ORIGINAL ARTICLE

Androgens concentrations and second-to fourth-digit ratio (2D:4D) in girls with congenital adrenal hyperplasia (21-hydroxylase deficiency)

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Submitted: 2012-09-05 Accepted: 2012-11-25 Published online: 2012-12-28

Key words: **congenital adrenal hyperplasia; androgens; testosterone; androstenedione; dehydroepiandrostenedione sulphate; autonomization; second-to-fourth-digit ratio**

Neuroendocrinol Lett 2012; **33**(8):787–791 **PMID:** 23391972 NEL331112A02

Abstract

OBJECTIVES: Excessive hyperandrogenism, though proper hydrocortisone supplementation is a frequent clinical problem in girls with congenital adrenal hyperplasia (CAH). This may result from autonomic regulation of androgen production established in prenatal life. It has been suggested that the length of the second finger relative to the length of the fourth finger (2D;4D ratio) is negatively related to prenatal testosterone concentration. **DESIGN AND SETTING:** The retrospective study aimed to establish the relationship between the level of androgenization in utero determined using 2D:4D ratio and serum androgen concentrations in treated girls with CAH (21-OH deficiency) has been performed on 19 girls with CAH (21-OH deficiency) at the age of 3.7–19 years (mean 13.8 ± 4.07 years). All subjects were adequately treated with hydrocortisone (10–19 mg/m2; mean 13.81 ± 4.07 mg/m2). Anthropometric measurements of digits length were performed in all girls on X-rays obtained for bone age estimation. Apart from it, serum androgens concentrations (testosterone, androstenedione, s-DHEA) and 17-OH-progesterone (17-OHP) were assayed. **RESULTS:** Mean androgens serum concentrations in examined group were: testosterone 150.21 ± 155.44 ng/ml; androstenedione 4.15 ± 5.32 ng/ml, s-DHEA 70.39 ± 85.52 μg/dl. Mean 2D:4D ratio was 0.96 ± 0.04. Analysis of correlation showed positive linear correlations between testosterone, s-DHEA and 2D:4D ratio (r=0.53, p=0.023 and r=0.53; p=0.019, respectively). **CONCLUSIONS:** 2D:4D ratio parameter may be a simple test in indentification of female CAH patients prone to excessive androgen secretion despite proper treatment. The autonomization of adrenal androgens production in foetal life may cause its elevated levels in female patients with CAH although treated adequately.

Abbreviations:

11β-OH	- 11β-hydroxylase
17-OHP	- 17-hydroxyprogesterone
21-OH	- 21-hydroxylase
2D:4D	- second-to-fourth finger relative ratio
ACTH	- adrenocorticotrophic hormone
AR	- androgen receptor
CAH	- congenital adrenal hyperplasia
ER	- estrogen receptor
HPA	- hypothalamic-pituitary-adrenal axis
s-DHEA	- dehydroepiandrostenedione sulphate
SD	- standard deviation

INTRODUCTION

Recent studies have demonstrated that the ratio of the length of the second finger relative to the length of the fourth finger (2D:4D) is determined in the 14 week of foetal life and remains unchanged at puberty (Koehler *et al.* 2004). It may be a retrospective, morphological marker of foetal growth which persists into adult life (Fink *et al.* 2003; Ronalds *et al.* 2002). It is suggested that 2D:4D ratio is predictive of some diseases and may be used in diagnosis, prognosis and in early life-style interventions which may delay the onset of disease or facilitate its early detection (Manning *et al.* 2000). It has been reported to be related to hand preference (Fink *et al.* 2004), autism (Manning *et al.* 2001), Asperger's syndrome, sperm counts, family size, age at myocardial infarction in men and breast cancer in women (Lutchmaya *et al.* 2004), schizophrenia (Walder *et al.* 2006), dyslexia, migraine, stammering and immune dysfunction (Manning *et al.* 2000).

There is an indirect evidence that 2D:4D is negatively related to prenatal testosterone concentration and positively with prenatal estradiol concentration, so that 2D:4D is lower in men than in women (Galis *et al.* 2010; Lutchmaya *et al.* 2004; Manning *et al.* 2000; Neave *et al.* 2003). This is explained by the observation that 2D:4D ratio is influenced by the expression of *HOX* genes (*HOXA, HOXD*), which during vertebrate development controls the differentiation of the digits as well as the urogenital system and sex hormones activity (Buck *et al.* 2003; Firman *et al.* 2003; Wood *et al.* 2003).

The term congenital adrenal hyperplasia (CAH) refers to the group of autosomal recessive disorders resulting from mutations of genes for enzymes mediating the biochemical steps of production of cortisol from cholesterol by the adrenal glands (steroidogenesis) (Merke 2002). CAH due to 21-hydroxylase deficiency accounts for about 95% of diagnosed cases (classical form of this defect occurs in 1/14200 live births), 5% results from 11β – hydroxylase (11β-OH) depletion (Malasa *et al.* 2006; Merke 2002; Tajima et al.1999).

Cortisol production begins in the second month of foetal life. Inefficient cortisol production results in rising levels of ACTH, which in turn induces overgrowth and overactivity of the steroid-producing cells of the adrenal cortex. (White & Speiser 2000).

The conventional treatment of CAH consists of replacing both glucocorticoids and mineralocorticoids to reduce hyperplasia and overproduction of ACTH and adrenal androgens. Excessive hyperandrogenism, though proper hydrocortisone supplementation is a frequent clinical problem in girls with CAH, especially during puberty. At physiological doses, hydrocortisone prevents adrenal insufficiency, but does not inhibit ACTH and androgens production, and frequently higher doses of it are necessary to obtain adequate suppression in these subjects. Such doses expose patients to excessive levels of glucocorticoids causing iatrogenic hypercortisolism (Merke 2002; Tajima *et al.* 1999).

It is suggested, that this phenomenon is a result of foetal autonomization of adrenal glands in CAH (Tajima *et al.* 1999). Human females with this condition are exposed prenatally to higher than normal levels of adrenal androgens. Since Brown et al (2002) raised the hypothesis that prenatal androgen exposure reduces the 2D:4D ratio, therefore low values of this parameter may be the indicator of high expression of androgen-responsive genes (Manning *et al.* 2002).

The aim of the study was to establish the relationship between the level of androgenization *in utero* determined by 2D:4D ratio and serum androgen concentrations in girls with CAH due to 21-OH deficiency.

MATERIAL AND METHODS

The study was performed on 19 girls with CAH due to 21-OH deficiency at the age of 3.7–19 years (mean 13.8 ± 4.07 years) treated at the Paediatric Endocrinology Unit (Department of Paediatrics in Zabrze, Medical University of Silesia in Katowice, Poland). All of them received proper hydrocortisone supplementation at a dosage of 10–19 mg/m^2 (mean 13.81 ± 4.07 mg/m^2). Anthropometric measurements of II and IV digit's lengths of the left hand using vernier calipers were performed in all girls on current X-rays obtained for standard bone age estimation (Buck *et al.* 2003). Apart from it testosterone, s-DHEA (ECLIA, Roche Diagnostics), androstenedione (RIA, Immunotech SA, France and 17-OHP (RIA, IBL-International, Germany) serum concentrations were assayed. These procedures are a part of routine medical care in patients with CAH .

Statistical analyses were performed using the licensed version of STATISTICA 6.0 software. Mean androgens serum concentrations and mean 2D:4D ratio were expressed as means ± standard deviations (SDs). Correlations were analyzed by Pearson linear correlation. The p-value of <0.05 was considered to be statistically significant.

RESULTS

Mean androgens serum concentrations in examined group are presented in Table 1. Increased for age and sex level of serum testosterone concentration was found

in 8 patients (42%). Elevation for age and sex of serum androstenedione concentration concerned of 7 patients (36.8%). Levels of 17-OHP were typical for treated patients with 21-OH deficiency.

Mean 2D:4D ratio was 0.96 ± 0.04. Analysis of correlation showed positive linear correlations between testosterone (Figure 1) or s-DHEA (Figure 2) and 2D:4D ratio (r=0.53, p=0.023; r=0.53; p=0.019, respectively).

DISCUSSION

CAH due to 21-OH deficiency results in excessive androgen exposure in the gestational period and various degrees of masculinazation of the external genitalia in female foetuses. Intrauterine gonadal steroids are not only essential for the development of the genital organs, but also affect some other extragenital organ development. 2D:4D ratio shows a sexually dimorphic pattern with longer fourth digit from second digit in men compared to women (Okten *et al.* 2002). Fink *et al.* (2003) suggest that finger length ratio may provide a "window" into prenatal hormone exposure. Moreover, 2D:4D ratio is thought to be a better indicator of prenatal testosterone than of adult testosterone (Koehler *et al.* 2004). In the study by Berenbaum *et al.* (2006) individuals with 46,XY karyotype, but no effective prenatal androgen exposure due to complete androgen insensitivity syndrome had digit ratios that were feminized: they were higher than those of typical men and similar to those of typical women. Nevertheless, this effect was modest in size, and there was considerable within-group variability and between-group overlap (Berenbaum *et al.* 2006).

Recently Zheng and Cohn (2011) identified the molecular mechanism determining sexual dimorphism of digit ratios in mice. These authors demonstrated that androgen receptor (AR) and estrogen receptor α (ER-α) activity is higher in digit 4 than in digit 2. Inactivation of AR decreases growth of digit 4, which causes a higher 2D:4D ratio, whereas inactivation of ER-α increases growth of digit 4, which leads to a lower 2D:4D ratio. Additionally they also showed that addition of androgen has the same effect as inactivation of ER and that addition of estrogen mimics the reduction of AR. Therefore androgen and estrogen differentially regulate the network of genes that controls chondrocyte proliferation, leading to differential growth of digit 4 in males and females (Zheng & Cohn 2011).

Despite progress made in its recognition and treatment, diagnosis and management of 21-OH deficiency is still the subject of many debates and controversies (Garn *et al.* 1975, Malasa *et al.* 2006). The positive correlation between foetal androgenization and adrenal androgens concentrations in extrauterine life in the analyzed group confirms the view demonstrated in the study performed in 21-OH deficient mice (Tajima *et al.* 1999), suggesting that intrauterine glucocorticoid deficiency in CAH causes hyperactivity of the HPA axis and insensivity to glucocorticoid feedback. The dissociation between hypothalamic and pituitary inhibition

Tab. 1. Mean serum concentrations of androgens (testosterone, androstenedione, dihydroepiandrostenedione sulphate) and 17-hydroxyprogesterone in examined female patients with congenital adrenal hyperplasia (21-hydroxylase deficiency).

Parameter	Mean ± SD	Unit
testosterone	150.21 ± 155.44	ng/dl
androstenedione	4.15 ± 5.32	ng/ml
s-DHEA	70.39 ± 85.52	µg/dl
17-OHP	18.95 ± 11.77	ng/ml

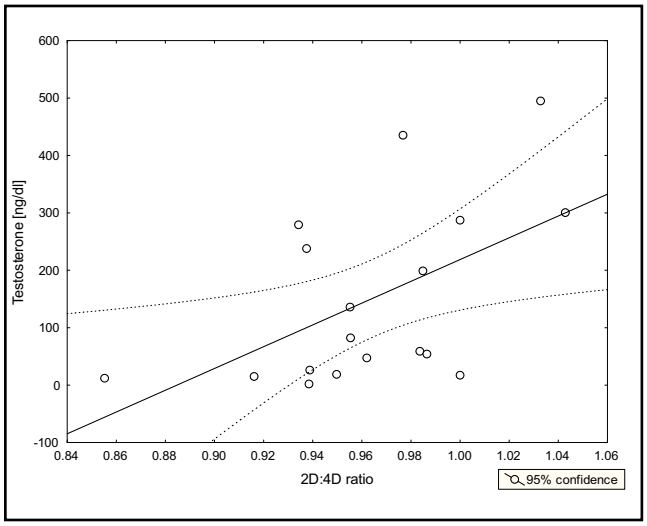

Fig. 1. Linear correlation between 2D:4D ratio and serum testosterone concentrations in examined female patients with congenital adrenal hyperplasia (n=19, r=0.53; p=0.023).

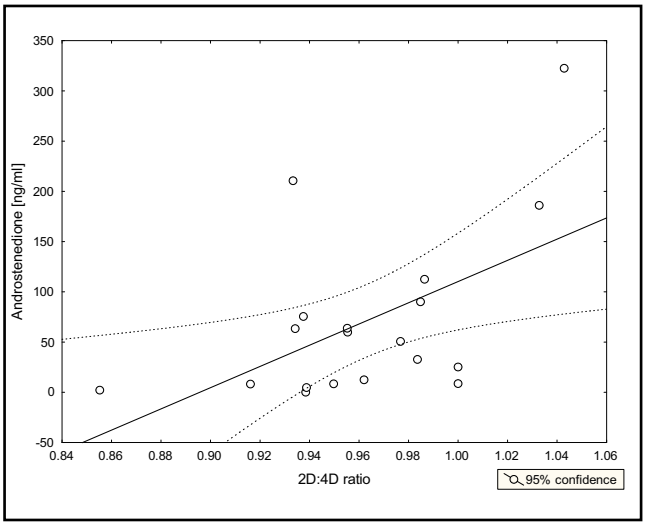

Fig. 2. Linear correlation between 2D:4D ratio and serum androstenedione concentrations in examined female patients with congenital adrenal hyperplasia (n=18, r=0.53; p=0.019).

following prenatal dexamethasone treatment revealed that ACTH production is not solely dependent on hypothalamic hyperactivity and the adrenal abnormalities in this animal model cannot be explained only by overproduction of ACTH (Tajima *et al.* 1999). Therefore, the current approach of prenatal glucocorticoid administration in humans with CAH may not be sufficient to suppress the foetal HPA axis and may provide a useful model to understand later clinical manifestations of this condition.

Glucocorticoid deficiency and excessive stimulation of the hypothalamus and pituitary concerning CRH and ACTH during foetal life may permanently impair the reactivity of pituitary glucocorticoid receptors in extrauterine life. The effect of this fact is the reduction of suppressive activity of the administered cortisol that results in elevation of ACTH and suprarenal androgens concentrations despite the proper postnatal treatment (Tajima *et al.* 1999).

Another demonstration of early developmental impairment of HPA function may by the incidence of adrenal rest tumors in patients with CAH. Ectopic adrenal rest tissue is biochemically identical to that of the adrenal cortex and can function like adrenal tissue; its function is increased by corticotrophin and decreased by high doses of glucocorticoids. These tumors embryologically originate from the mesoderm of urogenital ridge, therefore are most frequently found in the testes; however they may occur also in the celiac plexus, broad ligaments and ovaries (Merke *et al.* 2002). It is believed, that these aberrant adrenal cells having receptors for both ACTH and angiotensin II most likely vanish if not stimulated. However in CAH it is not always effective to reduce tumor size by suppressing ACTH secretion by intensifying corticosteroid supplementation and even in well-controlled CAH males with normal or suppressed plasma ACTH levels, testicular adrenal rest tumors have grown (Claahsen-van der Grinten, *et al.* 2007; Stikkelbroeck *et al.* 2004).

Standard medical treatment consists of oral glucocorticoid and mineralocorticoid administration in order to suppress adrenal androgens and to compensate for adrenal steroid deficiencies. However, available treatment is far from ideal, and not much is known about the long-term outcome in CAH as trials in patients in adulthood or old age are rare (Malasa *et al.* 2006). In our study we found a significant correlation between foetal androgenization measured by 2D:4D ratio and adrenal androgens concentrations in the extrauterine life in female patients. Thus, we conclude that this parameter may be a simple test in indentification of female CAH patients who will need more intensive follow-up during the treatment. Our observations also suggest that the autonomization of adrenal androgens production in foetal life may be the cause of excessive androgen secretion in female patients with CAH despite adequate hydrocortisone substitution therapy.

REFERENCES

1 Berenbaum SA, BRyk KK, Nowak N *et al.* (2006). Fingers as a marker of prenatal androgen exposure. Endocrinology. **150**: 5119–5124.
2 Brown WM, Hines M, Fane BA *et al.* (2002). Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. Horm Behav. **42**: 380–386.
3 Buck JJ, Williams RM, Hughes IA, Acerini CL (2003). In-utero androgen exposure and 2nd to 4th digit length ratio—comparisons between healthy controls and females with classical congenital adrenal hyperplasia. Hum. Reprod. **18**: 976–979.
4 Claahsen-van der Grinten HL, Otten BJ, Sweep FC *et al.* (2007). Testicular tumors in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency show functional features of adrenocortical tissue. J. Clin. Endocrinol. Metab. **92**: 3674–3680.
5 Fink B, Neave N, Manning JT (2003). Second to fourth digit ratio, body mass index, waist-to-hip ratio, and waist-to-chest ratio: their relationships in heterosexual men and women. Ann Hum Biol. **30**: 728–738.
6 Fink B, Manning JT, Neave N *et al.* (2004). Second to fourth digit ratio and hand skill in Austrian children. Biol Psychol. **67**: 375–384.
7 Firman RC, Simmons LW, Cummins JM *et al.* (2003). Are body fluctuating asymmetry and the ratio of 2nd to 4th digit length reliable predictors of semen quality? Human Reproduction. **18**: 808–812.
8 Galis F, Ten Broek CMA, Van Dongen S *et al.* (2010). Sexual Dimorphism in the Prenatal Digit Ratio (2D:4D). Arch Sex Behav. **39**: 57–62.
9 Garn SM, Burdi AR, Babler WJ *et al.* (1975). Early prenatal attainment of adult metacarpal-phalangeal rankings and proportions. Am J Phys Anthropol. **43**:327–332.
10 Koehler N, Simmons LW, Rhodes G (2004). How well does second-to-fourth-digit ratio in hands correlate with other indications of masculinity in males? Proc Biol Sci. **271**(Suppl 5): S296–S298.
11 Lutchmaya S, Baron-Cohen S, Raggatt P, Knickmeyer R, Manning JT (2004) 2nd to 4th digit ratios, fetal testosterone and estradiol. Early Hum Dev. **77**: 23–28.
12 Malasa MA, Doganb S, Evcila EH *et al.* (2006). Fetal development of the hand, digits and digit ratio (2D:4D). Early Human Development . **82** : 469–475.
13 Manning JT, Bundred PE (2000). The ratio of 2nd to 4th digit length: a new predictor of disease predisposition? Med Hypotheses. **54**: 855–857.
14 Manning JT, Baron-Cohen S, Wheelwright S *et al.* (2001). The 2nd to 4th digit ratio and autism. Dev Med Child Neurol. **43**: 160–164.
15 Manning JT, Bundred PE, Flanagan BF (2002). The ratio of 2nd to 4th digit length: a proxy for transactivation activity of the androgen receptor gene? Med Hypotheses. **59**: 334–336.
16 Merke DP (2002). Future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Ann Intern Med. **136**: 320–334.
17 Neave N, Laing S, FinkB , *et al.* (2003). Second to fourth digit ratio, testosterone and perceived male dominance. Proc Biol Sci. **270**: 2167–2172.
18 Okten A, Kalyoncu M, Yaris N (2002). The ratio of second- and fourth- digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Early Hum Dev. **70**: 47–54.
19 Ronalds G, Phillips DI, Godfrey KM *et al.* (2002). The ratio of second to fourth digit lengths: a marker of impaired fetal growth? Early Hum Dev. **68**: 21–26.
20 Stikkelbroeck NM, Hermus AR, Suliman HM *et al.* (2004). Asymptomatic testicular adrenal rest tumours in adolescent and adult males with congenital adrenal hyperplasia: basal and follow-up investigation after 2.6 years. J. Pediatr. Endocrinol. Metab. **17**: 645–653.
21 Tajima T, Ma X-M, Bornstein SR *et al.* (1999). Prenatal dexamethasone treatment does not prevent alterations of the hypothalamic pituitary adrenal axis in steroid 21-hydroxylase deficient mice. Endocrinology. **140**: 3354–3362.

22 Walder DJ, Andersson TL, McMillan AL *et al.* (2006). Sex differences in digit ratio (2D:4D) are disrupted in adolescents with schizotypal personality disorder: Altered prenatal gonadal hormone levels as a risk factor. Schizopr Res. **86**: 118–122.
23 White PC, Speiser PW (2000). Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr. Rev. **21**: 245–291.
24 Wood S, Vang E, Manning J, Walton J, Troup S, Kingsland C, Lewis-Jones ID (2003). The ratio of second to fourth digit length in azoospermic males undergoing surgical sperm retrieval: predictive value for sperm retrieval and on subsequent fertilization and pregnancy rates in IVF/ICSI cycles. Journal of Andrology. **24**: 1–7.
25 Zheng Z, Cohn MJ 92011). Developmental basis of sexually dimorphic digit ratios. Proc Natl Acad Sci USA. **108**: 16289–16294.