 43 healthy newborns (22 females) at 13 time points from birth to one year of age in an ambulatory setting, and performed *in house* GC/MS steroid profiling for 67 steroid metabolites. Data were analyzed for androgen production through the classic and backdoor pathway and calculations of diagnostic ratios for steroid enzyme activities were performed. Analysis revealed that during minipuberty androgen production is much higher in boys than in girls (e.g. androsterone (An)), originates largely from the testis (An^{boys}-An^{girls}), and uses predominantly the alternative backdoor pathway (An/Et; $\Delta5<\Delta4$ lyase activity). Modelling of steroid enzyme activities showed age-related effects for 21-, 11-, 17-hydroxylase and P450 oxidoreductase activities as well as 3β-hydroxysteroid dehydrogenase, 11β-hydroxylase type 1/2 and 5α-reductase activities. Sex-related characteristics were found for 21-hydroxylase and 5α-reductase activities. Overall, our study shows that androgen biosynthesis during minipuberty favors the backdoor pathway over the classic pathway. Calculations of specific diagnostic ratios for enzyme activities seem to allow the diagnosis of specific steroid disorders from the urinary steroid metabolome.

Keywords: newborn infants, GC-MS, urinary steroid profile, urinary steroid metabolome, minipuberty, androgens, steroid enzyme activity, development

1. Introduction

During the first year of life the steroid metabolome changes remarkably, mainly due to three developmental events (1). First, the fetal-placental-maternal unit, which is a steroid forming and metabolizing unit during pregnancy, is disrupted at birth. Second, the fetal adrenal, which produces predominantly dehydroepiandrosterone (DHEA) involutes in the first 3-6 months; and third, steroid organs develop. Within months postnatally the adult adrenal cortex is ready to produce mineralocorticoids and glucocorticoids, while the production of adrenal C19 steroids starts very slowly after birth and becomes clinically apparent only after 6-8 years of age at adrenarche (2). Similarly, androgen production in the testis, which is highly active in mid-gestation, decreases after birth, but reveals a postnatal surge during the so-called minipuberty. By contrast, the human ovary is thought to be steroidogenically quiescent during pregnancy and prepubertal years (3).

Minipuberty, characterized by a transient surge of testosterone and its precursor androstenedione due to a transient activation of the hypothalamic-pituitary-gonadal axis, has been described in male neonates aged 1-3 months many years ago (4), but its role remains unclear. Minipuberty may be important for early and late postnatal sexual differentiation in males. This differentiation includes, first, postnatal phallic growth (5) and an increase in testicular volume due to an increase in seminiferous tubules (6), Sertoli cell numbers and the number of germ cells (7,8) in preparation for future spermatogenesis (9). Second, during minipuberty masculinization of the brain is modulated. This is illustrated by studies showing an association between testosterone levels at 3-4 months of age and emotional regulation in early infancy (10), a relation between testosterone levels in the first six months of life with neurobehavioral sexual differentiation at 14 months (11), and an effect of testosterone on language function, hemispheric organization and lateralization of the brain as early as 4 weeks after birth (12). Third, minipuberty seems to correlate with somatic development, as testosterone and luteinizing hormone (LH) levels at 8 weeks of life correlate with body weight and body mass index (BMI) at six years of age (13). By contrast, the hormonal pattern during the time of minipuberty is highly variable and less clear in girls (14). Thus, further characterization of the event minipuberty is needed.

Recently, an alternative backdoor pathway for dihydrotestosterone (DHT) synthesis has been described, first in marsupials (15), then in humans (16). It has been suggested that this pathway is important for male sexual differentiation *in utero* and that it is functional in the fetal testis. We have shown that the genes of this backdoor pathway are differently expressed in the fetal compared to the adult testis (17), likely determining the flow through the classic and the alternative androgen biosynthetic pathways. The role of the backdoor pathway and its relationship to the classic pathway in minipuberty is unknown, but can be investigated by studying the profile of androgen metabolites excreted in the urine during the first 3-6 months of life.

Inborn errors of steroid biosynthesis and sex development are rare disorders. Steroid measurements are first line investigations for diagnosing specific disorders before performing genetic analysis (1,18). For many steroid biosynthetic defects caused by monogenic disorders, the steroid profile reveals characteristic changes, which may be recognized as a diagnostic pattern or as alterations of substrate to product conversion ratios correlating to specific enzyme activities and thus genes. For instance, the urine steroid profile of P450 oxidoreductase deficiency shows a pattern of increased 17-hydroxyprogesterone and 21-deoxycortisol metabolites (due to 21-hydroxylase deficiency), increased corticosterone metabolites (due to 17-hydroxylase deficiency), and decreased excretion of androgen metabolites (19,20). However, pathologic steroid patterns and ratios as surrogate markers of enzyme activities may only be recognized with the knowledge of normal physiology. As genetic disorders of steroidogenesis mostly manifest in the first year of life, knowledge on normal changes of the steroid profile, the steroid patterns and the substrate to product ratios during this time period are essential to use urinary steroid profiling as a diagnostic tool.

Therefore, the purpose of this study was twofold. First, to describe the characteristics of the urinary androgen metabolome during the time of minipuberty. Specifically, we aimed to investigate the possible role of the backdoor androgen biosynthesis pathway during minipuberty. Second, we analyzed the physiologic development of enzyme activities of steroidogenesis by calculating conversion ratios from urine metabolites during the first year of life. In a recent project, we have measured 67 steroids in the spot urine of 43 healthy, term-born neonates at 13 time points during the first year of life by gas GC-MS (21). This big, normative dataset was now analyzed to solve our specific questions.

2. Materials and Methods

2.1. Study population and urine collection procedures

The study was approved by the medical ethics committee of the Kanton Bern, Switzerland. Parents gave written informed consent. In brief, 43 healthy Caucasian girls and boys born at term with normal weight and length were recruited. Spot urines were collected at weeks 1, 3, 5, 7, 9, 11, 13, 17, 21, 25, 33, 41 and 49 of life. Details are described in (21).

2.2. Measurement of urinary steroid metabolites by GC-MS and quality assessment

Quantitative analysis of 67 urinary steroid hormone metabolites was performed by an in-house GC-MS method (21), adapted from reported methods (22). In brief, after medroxyprogesterone was added as a recovery standard, the urine sample was extracted on a Sep-Pak C18 column, then hydrolyzed with sulfatase and β-glucuronidase/arylsulfatase and free steroids were again extracted on a Sep-Pak C18 cartridge. The two standards Stigmasterol and 3β5β-TH-aldosterone were added to the extract, then methoxyamine HCl 2% in pyridine was added and the sample was heated at 60°C for one hour. After evaporation of the solvent, trimethylsilylimidazole (TMSI) was added and the extracts were heated at 100°C for 16 hours and then purified by gel filtration on Lipidex 5000 columns to remove the excess of derivatization reagent. The derivatized samples were analyzed by mass spectrometric analyses on a gas chromatograph 7890A from Agilent Technologies (La Jolla, California, USA) coupled to a mass selective detector Hewlett-Packard 5975C providing selected ion monitoring (SIM). For all steroids the signal-to-noise-ratio was ≥3. Intra- and inter-assay variations are reported in Appendix Table B of (21). The QuantiChrom Creatinine Assay (DICT-500; BioAssay Systems, Hayward, CA, USA) was used to measure urinary creatinine by quantitative colorimetry. Measured steroids were standardized by urinary creatinine concentration and expressed in µg/mmol creatinine. Minimal urine volume required for steroid analysis was 200 µl, standard volume was 1.5 ml; for creatinine measurement 5 µl urine was used. The reproducibility of our in-house GC-MS method is continuously monitored by an internal quality control. In addition, our laboratory participates in regular external quality controls organized by the University

College London Hospitals (London, United Kingdom) and by the Foundation for Quality Medical Laboratory Diagnostics skml (Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek, Nijmegen, The Netherlands).

2.3. **Data analysis and statistics**

All calculations and statistical analyses were conducted using the R software, version 3.2.2 (23). The urinary steroid metabolites µg/mmol creatinine were converted to µmol/mol creatinine using the molar mass of each steroid compound (see Table 1 of Ref. (21)).

To explore the role of the backdoor pathway for androgen biosynthesis, age- and sex-related changes of androsterone and of the steroid ratios of **Table 1** were modeled by multivariable linear quantile mixed regression taking subject as random effect into account (24). Sex and age were included as fixed effects. We considered five age-effects: constant (corresponding to no age effect), linear and natural quadratic, natural cubic and natural quartic splines. Since we considered the presence of a sex effect, we fitted a total of 10 possible models for androsterone and each steroid ratio and selected that model minimizing the Akaike information criterion (AIC). Quantile regression makes no distributional assumption, thus, no transformation of the steroid ratio values were necessary. Continuous conditional 25^{th}, 50^{th} and 75^{th} quantiles were plotted by combined use of quantile regression and natural splines according to the selected model (**Figure 1**) (25). As the adrenals and the testes are active in producing androgens during minipuberty, while the ovaries seem rather quiescent, the contribution of the testes to androgen production was calculated by subtracting the median values in girls from the median values in boys and continuous conditional 25^{th}, 50^{th} and 75^{th} quantiles were plotted (**Figure 1**). The median values of androsterone and of the steroid ratio androsterone/etiocholanolone in male infants were compared to female infants by Mann-Whitney U test for each time point.

Urinary steroid metabolites expressed in µmol/mol creatinine were used to calculate the urinary steroid metabolite ratios or fluxes listed in **Table 2**. Sex and age specific dependencies were modeled by multivariable linear quantile mixed regression and were visualized by quantile regression and natural splines as described before (**Figure 2** and **Supplement Figures**). The maximum value and the 97.5^{th}

quantile were determined for each ratio at each week of life and, in case of a sex difference derived from the quantile regression mixed model, the values were calculated separately for boys and girls (**Table 3**).

3. Results

3.1. Androgen production through the backdoor pathway predominates during minipuberty

To characterize the androgen biosynthesis from birth to one year of life, we analyzed the urinary steroid metabolome of 43 infants for sex- and age-dependent androsterone excretion (15,26-28). In a multivariable linear quantile mixed model, we found both a sex- and age-dependency. The continuous conditional 25th, 50th and 75th quantiles were plotted using quantile regression and natural splines (**Figure 1A)**. Urinary excretion of androsterone was similar between boys and girls at week 1, but increased significantly during minipuberty only in boys to a maximum at week 7, before decreasing to a baseline level as found in girls at week 17. Accordingly, higher median values of androsterone [µmol/mol creatinine] in male infants compared to female infants by Mann-Whitney U test were found at week 3 (59.8 vs. 37.3; p=0.011), week 5 (59.9 vs. 48.6; p=0.11), week 7 (85.4 vs. 35.6; p=0.0083), week 9 (57.4 vs. 33.5; p=0.012), week 11 (49.3 vs. 21.9; p=0.029) and week 13 (45.2 vs. 30.0; p=0.13). To assess the amount of androgen production that arises from the backdoor pathway in comparison to the classic pathway, we calculated the androsterone/eticholanolone ratio, which represents the backdoor pathway androgen synthesis after the 17,20-lyase reaction (27-29). This ratio showed a sex- and age-dependency. The quantile regression/natural splines plot showed no sex difference for the ratio at week 1, but an increase for boys until week 7 and a decline thereafter (**Figure 1B)**. Higher median values in males were found at week 5 (6.1 vs. 2.8; p=0.19), week 7 (10.1 vs. 4.6; p=0.014), week 9 (7.7 vs. 4.3; p=0.18), week 11 (4.5 vs. 3.2; p=0.16) and week 13 (5.6 vs. 3.5; p=0.12).

Generally, both the gonads and the adrenals contribute towards the urinary metabolome. However, the prepubertal ovary is inactive. Thus, subtracting the androgen production found in females from the production in males (in the first year of life), will subtract the adrenal contribution and reveal the androgen production by the testis only. This calculation showed a significant increase of androsterone

production (**Figure 1 C**) and an increased ratio of androgen production through the backdoor pathway compared to the classic pathway during the time of minipuberty for the testis **(Figure 1D).**

We also performed calculations for the 17,20 lyase activity, which is essential for any androgen production, but may follow the so-called Δ^5- or Δ^4-steroid pathway. The Δ^5-pathway leads from pregnenolone to 17-hydroxy-pregnenolone to DHEA and thus directly to the classic androgen biosynthetic path; the Δ^4-pathway leads to 17-hydroxyprogesterone as precursor, which is hardly converted to androstenedione (30), but may readily feed into the backdoor path. Our analysis revealed a steep decrease from a high level for the Δ^5-pathway lyase activity after birth to week 11 (**Figure 1E)** and a steep increase from a very low level for the Δ^4-pathway lyase activity (**Figure 1F**). While the Δ^5-activity remained relatively low after week 11, the Δ4-activity showed a mild, continued increase beyond week 11, but at a low level (similar to Δ^5-activity).

3.2. Most steroid enzyme activities are age- but not sex-specific during the first year of life

Forty-one formula for the calculation of substrate to product conversion ratios representing specific steroid enzyme activities were created based on published literature (**Table 2**). The respective calculations using our dataset are shown in **Table 3**. The maximum value and the 97.5th quantile for each ratio at 13 time-points stratified by sex in case of sex difference are presented. For the vast majority of ratios the maximum value and the 97.5th quantile lied very close to each other, but in several cases the maximum value also exceeded the twofold or threefold of the 97.5th quantile. In a mixed effect quantile regression model, age-related characteristics were generally found for all analyzed enzyme activities. Only for 21-hydroxylase (21-OHase) and 3β-hydroxysteroid-dehydrogenase (3β-HSD) activities, some calculations did not reveal this effect (**Table 3**). In contrast, a sex-related effect was only found for 21-OHase and 5α-reductase activities. Only 2/9 calculations for 3β-HSD and 1/5 calculations for 17α-hydroxylase (17-OHase) activities revealed a sex effect, while no such effect was found for 11-hydroxylase, P450 oxidoreductase and 11β-hydroxysteroid-dehydrogenase type 1/2 **(Table 3)**.

In addition to the maximum value and the 97,5th quantile, the continuous conditional 25th, 50th and 75th quantiles of all 41 steroid ratios were visualized according to the selected model (**Supplemental Material**

Figure 1). **Figure 2** shows the developmental pattern of four ratios from birth to 12 months. **Figure 2A** represents the best ratio to discriminate 21-OHase deficiency from normal 21-OHase activity reported by Kamrath et al. using 6OH-THE as the denominator (31). The ratio shows an association with sex and age. Starting at a similar level after birth, the relative 21-OHase activity decreased faster in girls in the first three months of life (corresponding to an increase in the ratio), while the decrease in boys occurred later.

Figure 2B shows the pattern for the 3β-HSD activity in the first year of life using again 6OH-THE as the denominator of the ratio. For this ratio an association with age, but not with sex was found. After birth, the relative 3β-HSD activity rapidly declined to 50% by week seven, then increased to a relative maximum by week eleven, and then declined again.

A representative pattern for the 17-OHase activity is shown in **Figure 2C**. An age, but no sex effect was found for this ratio. The 17-OHase activity seemed to increase slightly after birth till week seven and decreased thereafter continuously.

Finally, a representative ratio for the 5α-reductase activity is given in **Figure 2D**. Its ratio showed an association with sex and age. Relative 5α-reductase activity increased massively after birth in both sexes and was found highest between week seven and 17. Overall, 5α-reductase activity was higher in boys compared to girls, which corresponds to a lower ratio.

4. Discussion

The backdoor pathway for androgen biosynthesis is relatively novel and its exact role unclear (1). In previous work, we have shown through studies of human mutations in genes involved in the backdoor pathway that it is needed for normal fetal male sexual development, and that the gene expression profile of backdoor pathway genes changes from fetal to adult life in the human testis (16,17). Similarly, the role of the event minipuberty, which occurs predominantly in males around postnatal days 30-100, remains unclear (3). Therefore, we aimed to model the androgen production from birth to one year of life and calculated the contribution of the backdoor path to overall androgen biosynthesis using our steroid metabolome databank (21). Interestingly, we were able to model the event minipuberty by tracking the specific androgen metabolites in the urine. As expected, the rise in androgen production during

minipuberty is much more significant in boys than in girls, and the androgen source seems confined to the testis. As novel information, we found that androgen production during minipuberty seems to occur rather through the backdoor pathway than through the classic pathway. This is supported by calculations for the precursor/product ratio androsterone/etiocholanolone (An/Et) and by flux calculations looking at 17,20 lyase activity, which is essential for any androgen production. Higher An/Et ratio during minipuberty suggests enhanced backdoor pathway activity. This An/Et ratio is also reported as a formula for assessing 5α-reductase activity in the first year of life and showed an identical pattern as seen in our cohort (32). While lyse activity in the Δ5-path rather leads to the classic androgen biosynthesis, the Δ4-path produces 17-hydroxyprogesterone, which rather feeds into the backdoor path. In our cohort, lyase activity in the Δ5-path was extremely high at birth and dropped massively after birth to 10 weeks of age. This likely reflects the involution of the fetal adrenal gland, which produces exclusively DHEA over the Δ5-path, while postnatally the adult adrenal cortex in the first year of life does not produce androgens. By contrast, a significant rise in lyase activity in the Δ4-path within the first 10 weeks postnatally in our cohort may reflect higher androgen biosynthesis through the backdoor pathway in the testis during minipuberty. Overall, our data suggest that during the first three months of life the human testis favors the backdoor over the classic pathway for producing androgens. As androgen production during minipuberty is needed for normal postnatal male sexual development (3), the backdoor pathway is not only crucial for prenatal male sexual development (16,17), but also plays an important role after birth.

The second aim of this study was to model the development of steroid enzyme activities implicated in human disorders of steroidogenesis (e.g. congenital adrenal hyperplasia (CAH)) from data collected in our urinary steroid databank (21). The purpose of the calculation of a specific precursor to product ratio (as surrogate marker for an enzyme activity) is to obtain reliable cut-offs for diagnosing steroid disorders from the urinary steroid profile. An ideal diagnostic ratio should be able to discriminate a deficient enzyme activity from a normal one. As the calculated steroid metabolite ratios usually show a wide variability especially in the upper range, which represents a low enzyme activity, it has been suggested in the literature to describe the diagnostic ratios by the maximum values and the 97.5th quantiles found in

controls (31). We did that accordingly and summarized our data of diagnostic ratios in **Table 3**. By contrast, to illustrate the development of the enzyme activities during the first year of life, we assessed the highly skewed distributed data by the median and IQR (Supplemental Figures). In principal, all ratios assessing 21-OHase, 3β-HSD, 11-OHase, 17-OHase, POR, 11-HSD1/2 and 5α-reductase activities are age-dependent in the first year of life. In addition, 21-OHase and 5α-reductase seem to be sex-dependent.

For some ratios, we were able to find normative data for comparison in the literature (29,31-33). In general, calculated ratios for steroid enzyme activities of our study compared very well with other studies, indicating that comparisons of data between laboratories and methods are possible when using ratios. However, only for ratios describing the 21-OHase activity, we found two studies, in which data of controls were assessed in comparison with a group of affected CAH patients (31,33). In the bigger and more recent study comparing 21-OHase deficient patients (n=95) to controls (n=261), it has been shown that only steroid ratios with the 21-deoxycortisol metabolite pregnanetriolone (PTO) as the numerator in combination with urinary glucocorticoid metabolites as the denominator where able to discriminate 21-OHase deficiency from controls (31). The best diagnostic ratio was PTO to 6α-OH-tetrahydrocortisone, which was >8.5 fold higher in 21-OHase deficiency. Compared to this excellent study, which clearly sets the standard for future use of diagnostic ratios, our data are well in line with the control group. Thus, using our data, we should be able to diagnose 21-OHase deficiency from the urinary steroid profile unambiguously. Furthermore, it appears that once established, diagnostic ratios can be applied between labs and methods for the analysis of the urinary steroid profile with respect to steroid enzyme deficiencies.

Unfortunately, there are no larger studies available assessing the specificity and the predictive value of diagnostic ratios for 3β-HSD, 11-OHase, 17-OHase, POR and 11-HSD1/2 deficiencies. Although many reported ratios have been labeled as being diagnostic in single patients, their discriminating value awaits testing in larger groups. This difficult task might only be solved through collaborations between laboratories assessing urinary steroid profiles, because those steroid disorders are very, very rare. In addition, diagnostic urine samples are only available at the very beginning, as most patients require (immediate) steroid replacement therapy, which will mask the diagnostic pattern of the disorder in the

urinary steroid profile. Also, urinary steroid profiling by GC-MS is not widely established as a diagnostic method. Thus, in many patients with a genetic steroid disorder, no diagnostic urine sample and steroid profile has been collected before treatment. Taking a patient off treatment for diagnostic purpose bears a certain risk and, therefore, mostly leads to a direct genetic work-up in undiagnosed patients under steroid therapy. Aware of those difficulties, we are collecting GC-MS generated urine steroid profiles of rare patients with steroid disorders in a local databank and recommend colleagues to do the same.

Another limitation of studies in the field is that different formula for the estimation of enzyme activities are used according to individually measured urinary steroid metabolites. Although those formula may all characterize the same enzyme activity, they cannot be directly compared when not using identical precursors and products for the calculations. In our study, we encountered this problem for several ratios, which led to the creation of adapted ratios. In the future, it may be therefore recommended to define the diagnostic ratios precisely. This will require some standardization in GC-MS urinary steroid profiling, but will have the advantage that diagnostic ratios will be comparable between laboratories.

In conclusion, studies of the urinary steroid metabolome are valuable for solving specific questions on easily available biomaterial. We show that androgen biosynthesis through the backdoor pathway predominates during minipuberty. Additionally, we provide longitudinal normative data for diagnostic ratios for steroid enzyme activities.

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Figure Legends

Figure 1 Assessment of androgen production through the alternative backdoor pathway. All figures were created by the combined use of the quantile regression method and natural splines. The solid lines represent the 50th and the dashed lines the 25th and 75th quantiles. Figure A shows overall androgen production over the first 12 months of life using androsterone as a surrogate metabolite. Figure B shows the flux through the backdoor pathway using the established ratio androsterone/etiocholanolone (29). In Figure A and B black lines are used for boys and grey lines for girls. In Figure C and D the contribution to androgen production by testes only is depicted. In Figure E and F the 17,20 lyase activities of the Δ^5- and the Δ^4-steroid pathway are shown; age effects were found, but no sex differences. The ID number of the steroid compound /steroid ratio from **Table 1** is indicated in square brackets.

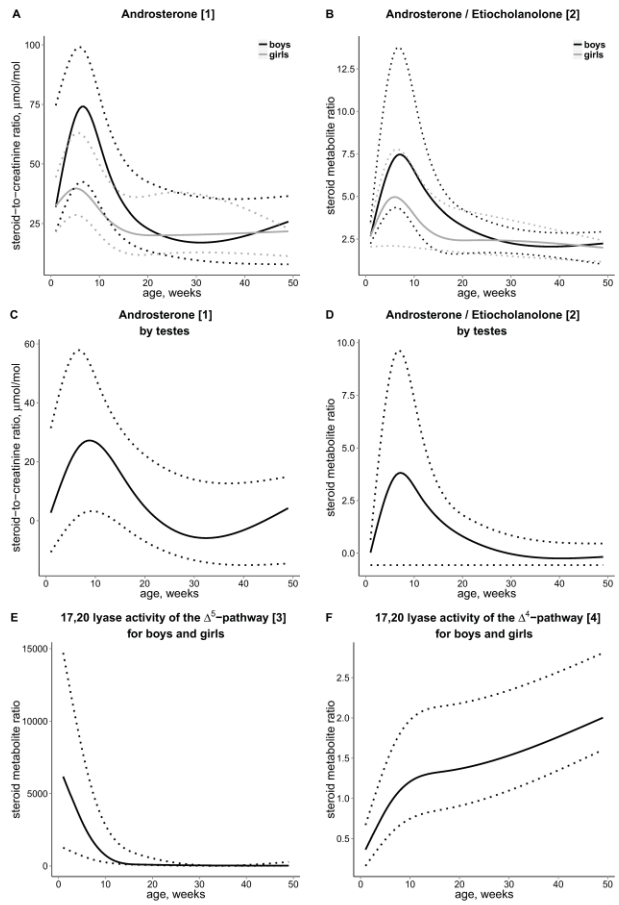

Figure 2 Assessment of sex- and age-specific dependencies of urinary steroid metabolite ratios corresponding to specific enzymes of steroidogenesis. All figures were created by the combined use of the quantile regression method and natural splines. The solid lines represent the 50th and the dashed lines the 25th and 75th quantiles. Black lines are used for boys and grey lines for girls. A, sex- and age-specific pattern for a specific ratio representing the 21-hydroxylase. B, age-dependent pattern for the 3β-hydroxysteroid dehydrogenase. C, age-dependent pattern for the 17α-hydroxylase. D, age- and sex-dependent pattern for the 5α-reductase during the first 12 months of life. The ID number of the steroid ratio from **Table 2** is indicated in square brackets.

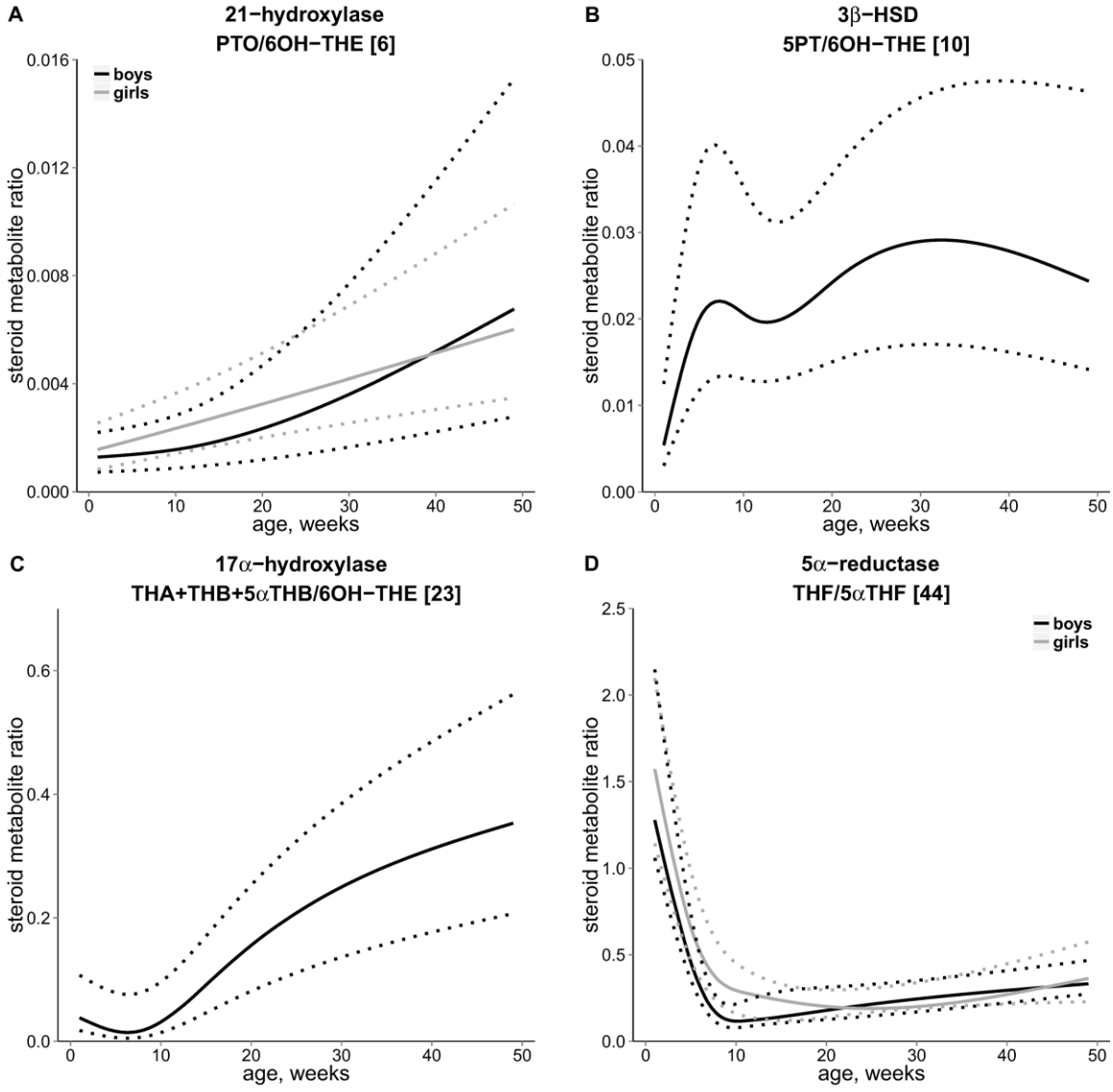

Table 1. Urinary steroid metabolites and ratios for the evaluation of the flux through the alternative backdoor pathway for androgen production. Established formula and calculations.

ID	Metabolites/Ratios	Abbreviation	Reference
	ANDROGEN GENERATED FROM THE CLASSIC AND THE ALTERNATIVE BACKDOOR PATHWAY		
1	androsterone	AT	Auchus et al. 2004 (15)
	ALTERNATIVE BACKDOOR PATHWAY AFTER THE 17,20 LYASE VS. CLASSIC PATHWAY ACTIVITY		
2	androsterone/etiocholanolone	AT/ET	Kamrath et al. 2012 (29)
	CYP17A1 (17,20 LYASE) ACTIVITY FOR THE Δ^5-STEROID PATHWAY		
3	(dehydroepiandrosterone+16α-OH-dehydroepiandrosterone+ androstenediol)/pregnenetriol	(DHEA+16OH-DHEA+ Δ5-diol)/5PT	Homma et al. 2006 (28)
	CYP17A1 (17,20 LYASE) ACTIVITY FOR THE Δ^4-STEROID PATHWAY		
4	11β-OH-androsterone/pregnanetriol	11β-OH-AT/PT	Homma et al. 2006 (28)

Table 2. Formula to calculate for steroid conversion ratios representing relative steroid enzyme activities involved in genetic steroid disorders.

ID	Disorders and pathways and their diagnostic ratios	Ratio abbreviation	Reference[a]
21-HYDROXYLASE DEFICIENCY (21OHD)			
5	pregnanetriolone/TH-cortisone	PTO/THE	Kamrath et al. 2016 (31)
6	pregnanetriolone/6α-OH-TH-cortisone	PTO/6OH-THE	Kamrath et al. 2016 (31)
7	pregnanetriolone/ (TH-cortisone+6α-OH-TH-cortisone)	PTO/(THE+6OH-THE)	Kamrath et al. 2016 (31)
8	pregnanetriolone/(TH-cortisone+ 6α-OH-TH-cortisone+6α-OH-β-cortolone)	PTO/(THE+6OH-THE+6OH-β-Cl)	Kamrath et al. 2016 (31)
3β-HSD DEFICIENCY (3HSDD)			
9	pregnenetriol/TH-cortisone	5PT/THE	Caulfield et al. 2002 (33) Kamrath et al. 2016 (31)
10	pregnenetriol/6α-OH-TH-cortisone	5PT/6OH-THE	Caulfield et al. 2002 (33) Kamrath et al. 2016 (31)
11	pregnenetriol/(TH-cortisone+6α-OH-TH-cortisone)	5PT/(THE+6OH-THE)	Caulfield et al. 2002 (33) Kamrath et al. 2016 (31)
12	pregnenetriol/(TH-cortisone+6α-OH-TH-cortisone+ 6α-OH-β-cortolone)	5PT/(THE+6OH-THE+6OH-β-Cl)	Caulfield et al. 2002 (33) Kamrath et al. 2016 (31)
13	dehydroepiandrosterone/TH-cortisone	DHEA/THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
14	dehydroepiandrosterone/6α-OH-TH-cortisone	DHEA/6OH-THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
15	dehydroepiandrosterone/(TH-cortisone+ 6α-OH-TH-cortisone)	DHEA/(THE+6OH-THE)	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
16	dehydroepiandrosterone/(TH-cortisone+ 6α-OH-TH-cortisone+6α-OH-β-cortolone)	DHEA/(THE+ 6OH-THE+6OH-β-Cl)	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
RATIO TO DISTINGUISH 3β-HSD DEFICIENCY FROM 21-HYDROXYLASE DEFICIENCY			
17	pregnenetriol/pregnanetriolone	5PT/PTO	Caulfield et al. 2002 (33)
11β-HYDROXYLASE DEFICIENCY (11OHD)			
18	TH-11-deoxycortisol/TH-cortisone	THS/THE	Caulfield et al. 2002 (33) Kamrath et al. 2016 (31)
19	TH-11-deoxycortisol/6α-OH-TH-cortisone	THS/6OH-THE	Caulfield et al. 2002 (33) Kamrath et al. 2016 (31)
20	TH-11-deoxycortisol/ (TH-cortisone+6α-OH-TH-cortisone)	THS/(THE+6OH-THE)	Caulfield et al. 2002 (33) Kamrath et al. 2016 (31)
21	TH-11-deoxycortisol/(TH-cortisone+ 6α-OH-TH-cortisone+6α-OH-β-cortolone)	THS/(THE+6OH-THE+6OH-β-Cl)	Caulfield et al. 2002, (33) Kamrath et al. 2016 (31)
17α-HYDROXYLASE DEFICIENCY (17OHD)			
22	(11-dehydro-TH-corticosterone+TH-corticosterone+ allo-TH-corticosterone)/TH-cortisone	THA+THB+5αTHB/THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
23	(11-dehydro-TH-corticosterone+TH-corticosterone+ allo-TH-corticosterone)/6α-OH-TH-cortisone	THA+THB+5αTHB/6OH-THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
24	(11-dehydro-TH-corticosterone+ TH-corticosterone+allo-TH-corticosterone)/ (TH-cortisone+6α-OH-TH-cortisone)	THA+THB+5αTHB/(THE+6OH-THE)	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
25	(11-dehydro-TH-corticosterone+TH-corticosterone+ allo-TH-corticosterone)/(TH-cortisone+ 6α-OH-TH-cortisone+6α-OH-β-cortolone)	THA+THB+5αTHB/ (THE+6OH-THE+6OH-β-Cl)	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
26	(11-dehydro-TH-corticosterone+ TH-corticosterone+allo-TH-corticosterone)/ (androsterone+etiocholanolone)	(THA+THB+5αTHB)/(AT+ET)	Krone et al. 2010 (34)
P450 OXIDOREDUCTASE DEFICIENCY (PORD)			
27	(17-OH-pregnanolone+pregnanetriol)/ (androsterone+etiocholanolone)	(17HP+PT)/(AT+ET)	Krone et al. 2010 (34)
28	(17-OH-pregnanolone+pregnanetriol)/TH-cortisone	(17HP+PT)/THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
29	(17-OH-pregnanolone+pregnanetriol)/ 6α-OH-TH-cortisone	(17HP+PT)/6OH-THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
30	(17-OH-pregnanolone+pregnanetriol)/ (TH-cortisone+6α-OH-TH-cortisone)	(17HP+PT)/(THE+6OH-THE)	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
31	(17-OH-pregnanolone+pregnanetriol)/ (TH-cortisone+6α-OH-TH-cortisone+ 6α-OH-β-cortolone)	(17HP+PT)/ (THE+6OH-THE+6OH-β-Cl)	Krone et al. 2010 (34) Kamrath et al. 2016 (31)

32	pregnanediol/TH-cortisone	PD/THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
33	pregnanediol/6α-OH-TH-cortisone	PD/6OH-THE	Krone et al. 2010 (34) Kamrath et al. 2016 (31)
34	pregnanediol/(TH-cortisone+6α-OH-TH-cortisone)	PD/(THE+6OH-THE)	Krone et al. 2010, (34) Kamrath et al. 2016 (31)
35	pregnanediol/(TH-cortisone+6α-OH-TH-cortisone+ 6α-OH-β-cortolone)	PD/(THE+6OH-THE+6OH-β-Cl)	Krone et al. 2010, (34) Kamrath et al. 2016 (31)
APPARENT MINERALOCORTICOID EXCESS (AME)/11β-HSD2 DEFICIENCY (11HSD2D)			
36	cortisol/cortisone	F/E	Krone et al. 2010 (34)
37	(TH-cortisol+allo-TH-cortisol)/TH-cortisone	(THF+5αTHF)/THE	Krone et al. 2010 (34)
38	(α-cortol+β-cortol)/(α-cortolone+β-cortolone)	(α-C+β-C)/(α-Cl+β-Cl)	Krone et al. 2010 (34)
39	(α-cortol+β-cortol)/(α-cortolone+β-cortolone+ 6α-OH-α-cortolone+1β-OH-β-cortolone+ 6α-OH-β-cortolone)	(α-C+β-C)/(α-Cl+β-Cl+6OH-α-Cl+ 1β-OH-β-Cl+6OH-β-Cl)	Krone et al. 2010 (34)
40	(cortisol+cortisone)/(TH-cortisol+allo-TH-cortisol+ TH-cortisone)	(F+E)/(THF+5αTHF+THE)	Krone et al. 2010 (34)
APPARENT CORTISONE REDUCTASE DEFICIENCY (ACRD)/HEXOSE-6-PHOSPHATE DEHYDROGENASE (H6PDH)/ CORTISONE REDUCTASE DEFICIENCY (CRD)/11β-HSD1 DEFICIENCY (11HSD1D)			
41	TH-cortisone/(TH-cortisol+allo-TH-cortisol)	THE/(THF+5αTHF)	Krone et al. 2010 (34)
42	(α-cortolone+β-cortolone)/(α-cortol+β-cortol)	(α-Cl+β-Cl)/(α-C+β-C)	Krone et al. 2010 (34)
43	(α-cortolone+β-cortolone+6α-OH-α-cortolone+ 1β-OH-β-cortolone+6α-OH-β-cortolone)/ (α-cortol+β-cortol)	(α-Cl+β-Cl+6OH-α-Cl+1β-OH-β-Cl+ 6OH-β-Cl)/(α-C+β-C)	Krone et al. 2010 (34)
5α-REDUCTASE DEFICIENCY (5ARD)			
44	TH-cortisol/allo-TH-cortisol	THF/5αTHF	Krone et al. 2010 (34)
45	TH-corticosterone/allo-TH-corticosterone	THB/5αTHB	Krone et al. 2010 (34)

[a]Glucocorticoid metabolites in some ratios indicated in the references were replaced by different combinations of fetal urinary glucocorticoid metabolites where appropriate by analogy with Kamrath et al. 2016 (31).

Table 3. Specific urine steroid metabolite ratios at 13 time-points in the first year of life representing specific enzyme activities of the steroid metabolism. The highest ratio and the 97.5th quantile in parenthesis are presented. Age and sex dependency of each steroid ratio were estimated by multivariable linear quantile mixed models and the respective results are indicated in the columns "Age" and "Sex".

ID	Steroid metabolite ratios		Week 1	Week 3	Week 5	Week 7	Week 9	Week 11	Week 13	Week 17	Week 21	Week 25	Week 33	Week 41	Week 49	Ag	Se
21-HYDROXYLASE DEFICIENCY (21OHD)																	
5	PTO/THE		0.0279 (0.0335)	0.062 (0.0434)	0.0478 (0.0468)	0.0369 (0.028)	0.04 (0.0301)	0.0639 (0.0264)	0.1198 (0.0954)	0.0293 (0.0154)	0.0227 (0.0167)	0.0862 (0.0508)	0.0475 (0.0364)	0.5318 (0.2335)	0.0987 (0.0928)	no	no
6	PTO/6OH-THE	boy	0.0047 (0.0044)	0.0145 (0.0107)	0.006 (0.0052)	0.0043 (0.0042)	0.0058 (0.0049)	0.0066 (0.0061)	0.0096 (0.0078)	0.0107 (0.0085)	0.009 (0.0086)	0.017 (0.0146)	0.0195 (0.0189)	0.0208 (0.019)	0.1153 (0.1093)	yes	yes
		girls	0.0056 (0.0053)	0.0035 (0.0035)	0.0057 (0.005)	0.006 (0.0057)	0.005 (0.005)	0.0261 (0.0175)	0.0705 (0.041)	0.0076 (0.0074)	0.0155 (0.0134)	0.0285 (0.0251)	0.0488 (0.0392)	0.057 (0.0406)	0.0282 (0.0267)		
7	PTO/(THE+6OH-THE)	boy	0.0035 (0.0034)	0.0107 (0.0094)	0.0037 (0.0037)	0.0039 (0.0036)	0.0036 (0.0031)	0.0039 (0.0038)	0.0089 (0.007)	0.0078 (0.0065)	0.0045 (0.0045)	0.0066 (0.0065)	0.012 (0.0109)	0.008 (0.0075)	0.0532 (0.0457)	no	yes
		girls	0.0043 (0.0043)	0.0033 (0.0039)	0.0039 (0.0036)	0.0045 (0.0039)	0.0036 (0.0036)	0.0185 (0.0122)	0.0402 (0.0245)	0.0046 (0.0045)	0.0092 (0.0077)	0.012 (0.0118)	0.0241 (0.018)	0.0515 (0.0351)	0.0175 (0.017)		
8	PTO/(THE+6OH-THE+6OH-β-Cl)	boy	0.0022 (0.0021)	0.0088 (0.0067)	0.0033 (0.0031)	0.0028 (0.0027)	0.0028 (0.0025)	0.0034 (0.0023)	0.0071 (0.0058)	0.0074 (0.0062)	0.0044 (0.0043)	0.0063 (0.0063)	0.0116 (0.0104)	0.0079 (0.0074)	0.0464 (0.04)	no	yes
		girls	0.0032 (0.0028)	0.0024 (0.0023)	0.0034 (0.0029)	0.0037 (0.0033)	0.0032 (0.0031)	0.0103 (0.0074)	0.0382 (0.0228)	0.0041 (0.004)	0.0086 (0.0072)	0.0116 (0.0115)	0.0233 (0.0175)	0.047 (0.033)	0.0169 (0.0162)		
3β-HSD DEFICIENCY (3HSDD)																	
0	5PT/THE		1.88 (1.786)	1.467 (0.5684)	1.498 (1.411)	0.8546 (0.7751)	0.8008 (0.5927)	0.9749 (0.3978)	0.5489 (0.2452)	0.3163 (0.1815)	0.3809 (0.2344)	0.9418 (0.439)	0.922 (0.2987)	0.3437 (0.2122)	0.5945 (0.2575)	no	no
1	5PT/6OH-THE		0.36 (0.304)	0.107 (0.0846)	0.13 (0.0967)	0.1575 (0.1328)	0.3249 (0.1672)	0.398 (0.1711)	0.175 (0.11)	0.169 (0.1323)	0.1847 (0.1393)	0.1325 (0.0974)	0.3785 (0.2123)	0.0881 (0.0873)	0.1116 (0.104)	yes	no
1	5PT/(THE+6OH-THE)		0.3021 (0.2598)	0.0778 (0.0466)	0.1196 (0.0904)	0.1186 (0.1021)	0.2311 (0.1387)	0.2826 (0.1187)	0.1092 (0.0727)	0.1101 (0.0723)	0.1244 (0.0726)	0.1162 (0.0717)	0.2683 (0.1321)	0.0502 (0.0446)	0.0817 (0.0681)	yes	no
2	5PT/(THE+6OH-THE+6OH-β-Cl)		0.2257 (0.2077)	0.0557 (0.0394)	0.0845 (0.0706)	0.0901 (0.078)	0.1732 (0.1031)	0.1566 (0.0826)	0.0907 (0.063)	0.0908 (0.0671)	0.1031 (0.0669)	0.0921 (0.0621)	0.2127 (0.1107)	0.0465 (0.0406)	0.0735 (0.0606)	yes	no
3	DHEA/THE		0.4194 (0.3393)	0.2101 (0.1853)	1.153 (0.6294)	0.2533 (0.2077)	0.2023 (0.1993)	0.0969 (0.0732)	0.3599 (0.1114)	0.062 (0.0476)	0.0637 (0.0499)	0.4753 (0.2553)	0.0887 (0.0485)	0.0899 (0.0476)	0.08 (0.0594)	no	no
4	DHEA/6OH-THE		0.0976 (0.091)	0.0466 (0.031)	0.0384 (0.034)	0.0373 (0.0314)	0.1024 (0.0545)	0.04 (0.0308)	0.2713 (0.0659)	0.0282 (0.0259)	0.0589 (0.039)	0.0669 (0.0521)	0.0713 (0.0563)	0.0322 (0.038)	0.0935 (0.0598)	yes	no
5	DHEA/(THE+6OH-THE)	boy	0.0737 (0.0593)	0.017 (0.0156)	0.0353 (0.0277)	0.0267 (0.0202)	0.0051 (0.0049)	0.0145 (0.0125)	0.0105 (0.0099)	0.0066 (0.0066)	0.0096 (0.0086)	0.0068 (0.0068)	0.0395 (0.0299)	0.0039 (0.0026)	0.0431 (0.0269)	no	yes
		girls	0.0626 (0.056)	0.0382 (0.0305)	0.0297 (0.0237)	0.024 (0.0228)	0.0654 (0.0533)	0.0244 (0.0225)	0.1547 (0.0918)	0.0179 (0.0176)	0.0306 (0.0255)	0.0586 (0.0454)	0.0133 (0.0126)	0.0109 (0.0102)	0.0066 (0.0063)		
6	DHEA/(THE+6OH-THE+6OH-β-Cl)	boy	0.0345 (0.0247)	0.0123 (0.0119)	0.0249 (0.0199)	0.0173 (0.0127)	0.0042 (0.0041)	0.0126 (0.0107)	0.0087 (0.0082)	0.0062 (0.0061)	0.0087 (0.0078)	0.0064 (0.0063)	0.0387 (0.0299)	0.0036 (0.0024)	0.0376 (0.0229)	no	yes
		girls	0.0379 (0.0294)	0.027 (0.0227)	0.0224 (0.0179)	0.0177 (0.0175)	0.0531 (0.0427)	0.0214 (0.0184)	0.1471 (0.0862)	0.0171 (0.016)	0.0277 (0.0226)	0.0465 (0.0362)	0.0126 (0.013)	0.0107 (0.01)	0.0063 (0.006)		
RATIO TO DISTINGUISH 3β-HSD DEFICIENCY FROM 21-HYDROXYLASE DEFICIENCY																	
7	5PT/PTO		405.4 (196.4)	203 (49.89)	82.18 (41.21)	77.77 (72)	88.23 (66.22)	41.86 (27.58)	33.15 (20.27)	76.52 (44.26)	47.08 (27.42)	26.51 (22.62)	69.24 (25.44)	18.09 (17.92)	12 (11.8)	yes	no
11β-HYDROXYLASE DEFICIENCY (11OHD)																	
8	THS/THE		0.1391 (0.1341)	0.0926 (0.0887)	0.1087 (0.1075)	0.0339 (0.0306)	0.0306 (0.0232)	0.0341 (0.025)	0.104 (0.0552)	0.048 (0.0275)	0.0757 (0.0613)	0.227 (0.0969)	0.0663 (0.0657)	0.0783 (0.0676)	0.1084 (0.103)	yes	no
9	THS/6OH-THE		0.0452 (0.0368)	0.0238 (0.03)	0.029 (0.0174)	0.0123 (0.0114)	0.0131 (0.0131)	0.0176 (0.0162)	0.0784 (0.0324)	0.0364 (0.0214)	0.0657 (0.0554)	0.0658 (0.061)	0.0833 (0.0703)	0.0817 (0.0724)	0.1283 (0.1071)	yes	no
0	THS/(THE+6OH-THE)		0.0341 (0.0211)	0.0187 (0.016)	0.0126 (0.0101)	0.0067 (0.0064)	0.0073 (0.0073)	0.0101 (0.0087)	0.0447 (0.0175)	0.0184 (0.0175)	0.0307 (0.0276)	0.0277 (0.0282)	0.0318 (0.0301)	0.0316 (0.0324)	0.0449 (0.0412)	yes	no
1	THS/(THE+6OH-THE+6OH-β-Cl)		0.016 (0.0144)	0.0135 (0.0103)	0.011 (0.0084)	0.0062 (0.0058)	0.0068 (0.0067)	0.0092 (0.0091)	0.0425 (0.0164)	0.0172 (0.0164)	0.0286 (0.0286)	0.0268 (0.0231)	0.0309 (0.0276)	0.0311 (0.029)	0.0441 (0.0309)	yes	no
17α-HYDROXYLASE DEFICIENCY (17OHD)																	
2	THA+THB+5αTHB/THE		1.592 (1.16)	0.814 (0.8001)	1.608 (0.9228)	1.172 (0.6845)	1.261 (0.7656)	0.7127 (0.506)	0.6943 (0.6644)	1.126 (0.6883)	1.133 (0.8644)	1.2 (1.155)	1.311 (1.178)	1.054 (0.9272)	1.208 (1.003)	yes	no
3	THA+THB+5αTHB/6OH-THE		0.5168 (0.3189)	0.1942 (0.19)	0.2094 (0.1765)	0.2195 (0.2131)	0.5646 (0.4202)	0.467 (0.3039)	0.4984 (0.387)	0.5595 (0.334)	0.7406 (0.5888)	1.05 (0.7068)	1.287 (1.19)	1.396 (1.134)	1.898 (1.578)	yes	no

24	THA+THB+5αTHB/(THE+6OH-THE)		0.3901 (0.2378)	0.1551 (0.1498)	0.1645 (0.1531)	0.179 (0.15)	0.39 (0.2779)	0.2821 (0.1999)	0.2842 (0.221)	0.3738 (0.2172)	0.3815 (0.2326)	0.5201 (0.2898)	0.6497 (0.5141)	0.5179 (0.4412)	0.7382 (0.6428)	yes	no
25	THA+THB+5αTHB/ (THE+6OH-THE+6OH-β-Cl)		0.1827 (0.1431)	0.1185 (0.1063)	0.1377 (0.1182)	0.1231 (0.1084)	0.3328 (0.2276)	0.2535 (0.1668)	0.2702 (0.2049)	0.3239 (0.1964)	0.3618 (0.3197)	0.4892 (0.3666)	0.6175 (0.4943)	0.4959 (0.4219)	0.7103 (0.6157)	yes	no
26	(THA+THB+5αTHB)/(AT+ET)	boy	47.83 (41.6)	22.25 (18.02)	46 (37.85)	19.26 (17.85)	34.34 (25.02)	22.51 (20.89)	23.8 (21.28)	26.25 (26.04)	38.17 (28.15)	65.24 (60.86)	60.72 (56.8)	103.9 (96.52)	131.3 (119.2)	yes	yes
		girls	18.99 (17.16)	26.23 (25.11)	26.62 (24.91)	37.15 (24.29)	119.8 (75.28)	68.22 (62.45)	96.02 (89.17)	67.67 (54.22)	93.58 (78.06)	123.6 (93.42)	52.84 (48.68)	52.71 (43.24)	119.4 (95.82)		
P450 OXIDOREDUCTASE DEFICIENCY (PORD)																	
27	(17HP+PT)/(AT+ET)		3.995 (2.6)	5.826 (5.012)	4.228 (2.89)	3.086 (2.981)	6.044 (5.146)	5.2 (4.106)	4.528 (2.847)	6.938 (5.868)	7.227 (7.004)	6.678 (6.276)	7.536 (6.717)	5.363 (4.287)	6.62 (5.56)	yes	no
28	(17HP+PT)/THE		0.305 (0.2503)	0.8103 (0.3641)	0.4091 (0.3639)	0.3157 (0.2918)	0.276 (0.1115)	0.2231 (0.1879)	0.5293 (0.5015)	0.342 (0.2918)	0.2763 (0.2308)	0.3979 (0.3963)	0.3015 (0.2118)	0.8954 (0.4857)	0.7659 (0.6574)	yes	no
29	(17HP+PT)/6OH-THE		0.0787 (0.027)	0.0788 (0.0544)	0.0508 (0.0437)	0.0407 (0.0284)	0.065 (0.0412)	0.0911 (0.0862)	0.3764 (0.1391)	0.1574 (0.1317)	0.1882 (0.1406)	0.1821 (0.1522)	0.2658 (0.2102)	0.152 (0.1446)	0.3632 (0.2785)	yes	no
30	(17HP+PT)/(THE+6OH-THE)		0.0594 (0.0217)	0.0537 (0.0403)	0.0339 (0.0231)	0.0313 (0.0248)	0.0526 (0.0277)	0.0647 (0.0546)	0.2146 (0.0946)	0.1006 (0.0923)	0.112 (0.0843)	0.079 (0.077)	0.0831 (0.0814)	0.0867 (0.0825)	0.1675 (0.1516)	yes	no
31	(17HP+PT)/ (THE+6OH-THE+6OH-β-Cl)		0.0278 (0.0178)	0.0414 (0.0371)	0.0266 (0.0256)	0.0254 (0.0205)	0.0441 (0.0227)	0.0453 (0.038)	0.2041 (0.0832)	0.0874 (0.0863)	0.1043 (0.0803)	0.0743 (0.0734)	0.0785 (0.0774)	0.0792 (0.0759)	0.146 (0.1363)	yes	no
32	PD/THE		0.3413 (0.2859)	0.1726 (0.1251)	0.2996 (0.146)	0.0787 (0.054)	0.1221 (0.1171)	0.0425 (0.0242)	0.7094 (0.4789)	0.1661 (0.0676)	0.1036 (0.0837)	0.1208 (0.1106)	0.0521 (0.0456)	0.0616 (0.0515)	0.1044 (0.1024)	no	no
33	PD/6OH-THE		0.0489 (0.0375)	0.0336 (0.0181)	0.0342 (0.0237)	0.008 (0.0078)	0.0556 (0.0539)	0.0171 (0.0165)	0.3912 (0.3525)	0.1065 (0.0527)	0.0709 (0.0619)	0.1044 (0.0624)	0.0514 (0.0421)	0.0641 (0.0503)	0.0503 (0.0437)	yes	no
34	PD/(THE+6OH-THE)		0.0428 (0.0322)	0.0262 (0.0146)	0.0228 (0.017)	0.0063 (0.0058)	0.0377 (0.0347)	0.0122 (0.0105)	0.2521 (0.2059)	0.0649 (0.0286)	0.0421 (0.0295)	0.0414 (0.029)	0.0227 (0.0219)	0.0217 (0.0186)	0.0251 (0.021)	yes	no
35	PD/(THE+6OH-THE+6OH-β-Cl)		0.0354 (0.0172)	0.021 (0.0107)	0.017 (0.0122)	0.0052 (0.0049)	0.0338 (0.0286)	0.0111 (0.0093)	0.2325 (0.1947)	0.055 (0.0254)	0.0405 (0.0296)	0.0399 (0.0279)	0.0223 (0.0213)	0.0213 (0.018)	0.0237 (0.0201)	yes	no
APPARENT MINERALOCORTICOIDEXCESS (AME)/11β-HSD2 DEFICIENCY (11HSD2D)																	
36	F/E		76.61 (54.07)	38.95 (13.09)	10.19 (4.742)	14.66 (3.681)	28.3 (2.242)	35.22 (6.76)	51.993 (18.712)	16.349 (6.801)	1.681 (1.555)	11.061 (5.217)	3.019 (2.689)	20.805 (10.004)	4.239 (2.869)	yes	no
37	(THF+5αTHF)/THE		0.27 (0.278)	0.2815 (0.1126)	0.2269 (0.1087)	0.2513 (0.213)	0.4203 (0.2314)	0.798 (0.6606)	1.356 (1.269)	2.105 (1.904)	2.162 (1.623)	3.614 (3.455)	4.331 (3.878)	2.609 (2.459)	4.999 (4.166)	yes	no
38	(α-C+β-C)/(α-Cl+β-Cl)		2.746 (1.222)	1.1 (0.7439)	2.408 (1.492)	0.9456 (0.941)	1.232 (1.099)	0.5002 (0.4655)	0.8667 (0.8632)	0.8523 (0.7477)	0.6627 (0.5625)	0.8173 (0.7328)	1.424 (1.085)	0.6541 (0.6307)	0.8764 (0.8214)	yes	no
39	(α-C+β-C)/(α-Cl+β-Cl+6OH-α-Cl+ 1β-OH-β-Cl+6OH-β-Cl)		0.0542 (0.0362)	0.0391 (0.0275)	0.0677 (0.0488)	0.0598 (0.0586)	0.0808 (0.0414)	0.0507 (0.0358)	0.2061 (0.1114)	0.075 (0.0522)	0.0726 (0.0553)	0.0524 (0.0497)	0.1359 (0.105)	0.0715 (0.0684)	0.1059 (0.0988)	yes	no
40	(F+E)/(THF+5αTHF+THE)		0.8312 (0.8189)	0.4776 (0.4274)	1.032 (0.8279)	1.051 (0.6255)	1.033 (0.4706)	0.3264 (0.2954)	0.5463 (0.4871)	0.2268 (0.2013)	0.3098 (0.2645)	0.4643 (0.2987)	0.5877 (0.2672)	0.1251 (0.1024)	0.1288 (0.1262)	yes	no
APPARENT CORTISONE REDUCTASE DEFICIENCY (ACRD)/HEXOSE-6-PHOSPHATE DEHYDROGENASE (H6PDH)/ CORTISONE REDUCTASE DEFICIENCY (CRD)/11β-HSD1 DEFICIENCY (11HSD1D)																	
41	THE/(THF+5αTHF)		128.7 (118.4)	108.2 (101.9)	92.34 (80.94)	65.76 (40.87)	48.39 (41.2)	23.37 (21.85)	19.13 (16.02)	6.33 (6.266)	5.69 (3.828)	35.866 (10.195)	12.04 (5.853)	1.452 (1.418)	1.393 (1.274)	yes	no
42	(α-Cl+β-Cl)/(α-C+β-C)		14.54 (13.19)	18.99 (14.15)	10.79 (9.641)	18.04 (13.58)	14.92 (14.5)	15.29 (11.95)	14.65 (13.03)	19.28 (13.88)	9.57 (8.913)	10.61 (8.578)	5.223 (4.688)	5.088 (4.933)	4.44 (4.047)	yes	no
43	(α-Cl+β-Cl+6OH-α-Cl+1β-OH-β-Cl+ 6OH-β-Cl)/(α-C+β-C)		617.3 (468.2)	433.5 (301.7)	260.4 (231.5)	295.6 (219.7)	313.6 (280.9)	231.7 (175.9)	258.4 (241.5)	273 (179.7)	117.7 (116.1)	149 (139.4)	143.7 (103.5)	82.23 (75.44)	49.12 (48.34)	yes	no
5α-REDUCTASE DEFICIENCY (5ARD)																	
44	THF/5αTHF	boy	4.164 (2.872)	3.094 (2.774)	1.093 (1.008)	0.4993 (0.4883)	0.5926 (0.5843)	0.508 (0.4209)	0.4859 (0.405)	0.4421 (0.4147)	0.384 (0.2723)	0.5647 (0.5486)	0.895 (0.7668)	0.4919 (0.4781)	0.6935 (0.6768)	yes	yes
		girls	3.285 (2.023)	3.831 (2.561)	4.385 (4.02)	0.9944 (0.9245)	0.6356 (0.5879)	0.567 (0.518)	0.9602 (0.7674)	0.3931 (0.2772)	0.4925 (0.474)	1.069 (0.9217)	0.4492 (0.4481)	1.084 (0.8829)	1.778 (1.53)		
45	THB/5αTHB	boy	222.2 (137.9)	26.28 (10.46)	8.744 (8.28)	61.89 (47.37)	95.86 (91.44)	202 (155)	105.7 (99.16)	480.9 (432.8)	131.9 (129.5)	207.5 (207.4)	193.9 (186.1)	307.9 (282.5)	136.9 (126.5)	yes	yes
		girls	152.5 (137.9)	34.75 (23.85)	60.63 (38.52)	15.1 (14.89)	103.9 (86.08)	82.07 (80.31)	142.9 (126)	202 (185.5)	327.6 (304.5)	246.2 (192.5)	263.8 (260.8)	169.4 (161)	147.2 (137)		

Number of calculated ratios per week: week 1: 34-36; week 3: 36-38; week 5: 32-35; week 7: 32-35; week 9: 36-38; week 11: 31-35; week 13: 34-38; week 17: 32-33; week 21: 30-32; week 25: 30-33; week 33: 26-28; week 41: 21-24; week 49: 19-20