DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numbers.

I Liivak K, Tobi S, Schlecht H, Tillmann V. Incidence of classical 21-hydroxylase deficiency and distribution of *CYP21A2* mutations in Estonia. Horm Res 2008;69:227–232.

II Liivak K, Foster PJ, Thalange N, Tillmann V. Short-Term Growth in Children with Congenital Adrenal Hyperplasia. Horm Res 2009;71:142–147.

III Liivak K, Tillmann V. 24-hour blood pressure profiles in children with congenital adrenal hyperplasia on two different hydrocortisone treatment regimens. Accepted for publication in the Journal of Pediatric Endocrinology and Metabolism on 9 November, 2008

Degree applicant's contribution to the preparation of the publications:

Paper I: Identification and recruitment of patients, blood sample collection, clinical data collection, data analysis, writing of the paper.

Paper II: Design of the study, recruitment of patients and their parents, education of parents, control of data collection by parents, data analysis, writing of the paper.

Paper III: Design of the study, recruitment of patients, data collection, data analysis, writing of the paper.

ABBREVIATIONS

11β-HSD2	11 β-hydroxysteroid dehydrogenase-2
17-OHP	17-hyproxyprogesteron
21-OHD	21-hydroxylase deficiency
3β-HSD	3β-hydroxysteroid dehydrogenase
8bp	8. base pare
ACTH	Adrenocorticotropic hormone
Arg	Arginine
ARMS	Amplification refractory mutation system
Asn	Asparagine
AUC	Area under curve
BA	Bone age
BMI	Body mass index
CA	Chronological age
CAH	Congenital adrenal hyperplasia
cAMP	cyclic adenosine monophosphate
CRH	Corticotropin releasing hormone
CYP21A1P	Cytochrome P450, family 21, subfamilyA, polypeptide 1 pseudogene
CYP21A2	Cytochrome P450, family 21, subfamilyA, polypeptid
dBP	Diastolic blood pressure
Del	Deletion
DHEA	Dehydroepiandrostenedion
DHEA-S	Dehydroepiandrostenedion-sulphate
DNA	Deoxyribonucleic acid
ESPE	European Society for Paediatric Endocrinology
FC	Fludrocortisone
GH	Growth hormone
Gln	Glutamine
GnRH	Gonadotropin releasing hormone
HC	Hydrocortisone
HLA	Human leukocyte antigen
HPA	Hypotalamic-pituitary adrenal
IL	Interleukin
Ile	Isoleucine
In2	Intron 2
IUGR	Intrauterine growth retardation
Leu	Leucine
LWPES	Lawson Wilkins Pediatric Endocrine Society
NC	Non-classic
PCR	Polymerase chain reaction
PRA	Plasma renin activity
Pro	Proline

SAS	Statistical analysis software
sBP	Systolic blood pressure
SD	Standard deviation
SV	Simple virilising
SW	Salt wasting
TNF	Tumor necrosis factor
Trp	Tryptophan
UK	United Kingdom
Val	Valine

1. INTRODUCTION

Congenital adrenal hyperplasia (CAH) arises from inherited defects in enzymes that are necessary for cortisol biosynthesis. In more than 90% of CAH cases, the defective enzyme is 21-hydroxylase. The disorder is characterised by cortisol deficiency, with or without aldosterone deficiency and androgen excess. The biosynthesis of all adrenal steroids is regulated by negative feedback, but among all of the steroid hormones produced by the adrenals, cortisol is the only one to exert significant feedback control on ACTH secretion. Accordingly, when cortisol secretion is insufficient, for whatever reason, the feedback loop opens and the ACTH rises (Forest 2004).

The quality of life of 21-OHD patients has improved markedly since cortisone treatment was introduced in 1951 (Crumbach *et al.* 1952). However, the available 21-OHD therapy does not achieve fully normal growth and puberty to the extent that it can effectively suppress hyperandrogenism without iatrogenic Cushing's syndrome (Riepe *et al.* 2007). Recent studies have shown that despite advances in the treatment and knowledge of the disease, many issues are still unresolved, including short adult height, infertility, adrenomedullary insufficiency, obesity and cardiovascular risk factors.

The incidence of 21-OHD has been studied in many regions, including Estonia's neighbouring countries, Finland and Sweden (Jääskelainen *et al.* 1997-a, Thilen *et al.* 1998). There has been no information about the incidence of 21-OHD in Estonia.

2. REVIEW OF LITERATURE

2.1. Definition and historical background

Congenital adrenal hyperplasia (CAH) is a group of inherited syndromes caused by deficient adrenal corticosteroid biosynthesis. Ninety percent of cases of CAH are due to 21-hydroxylase deficiency (21-OHD) (White and Speiser 2000). 21-OHD can be classified according to symptoms, signs and age of presentation into 'classical' and 'non-classical' forms. The classical type includes a severe salt-wasting (SW) form, which usually presents with acute adrenal crisis in early infancy, and a simple virilising (SV) form in which patients demonstrate masculinisation of external genitalia in females or virilisation in early life in males. The non-classic (NC) or 'late onset' form presents in females with signs and symptoms of mild androgen excess at or around the time of puberty. The other, less common, causes of CAH are 11β-hydroxylase, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase and StAR deficiency (lipoid CAH).

CAH was first described in 1865 by Neapolitan anatomist Luigi De Grecchio, who made an autopsy in a 40-year-old male (De Grecchio 1865). The cadaver had a beard, developed muscles, male-type pubic hair and penile length of 6 cm. De Grecchio first discovered uretral hypospadia and undescended testes, and upon further investigation he also found an uterus, ovaries and adrenal hyperplasia. The patient was described as a girl at birth but the sex was changed to male four years later. In adult life the patient considered himself a male both sexually and socially. The cause of death was acute illness with vomiting, diarrhoea and exhaustion. This case, from more than 140 years ago, accurately describes the clinical symptoms for CAH that are known today (New and Wilson 1999).

In 1950 Lawson Wilkins developed a theory that hyperplasia and over-production of adrenal androgens resulted from an impaired capacity to produce cortisol (Crumbach *et al.* 1952). He also suggested that such patients should be treated with corticosteroids. A more through understanding of the disease continued to develop until the second half of the twentieth century, when hormonal abnormalities and the recessive nature of the genetic traits of CAH were discovered (Bongiovanni *et al.* 1963). A clinical picture of the different forms of CAH was described exhaustively in the same period by Prader (1962) and Bongiovanni *et al.* (1963).

2.2. Normal physiology of adrenal cortex

2.2.1 Anatomy of adrenal cortex

The adrenal cortex consists of three anatomically distinct zones:

a) The outer zona glomerulosa, where mineralocorticoids (aldosterone) are produced. The production is regulated by angiotensin II, potassium and ACTH.
b) The central zona fasciculata, which is responsible for glucocorticoid synthesis. This zone is regulated by ACTH, cateholamines and several cytokines (IL-1, IL-6, TNF).
c) The inner zona reticularis is a site of adrenal androgen [dehydroepiandrostenedion (DHEA), DHEA-sulphate and anrostenedione] and some glucocorticoid secretion.

2.2.2. Normal steroid hormone synthesis

The main pathway of cortisol, aldosterone and testosterone synthesis is shown in Figure 1. All adrenal corticosteroids are synthesised from cholesterol through a series of enzyme-mediated transformations. Cholesterol in the adrenal tissue may be synthesised in situ from acetate or it may come from cholesterol produced in the liver and transported to the adrenal glands by low-density lipoprotein. Several of the reactions present in steroidogenesis involve cytochrome P-450 enzymes. The rate-limiting step in the synthesis of all steroids is the conversion of cholesterol to pregnenolone. This step is stimulated by ACTH in the zona fasciculata and the zona reticularis and by angiotensin II and III in the zona glomerulosa. The pathway leading to progesterone is common to both aldosterone and cortisol synthesis. In the zona reticularis and the zona fasciculata, progesterone is hydroxylated at the 17, 21, and 11 positions in order to form cortisol. Under normal circumstances, about 6 mg of cortisol per m^2 is synthesised per day (Linder *et al.* 1990, Kerrigan *et al.* 1993). The zona glomerulosa does not contain 17-hydroxylase activity; instead, hydroxylation occurs at positions 21, 11, and 18, and finally, aldosterone is formed by a dehydrogenase reaction.

The major androgens secreted by the adrenals are dehydroepiandrosterone (DHEA), DHEA sulphate (DHEA-S), and androstenedione (Lonchope 1986). Production of testosterone by these glands is minimal. DHEA and DHEA-S are mainly products of the zona reticularis, whereas androstenedione and testosterone are secreted by both the zona reticularis and the zona fasciculata (McKerns 1969).

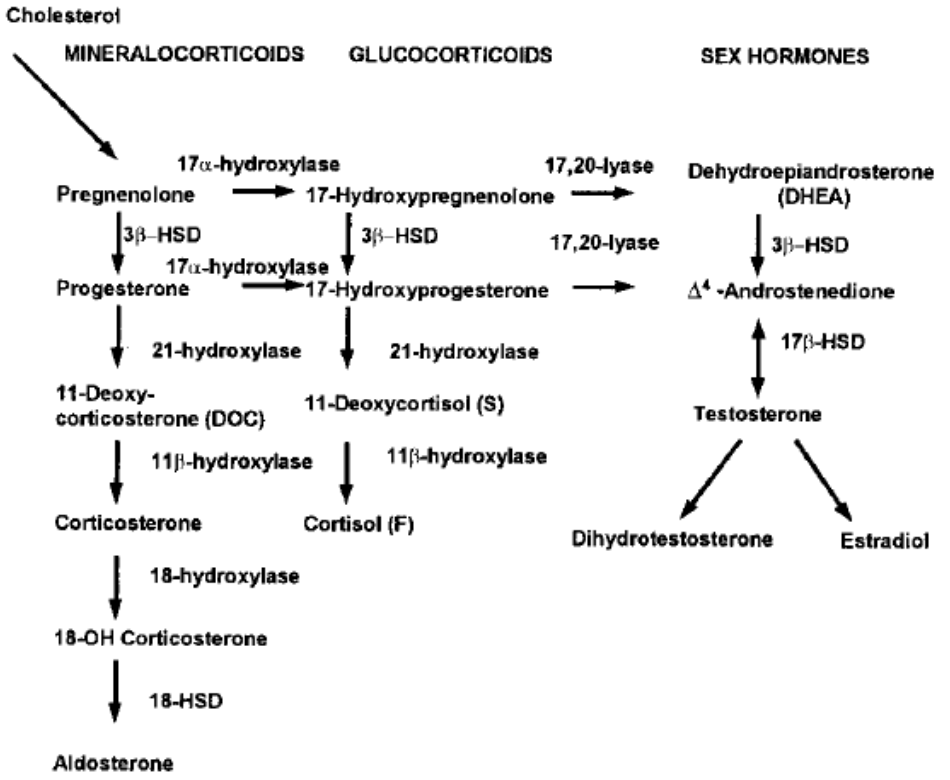

Figure 1. Pathways of steroid biosynthesis in the adrenal cortex

2.2.3. Regulation of steroidogenesis

2.2.3.1. Glucocorticoid secretion

Cortisol secretion is regulated mainly by ACTH, a peptide that is produced in the anterior pituitary, which also influences the remaining steps in steroidogenesis as well as the uptake of cholesterol from plasma lipoproteins. ACTH also maintains the size of the adrenal glands and stimulates melanocytes. This results in hyperpigmentation when secreted excessively, as occurs in Addison's disease.

Corticotropin releasing hormone (CRH) is the principal hypothalamic factor that stimulates the pituitary production of ACTH. Vasopressin, a peptide product in the posterior pituitary gland, also stimulates ACTH release by acting synergistically with CRH, and is an important physiological regulator of ACTH (Itoi 1998). CRH, which is produced in the paraventricular nuclei of the hypothalamus, activates ACTH secretion via a specific receptor coupled to cAMP-dependent signalling. CRH is secreted in a pulsatile fashion, which

results in the episodic secretion of ACTH and in the diurnal variation of cortisol secretion. The magnitude of the cortisol response to each ACTH burst remains relatively constant and, therefore, the total daily cortisol secretion is determined by the number of secretory periods, rather than the magnitude of each CRH and ACTH pulse (White and Speiser 2000).

Cortisol is the primary negative regulator of resting activity of the hypothalamic-pituitary-adrenal (HPA) axis through negative feedback on ACTH and CRH secretion.

2.2.3.2. Mineralocorticoid secretion

Mineralocorticoid secretion is regulated by the renin-angiotensin system (Hardman 1984). The primary stimuli for renin release include hypotension, sodium depletion, reduced intravascular volume and β-adrenergic stimulation. Renin enzymatically attacks its substrate angiotensin, thereby releasing angiotensin I. Cleavage by an angiotensin-converting enzyme forms angiotensin II, and this peptide plays a key role in the restoration of normal blood pressure by causing arteriolar vasoconstriction and stimulating aldosterone elaboration (Kramer *et al.* 1980). Aldosterone synthesis also increases as a result of falling sodium and rising potassium levels (McKenna *et al.* 1978, Farese *et al.* 1983).

2.2.3.3. Adrenal androgen secretion

The adrenal androgens (DHEA, DHEAS, androstenedione) are secreted in the zona reticularis from pregnenolone derived from side chain cleavage of cholesterol by the cytochrome P450scc enzyme. 17α-hydroxylase with 17,20-hydroxylase activity converts pregnenolone to DHEA, which can be converted to androstenedione by 3β-hydroxysteroid dehydrogenase (3β-HSD) or to androstenediol (in gonads) by 17β–hydroxysteroid oxioreductase. The latter enzyme can also convert androstenedione to testosterone (Parker 1991). Adrenal androgen production follows a characteristic age-related pattern. The foetal adrenal androgen synthesis is robust but remains negligible after the first year of life, during which a decrease occurs in the DHEAS level, while cortisol level remains constant (De Peretti and Forest 1978). The zona reticularis does not appear until three years of life and after that, during a peripubertal stage, elevation of adrenal androgens (adrenarche) occurs, which contributes to the development of pubic and axillary hair (Alesci *et al.* 2001). The secretion of GnRH at puberty activates the hypotalamo-pituitary-adrenal axis, causing an increase in testosterone and estrodiol levels in boys and girls, respectively. Around puberty there is also an increase in DHEA, DHEAS and androstenedione secretion, which continues through the third decade of life (Adams 1985, Parker 1991).

2.2.3.4. Diurnal rhythms of ACTH and cortisol

Plasma concentrations of ACTH and cortisol peak in the morning hours and wane in the afternoon and evening. ACTH and cortisol levels start to rise between two and three a.m., with ACTH reaching its peak between four and six a.m. (Brook and Hindmarsh 2001) and cortisol at around eight a.m. (Newell-Price *et al.* 2008). Both are released in pulses that are approximately 30–120 minutes apart. The amplitude and frequency of the cortisol or ACTH pulses are maximal in the morning hours. This is regulated by several factors, including a rhythmicity of CRH secretion, which itself has a diurnal pattern with a peak at four a.m. (Brook and Hindmarsh 2001). The other important factors in ACTH and cortisol circadian rhythmicity are feeding cycles (Follenius *et al.* 1982), light-dark cycles (Quigley *et al.* 1979, Wallace *et al.* 1991) and the inherent rhythmicity of the adrenal (Moore-Ede *et al.* 1983). It has also been shown that ACTH and cortisol release are stimulated by stress (Dempsher *et al.* 1983, Udelsman *et al.* 1987, Berkenbosh *et al.* 1989).

2.3. Patophysiology of 21-OHD

Patients with 21-OHD are unable to adequately synthesise cortisol and, therefore, the adrenal cortex is stimulated by CRH and ACTH, resulting in hyperplastic adrenal glands. This leads to the excessive synthesis of adrenal sex-hormone precursors and their by-products (Forest 2004). The aldosterone and cortisol synthesis is compromised because of the insufficient enzymatic conversion of progesterone to 11-deoxycortisone and 17-OHP to 11-deoxycortisol, respectively (see Figure 1). These conversions are catalysed by the enzyme 21-hydroxylase, which is primarily expressed in the adrenal cortex (Riepe *et al.* 2007). Studies have also shown, however, that 21-hydoxylase is also expressed in epidermis (Slominski *et al.* 1996), lymphocytes (Zhou *et al.* 1997), hippocampus (Beyenburg *et al.* 2001) and skin-keratocytes (Rogoff *et al.* 2001). The most characteristic biochemical marker in patients with 21-OHD is elevated serum17-hydroxyprogesterone (17-OHP) concentration. The highest levels of 17-OHP are observed in patients with the SW form of CAH, whereas patients with the SV form tend to show slightly lower levels. The medulla function of adrenals is also affected, which results in catecholamine, mainly epinephrine, deficiency (Merke *et al.* 2000). Along with hyperandrogenism, this can lead to various metabolic disturbances, including hyperinsulinism and hyperleptinemia (Weise *et al.* 2004).

2.4. Clinical picture in 21-OHD

2.4.1. Salt wasting 21-OHD.

Approximately 75% of patients with 21-OHD cannot adequately synthesise aldosterone. Elevated 21-hydroxylase precursors – progesterone and 17-OHP – may act as mineralocorticoid antagonists, exacerbating the effects of aldosterone deficiency (Oelkers *et al.* 1996). Renal salt wasting, together with fluid volume depletion in aldosterone deficiency, causes low sodium and high potassium concentration in serum, and high plasma renin activity (PRA) levels. Cortisol deficiency in these patients contributes to poor cardiac function, poor vascular response to catecholamines and increased secretion of antidiuretic hormone (Lamberts *et al.* 1997, Speiser and White 2003). Together with aldosterone deficiency, this may manifest at the age of 1–4 weeks old, thereby threatening an adrenal crisis characterised by vomiting, lethargy, weight loss and shock with hypotension, hyperkalaemia and hyponatremia (Forest 2004, Merke and Bornstein 2005).

The secretion of an excessive amount of androgens does not significantly influence male sexual differentiation during the prenatal period. In females, however, an excess of androgens can lead to prenatal virilisation, with ambiguous male external genitalia appearing at birth. This includes clitoral enlargement, fused labia major and a urogenital sinus. Ambiguity can be estimated on the five-point scale developed by Prader and Gutner (1955). Boys do not usually have changes in external genitalia except variable hyperpigmentation and, in some cases, penile enlargement.

Postnatal patients with the SW form exhibit signs of progressive virilism, rapid somatic growth and advanced bone age. The mean adult height in patients with SW remains about 1.4 standard deviation (SD) below the population mean (Eugster *et al.* 2001).

2.4.2. Simple virilising 21-0HD

Although cortisol synthesis is impaired in patients with SV form cortisol synthesis, they do have adequate aldosterone secretion and sodium balance is maintained. The clinical picture is due to androgen excess in pre- and post-natal periods. The diagnosis of a newborn female is usually made soon after birth because of the apparent genital ambiguity, but the early detection of newborn males is largely dependent on screening (Speiser and White 2003). Genitalia may continue to virilise post-natally due to an excess of adrenal androgens, and pseudoprecocious puberty can occur (Forest 2004). Signs of hyperandrogenism include facial, axillary and pubic hair, adult body odour, temporal balding and severe acne. The high androgen levels can also accelerate growth in early childhood followed by premature maturation of epiphyseal plates. In patients

with the SV form this can also lead to impaired final height (Van der Kamp *et al.* 2002). The mean final height corrected to target height SDS in patients with the SV form was –1.51 in males and –0.96 in females. The authors concluded that in the SV form the loss of final height potential was caused by the delay in diagnosis.

2.4.3. Non-classic 21-OHD

Patients with NC 21-OHD are born with normal genitalia and do not have cortisol deficiency (Lebovitz *et al.* 1984, Merke 2008), but in late childhood or early adulthood they have manifestations of hyperandrogenism. The most common symptoms are premature pubarche in children, severe acne, hirsutism and oligomenorrhea in teenage girls and young women (Sklar *et al.* 1980, Marnyck *et al.* 1983, Balduccy *et al.* 1994, Moran *et al.* 2000). Precocious pseudopuberty can also occur in both sexes but it is relatively rare (Forest 1996). Oligospermia has been found in males in some cases (New and Wilson 1999). Short adult stature, insulin resistance, severe cystic acne, and reduced fertility are seen more commonly in patients with the NC form, both females and males (New and Wilson 1999). However, studies by New *et al.* (1988) and Cameron *et al.* (1996) found that the adult height in patients with the NC form was not impaired.

2.4.4. Diagnosis of 21-OHD

The diagnosis of 21-OHD is based on a clinical picture and elevated serum 17-OHP levels. In addition, an ultrasound investigation of the pelvis and a rapid karyotype should be conducted (Clayton *et al.* 2002). An elevated serum 17-OHP level above the age-dependent reference values confirms the diagnosis of 21-OHD. Particular attention should be paid to premature babies in which the normal 17-OHP values are higher than those in full-term babies (Allen *et al.* 1996). Accordingly, premature newborns may need serial measurements of 17-OHP to differentiate false positive from affected infants (al Saedi *et al.* 1996).

In borderline cases a corticotropin stimulation test (Synachten test) and genetic analysis should be used to confirm the diagnosis (Clayton *et al.* 2002). Plasma renin activity (PRA) is markedly elevated in patients with the SW form, but in some cases it could also be higher in patients with the SV form (Nimkarn *et al.* 2007). Serum concentrations of androstenedione and progesterone are also elevated in patients with 21-OHD.

2.4.5. Newborn screening

Despite many contrarguments, newborn screening for CAH is recommended by the LWPES/ESPE working group (Clayton *et al.* 2002). Sampling of blood spots for 17-OHP in the first 48–72 hours of life helps to identify both male and female patients, prevents incorrect sex assignment and decreases mortality and morbidity (Pass *et al.* 2000, ESPE neonatal screening working group 2001, Therrell *et al.* 2001). The other potential benefits of newborn screening for CAH are related to improved final height, in both classical and non-classical forms (New *et al.* 2004). However, attention should be paid to the evaluation of 17-OHP screening results, as false positive results are common in premature, sick or stressed infants who show a tendency to have higher 17-OHP values (al Saedi *et al.* 1996, Allen *et al.* 1997). Accordingly, it has been suggested that weight (Balsamo *et al.* 1996, Allen *et al.* 1997) or gestational age (Gruneiro-Papendieck *et al.* 2001, Steigert *et al.* 2002) adjusted cut-off levels for 17-OHP should be used.

2.4.6. Treatment

2.4.6.1. Glucocorticoid replacement

All patients with classic 21-OHD and symptomatic patients with the non-classical disease should be treated with glucocorticoids (Speiser and White 2003). This suppresses the excessive secretion of CRH and ACTH in hypothalamus and pituitary and reduces the production of adrenal sex steroids. Despite more than 50 years of experience of treatment with glucocorticoids, the management of the disease is still controversial and the outcome is not always as predicted. Hydrocortisone is considered a drug of choice in children but, due to its short half-life, the doses must be given at least three times a day. According to an LWPES/ESPE consensus statement (Clayton *et al.* 2002), the optimal dosage of hydrocortisone is between 10 and 15 mg/m^2 divided three times daily. During infancy, patients may require doses of over 25 mg/m^2 per day because of the markedly elevated sex hormones (Clayton *et al.* 2002). The main goal in glucocorticoid treatment is to use the lowest dosage that can suppress adrenal androgens and maintain normal growth and weight gain. Older adolescents and adults may be treated with prednisolone (2–4 mg/m^2/day), divided twice daily, or dexamethasone (0.25–0.375mg/m^2/day), given once daily (Clayton *et al.* 2002). The monitoring of serum 17-OHP levels plays an important role in the management of CAH. The blood samples of 17-OHP should be taken early in the morning before medication. 17-OHP can also be measured from blood spots from filter paper. This allows parents to take blood samples at home at different time points of the day (usually three times per day) and post them to the laboratory.

Treatment with glucocorticoids can have many side effects. The biggest threat is growth suppression due to overtreatment, especially in infancy, which may have a negative effect on final adult height (Jääskelainen and Voutilainen 1997-b). Increased body mass index (BMI) is also a marker of overtreatment (Yu and Grant 1995, Jääskelainen and Voutilainen 1997-b). Treatment with doses that are too low raises the adrenal androgens, which is manifested in the long-term in the early maturation of epiphyseal growth plates and short final height (Manoli *et al.* 2002). If the patients are treated with prednisolone or dexamethasone, they must be carefully monitored for signs of iatrogenic Cushing syndrome (White and Speiser 2000).

Because cortisol levels increase during stress, patients need additional doses of glucocorticoids during febrile illness, vomiting, after trauma or before surgery (Clayton *et al.* 2002). The stress dosing is two to three times that of usual maintenance therapy. When a patient is unable to take medicines orally (vomiting, surgery), hydrocortisone should be given rectally, intramuscularly or intravenously (Clayton *et al.* 2002).

Some studies have looked at the impact of different glucocorticoid regimens on 24-hour cortisol and 17-hydroxyprogesterone (17-OHP) profiles (Winterer *et al.* 1985, Plat *et al.* 1999, Charmandari *et al.* 2001-a, Charmandary *et al.* 2001-b). Most of these studies have found that a higher initial glucocorticoid dose in the morning provides better control of CAH, whereas some authors (van der Kamp *et al.* 2002, Rosenfield 2002) prefer to use the reversed pattern, i.e. administering the largest glucocorticoid fraction at bedtime. A study by Ross *et al.* (2005) revealed that 60% of clinicians used a reversed pattern of glucocorticoid treatment among adult patients in 30 UK teaching centres. The main goal of glucocorticoid replacement therapy is to mimic diurnal cortisol rhythms and to normalise the function of the hypothalamo-pituitary-adrenal axis. However, this is very difficult to achieve in practice since physiological serum ACTH and cortisol maximum levels occur between around four and eight a.m., and current treatment regimens cannot provide this.

2.4.6.2. Mineralocorticoid replacement

Patients with the SW form require mineralocorticoid replacement and, in infancy, additional sodium replacement therapy is recommended. According to an LWPES/ESPE consensus statement (Clayton *et al.* 2002), patients in early infancy require between 0.05 and 0.3 mg of fludrocortisone daily, but the typical maintenance dose is between 0.05 and 0.2 mg daily, depending on the sodium intake. Plasma renin activity level and blood pressure should be monitored during the mineralocorticoid therapy. In addition, infants should receive an extra salt supplement, between one and two grams of sodium chloride per day (Clayton *et al.* 2002, Merke *et al.* 2005). Older children have

usually acquired the taste of saltier food and do not require additional supplements of sodium chloride tablets.

2.4.6.3. Other therapeutic approaches

When conventional therapy fails, patients with 21-OHD may need laparoscopic adrenalectomy to suppress adrenal androgens (Zachmann *et al.* 1984, Van Wyk and Ritzen 2003). However, the risk of surgery and anesthesia is too high to make this part of conventional therapy.

There are also some studies regarding the use of growth hormone (GH) together with gonadotropin releasing hormone (GnRH) analogue in the treatment of CAH (Quintos *et al.* 2001, Lin-Su *et al.* 2005). The final height of children with CAH who were treated with growth hormone and GnRH analogue was significantly higher (–0.4 SDS) than in non-treated patients (–1.5 SDS) (Lin.Su *et al.* 2005).

2.5. Epidemiology

The majority of studies regarding the incidence of CAH are based on the classical forms of CAH diagnosed either by neonatal screening or clinically (Table 1). The study performed in three US states (Brosnan *et al.*1999) showed that the incidence of classic CAH was not statistically significant between the screened and the unscreened groups collected simultaneously over a five-year period.

The data from 6.5 million newborns screened worldwide showed that the average incidence of classic CAH was one per 15,000 live births (Pang and Clark1993, Therell *et al.* 2001). The incidence of CAH shows high variability according to ethnicity and geographical area. The highest incidences have been reported in two population groups: among Alaskan Yupic Eskimos (1:280) (Pang *et al.* 1982) and on the French island of La Reunion (1:2100) (Pang *et al.* 1988). The lowest incidences have been reported in New Zealand (1:23,000) (Cutfield 1995) and among African-Americans in the United States (1:42,000) (Therrell *et al.* 1998).

These studies are based on neonatal screening programmes and, therefore, do not account for cases of the NC form. However, some cases of the NC form of CAH can be detected by neonatal screening. The estimated rate of detection of NC 21-OHD cases in neonatal screening was 1:1,100,000 in Japan (Tajima *et al.* 1997) and 1:333,000 in Switzerland (Steigert *et al.* 2002).

Table 1. Incidence of classical form of CAH diagnosed either by neonatal screening[1] or clinically[2] in various countries

Region	Incidence	Reference
Sweden	1:9800	Thilen *et al.*1998[1]
Finland	1:15,000	Jääskelainen *et al.* 1997–a[2]
New Zealand	1:23,300	Cutfield 1995[1]
Japan	1:18,000	Tajima *et al.*1997[1]
France	1:13,000	Cartigny-Maciewski *et al.*1999[1]
Switzerland	1:11,000	Steigert *et al.*2002[1]
Italy	1:21,300	Cavarzere *et al.* 2005[1]
Germany	1:11,200	Olgemöller *et al.* 2003[1]
USA, Texas	1:16,000	Therrell *et al.*1998[1]
USA, Arkansas and Oklahoma	1:17,300	Brosnan *et al.* 1999[2]
USA, Wisconsin	1:11,000	Allen *et al.* 1997[1]
Romania	1:14,300	Grigorescu-Sido *et al.* 2005[2]
Scotland	1:17,000	Pang 1988[1]

The frequency of NC CAH is considered to be higher than in classic forms. The prevalence of NC CAH in the white population living in New York City was 1:100 (Zerah *et al.* 1990). These cases were found by early morning salivary 17-OHP screening in a randomly selected study group. In Ashkenazic Jews of Eastern European origin living in New York City, the NC form has been reported as high as 1:27 (Speiser *et al.* 1985). The other New York populations with a high prevalence of the NC form are Hispanics (1:40), Yugoslavians (1:50) and Italians (1:300) (Speiser *et al.* 1985, Sherman *et al.* 1988).

2.6. Genetics

2.6.1. Inheritance and gene locus

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is inherited as an autosomal recessive trait that is closely linked to the HLA class 3 complex (Pollac *et al.* 1979). Before *CYP21A2* genotyping, HLA markers were major tools in CAH prenatal diagnostics (White and New 1984). The genes encoding human *CYP21A2* and a pseudogene, *CYP21A1P* are located on chromosome 6p21.3 (Figure 2). The gene structure consists of 10 exons spread over 3.4 kb. The short arm of chromosome 6 also includes the major histocompatibility complex with genes crucial for conferring self versus non-self immunologic recognition, as well as genes causing dyslexia, celiac disease, hemachromatosis, and susceptibility to type 1 diabetes mellitus, among others (Speiser 2005). The two 21-hydroxylase genes are arranged in tandem with two highly homologous genes for the fourth component of serum complement (*C4A*, *C4B*).

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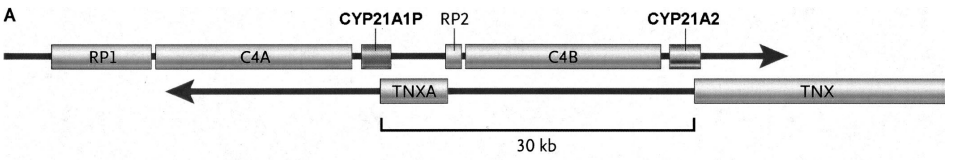

Figure 2. The chromosomal region of 6p21.3 containing the 21-hydroxylase genes

2.6.2. Mutations

The active *CYP21A2* and pseudogene *CYP21A1P* share almost 98% of nucleotidide sequences. Most of the common mutations in CAH patients are caused by two types of recombinations between *CYP21A2* and *CYP21A1P*. The most frequent type of mutation is a gene conversion in which the pseudogene sequence is transferred into *CYP21A2* (Harada *et al.* 1987). These are mostly point mutations or nucleotide sequence changes, but some chromosomes do undergo large gene rearrangements with contiguous pseudogene sequences. About 20% of mutations occur as a result of meiotic recombination deleting a 30-kilobase gene segment, producing a non-functional chimeric pseudogene (White *et al.* 1988). One to two percent of affected alleles are spontaneous mutations, not carried by either parent. According to the Human Gene Mutation Database there are more than 100 known mutations causing 21-OHD, but in most populations only 8–10 of them comprise 80–90% of alleles (Speiser 2005).

Figure 3 shows the position of mutations normally found in the pseudogene. Any of these mutations can be transferred to the active gene in gene conversion events.

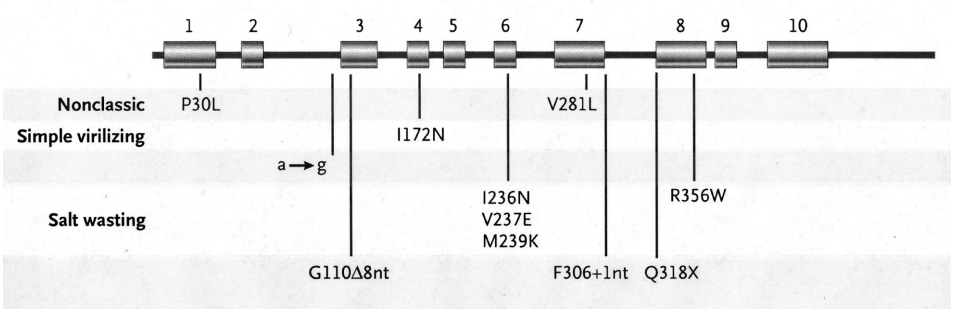

Figure 3. Mutations in steroid 21-hydroxylase causing congenital adrenal hyperplasia

2.6.3. Genotype-phenotype correlations

Most patients with CAH are compound heterozygotes (i.e. they have different mutations in different alleles) and the clinical phenotype is usually related to the less severely mutated allele, which leaves some residual 21-hydroxylase activity (Speiser *et al.* 1992, Wilson *et al.* 1995, Krone *et al.* 2000). Several studies have shown a close linkage between genotype and phenotype in patients with the most severe and mild forms of the disease, while this relationship is not so strong in moderately affected patients (Wilson *et al.* 1995, Jääskelainen *et al.* 1997-a).

As with phenotype *CYP21A2* mutations can be divided into three different groups (Higashi *et al.* 1988, Wedell *et al.* 1994). This categorisation is made by predicted enzymatic activity from in vitro mutagenesis and expression studies (Amor *et al.* 1988, Tusie-Luna *et al.* 1990, Tusie-Luna *et al.* 1991). According to Wedell *et al.* (1994), the first group is associated with the SW form and is caused by large deletions or some nonsense mutations. On this occasion there is no enzyme activity. The second group includes mutation Ile172Asn, which leaves 1–2% of the normal enzyme activity. This is still enough to adequately synthesise aldosterone, and patients with this mutation usually have the SV form of the disease. The third group consists of mutations as Val281Leu and Pro30Leu. These mild mutations produce enzymes retaining 60–70% of normal activity and are usually associated with the NC form.

There are also "leaky" splice mutations that can raise the variability of genotype-phenotype correlations. A mutation in the second intron (nucleotide 656A–>G; aka. Intron 2 splice mutation) comprises about 25% of classic 21-OHD alleles in most populations (Speiser 2005). This also leaves 1–2% of normal residual enzyme activity, and patients with this mutation usually have the SV form (Higashi *et al.* 1988, Higashi *et al.* 1991).

2.7. Growth of patients with 21-OHD

2.7.1. Long-term growth

Linear growth is severely affected in patients with 21-OHD. The following reasons were suggested as the causes of short adult height: elevated adrenal androgens that cause advanced epiphyseal maturation and premature epiphyseal fusion, early or precocious puberty, and treatment with glucocorticoids (David *et al.* 1994, Allen *et al.* 1996, Rivkees *et al.* 2000).

A meta-analysis that included data from 18 centres showed that the mean adult height in 21-OHD patients was –1.4 SD below the population mean (Eugster *et al.* 2001). Treatment during the first two years of life and puberty were the most important factors influencing adult height (Young and Hughes 1990, Rasat *et al.* 1995, Manoli *et al.* 2002). The best adult height outcome was

achieved in those diagnosed and treated early (David *et al.* 1994, Jääskelainen and Voutilainen 1997-b). Some papers have shown that final height was reduced when higher glucocorticoid doses were used during the first two years of life (New *et al.* 1988, Rasat *et al.* 1995). Recent data from Bonfig *et al.* (2007) revealed that pubertal growth was significantly decreased in patients with CAH and treatment with prednisolon during childhood resulted in short adult height. Similar results have also been found by other authors (Van der Kamp *et al.* 2002, Pinto *et al.* 2003).

The study by Bonfig *et al.* (2007) looked at the differences in growth between SW and SV forms. They revealed that patients with the SW form were slightly taller than those with the SV form, but when final height was adjusted to mid-parental height, the results were no more statistically significant.

2.7.2. Short-term growth

Short-term growth is usually defined as a growth event that occurs within one calendar year. When measurement intervals decrease, incremental patterns appear more irregularly and a number of short-scale components become apparent that are difficult to distinguish from measurement errors (Hermanussen *et al.* 1998). Most investigators agree that short-term growth is non-linear, but a concrete pattern is disputed. Many growth models have been proposed, such as the "mini growth spurts model" by Hermanussen *et al.* (1988), the "pulsatile growth model" by Greco *et al.* (1990), the "saltation and stasis model" by Lampl *et al.* (1992) and the "spurts and stasis model" by Thalange *et al.* (1996). The latter describes growth over a year as a biphasic process composed of growth spurts that last an average of eight weeks each and are separated by periods of very slow growth or stasis.

Children with growth disorders also grow in spurts and stases and the concrete pattern is dependent on ethiology. For example, growth hormone deficiency and Turner syndrome patients showed an increased time in stasis and reduced growth spurt amplitudes, while patients with intrauterine growth retardation (IUGR) had only reduced amplitude and length of growth spurts, but the time spent in stasis was similar to normal children (Tillmann *et al.* 2002).

No data exists regarding the short-term pattern of growth in children with CAH. It is not known what aspects of the growth pattern (e.g. number, size and duration of growth spurts, number and duration of stases) are affected in CAH and how treatment can affect this.

2.8. BMI and blood pressure

Long-term cardiac complications have become an important issue in managing CAH patients (Ogilvie *et al.* 2006, Sartorato *et al.* 2007). Higher BMI, body fat mass, insulin levels and blood pressure have been described in those patients (Cornean *et al.* 1998, Charmandary *et al.* 2002, Stikkelbroek *et al.* 2003, Völkl *et al.* 2006-a, Völkl *et al.* 2006-b). Obesity is a known risk factor for increased blood pressure and other cardiovascular diseases. Children with CAH have a tendency to be obese (Cornean *et al.* 1998, Völkl *et al.* 2006-a) and have decreased insulin sensitivity compared to normal children (Charmandary *et al.* 2002). Studies by Roche *et al.* (2001) and Volkl *et al.* (2006-a) have looked at blood pressure in patients with CAH. Both studies found elevated blood pressure and a lack of physiological nocturnal dip, which has also been considered as a relevant risk factor for future cardiac disease (Verdeccia *et al.* 1994). The exact mechanism of why CAH patients already have higher blood pressure in childhood is not known, but there are theories involving higher BMI (Cornean *et al.* 1998), glucocorticoid treatment (Benedikson *et al.* 1993) and impaired adrenomedullary function (Rahmouni *et al.* 2005). Glucocorticoids are also necessary for the correct functioning of the adrenal medulla, and patients with CAH have compromised function of the adrenomedullary system (Merke *et al.* 2000). There is some evidence that excessively elevated glucocorticoid levels also affect mineralocorticoid receptors by saturating the 11β-HSD2 enzyme (Dötch *et al.* 2001, Chemaitilly *et al.* 2003). However, conclusive data for substitution therapies involvement in elevated blood pressure in CAH patients is absent.

3. AIMS OF THE STUDY

1. To determine the incidence of classical form of 21-OHD in Estonia
2. To describe the phenotype and genotype in patients with classical form of 21-OHD in Estonia
3. To describe short-term growth patterns in children with CAH and to compare their growth characteristics with those of normal children
4. To evaluate the effects of two different hydrocortisone treatment regimens on 24-hour blood pressure profiles and biochemical control of the disease in children with the classical form of 21-OHD.

4. PATIENTS AND METHODS

The theses are based on three different studies:
1. Epidemiology, phenotype and genotype
2. Short-term growth
3. Twenty-four-hour blood pressure in two different hydrocortisone treatment regimens.

4.1. Study I

Patients
All members of the Estonian Endocrine Society were asked by E-mail (if no response, then by phone) to inform us about their patients with CAH. Clinical picture, time of diagnosis and elevated serum 17-OHP levels confirmed the diagnosis of classical forms of CAH in 20 patients. Age at diagnosis, clinical picture and maximum 17-OHP values at diagnosis (n=14) or later (n=6) were collected from the notes. A patient was classified as of Estonian origin, if both parents and all the grandparents considered themselves Estonian. All the other patients were studied as a group of non-Estonians, the majority of them of Slavic origin. The number of live births per year from 1978–2004 were taken from the Statistics Estonia website.

Mutation analysis
Blood samples of all 20 patients were screened for 8 common mutations using a panel of PCR ARMS tests. DNA was extracted from blood samples using the Puregene kit (Gentra Sytems Inc.) and tested for six common *CYP21A2* point mutations [p.Pro30Leu, g.655A/C>G (intron2 splice site), p.Ile172Asn, p.Val281Leu, p.Gln318X and p.Arg356Trp] using a series of ARMS PCR assays (Wedell and Luthman 1993). A further two common mutations, an eight base pair deletion in exon 3 of *CYP21A2* (g.707_714delGAGACTAC) and a large deletion resulting in the formation of a "chimeric" sequence – a fusion of 5'pseudogene and 3'functional gene *(5'CYP21A1P: 3'CYP21A2)* sequence were screened for by using two ARMS tests designed in-house at the National Genetics Reference Laboratory in Manchester, UK. Briefly, because the 5' pseudogene sequence of the "chimeric" sequence includes the exon 3 8bp deletion, the method is based on the detection of the eight-base pair deletion by ARMS primers either in the context of the pseudogene sequence (chimeric) or in isolation in the functional *CYP21A2* gene (8bp deletion). Two primary PCRs are performed in tandem using either a forward primer common to both the functional and pseudogene sequences (to detect the chimeric) (5'-GGTGCTGAACTCCAAGAGG-3'), or a primer annealing to only the functional gene (to detect 8bp del) (5'-CAGGCTGGTCTTAAATTCATA-3'). In both cases, the reverse primer binds only the 3' functional *CYP21A2* sequence and is designed to span the sequence in exon 6, which in the pseudogene contains a cluster of three point mutations (5'-CCTCAGCTGCATCTCCATGA-3').

Both secondary PCRs are identical involving ARMS primers to detect either the wild-type sequence in exon 3 (5'-AAAAAAAAAAAAGCTTTCCAGAGCAGRGACC-3') or the eight base-pair deletion (5'-CCGCTTTCCAGAGCAGRGACC-3'). R indicates an A/G: a "wobble" position in the primers. The common *CYP21A2* forward primer for the secondary ARMS reactions was 5'-TCAGTTCCCACCCTCCAGC-3' and the internal control reverse primer used to give an amplimer with the common primer of higher molecular weight to the ARMS products was 5'-CTCACAGAACTCCTGGGTC-3'). 20µl PCR reactions were carried out in Replinase buffer (1M Tris-HCl, pH9.0, 400mM ammonium sulphate, 30mM magnesium chloride) using Platinum Taq (Invitrogen) for the ARMS reactions. Reaction conditions for both primary and secondary PCRs were 94^0C, for 15 minutes, followed by either 20 cycles (primary reaction) or 30 cycles (secondary reaction) of 96°C for 12 seconds, 60°C for 1 minute, 72°C for 1 minute, followed by a final extension of 72°C, for 5 minutes. Thus a mutant band detected for the "chimeric" reaction but not 8bp reaction indicates a chimeric sequence, whereas a mutant band in both reactions indicates 8bp deletion in functional *CYP21A2* sequence. The allele frequency has been calculated from all 17 unrelated patients equalling 34 unrelated alleles. Due to insufficient DNA quality and/or quantity in the 7 unrelated patients we could not perform the Southern blot analysis to determine whether the second allele has the same mutation as on the first allele (homozygosity) or if it carries a deletion/ conversion (hemizygosity) which is not detected by our test.

Genotype groups

All patients were divided into 3 different genotype groups proposed by Wedell *et al.* (1994). Group 0 contained mutations with complete loss of enzyme activity (deletions, conversions, deletion of 8 base pair in exon 3, p.Gln318X, p.Arg356Trp). Group A contained patients who are carrying the intron 2 splice mutation (g.655A/C>G), which has been shown to result in low but measurable enzyme activity. Group B contained the p.Ile172Asn mutation, resulting in about 2% of normal enzymatic activity.

4.2. Study 2

Patients

All six prepubertal patients (2 boys and 4 girls) with SW CAH followed at the Tartu University Children's Hospital agreed to participate in the study. Five patients completed the study. In one boy 1/3 of daily measurements were missing and therefore excluded from analysis. The diagnosis of CAH secondary to 21-OHD was confirmed by the clinical picture, elevated serum 17-hydroxy-progesterone (17-OHP) level and mutation analysis of the *CYP21A2* gene (Liivak *et al.* 2008). The genotypes of our patients were: subject 1–8bp del/chimeric (common deletion or conversion), subject 2 – p.Gln318X/chimeric,

subject 3 – In2 splice/chimeric, subjects 4 and 5 – p.Arg356Trp (hemi/ homozygous genotype i.e other large deletion or 2 copies of the mutation). The mean age at diagnosis was 5 days. The mean age at the beginning of the study was 5.8 years (range 3.9–9.7). All patients received hydrocortisone 3 times daily with higher doses in the morning, and fludrocortisone once daily. The 17-OHP values were taken in the morning (between 8–10 am.) at 3 months intervals over the study period and presented as a mean value. Bone age (BA), calculated by RUS method (Tanner *et al.* 2001) was taken from the case notes at the nearest time point (BA_1) before (chronological age CA_1) and after the study (BA_2 and CA_2). To estimate the possible effect of BA maturation on growth, change in bone age per year (Δ BA/year) was calculated: Δ BA/year = $(BA_2 - CA_2) - (BA_1 - CA_1)/(CA_2 - CA_1)$. Thus, positive Δ BA/year indicates increased bone age maturation. Auxological and clinical characteristics are given in Table 4. The study was approved by the Tartu University Ethics Committee. Informed consent was obtained from parents of all patients.

Growth Measurements

All subjects were measured daily by one parent before bedtime over the study period (260–470 days), using the stretched technique (gentle upward pressure on the mastoid processes). Triplicate height measurements were taken on each occasion using a Raven Minimeter. The mean of the triplicate measurements was used in analysis. In total of 4728 height measurements were taken from 5 patients. There was a 2-week learning period for measuring technique. At the beginning of the study each child had nine "blind" triplicate measurements taken by his/her parent who later undertook all the measurements. The measurement error expressed as a standard deviation of the differences between these nine "blind" triplicate measurements was between 0.08–0.14 cm.

Statistical analysis

Height standard deviation score (SDS) at the beginning of the study (SDS1) and at the end of the study (SDS2) for each patient was calculated from Estonian growth standards (Silla and Teoste 1989). To obtain a better estimate of growth and growth velocity as function of time, we constructed smooth estimates of individual height and height velocity profiles using locally weighted, least squares, kernel regression analysis (Rubert and Wand 1994), with a bandwidth of 20 days for the height regression and 60 days for the velocity regression. The bandwidths were determined by a predicted squared error criterion (Muller 1987), so as to minimise error. Growth spurts were identified by using local maxima and minima in the velocity curves. In keeping with our previous studies (Thalange *et al.* 1996, Tillmann *et al.* 1998) growth stasis was defined as any period in which the height velocity curve fell below 0.007 cm/day (< 0.5mm/week). Characteristics defined from the height velocity curves included: I) the number of growth spurts and stasis; II) the mean length (days) and amplitude (cm/day) of the growth spurts; III) the time spent in stasis (days)

as a percentage of the study period (days). Characteristics of the growth velocity curves were compared with those found in our previous study of 43 normal prepubertal children (17 boys, 26 girls) (Tillmann *et al.* 1998). The mean age of controls was 6.7 years (range 5.7–7.7) and mean height SDS calculated from the UK standards (Freeman *et al.* 1995) at the beginning of the study was 0.0 (range –2.9 – +2.3). Two-Sample T-Test was used to compare data with controls. $P < 0.05$ was considered to be statistically significant.

4.3. Study 3

Patients

We managed to recruit 6 out of all 7 prepubertal children (4 girls and 2 boys) aged at least 4 years, with the SW form of CAH living in Estonia at the time of the study (the seventh child refused to participate). The diagnosis of 21-OHD was confirmed by the clinical picture, elevated serum 17-OHP level and mutation analysis of the *CYP21A2* gene. Their mean age at the beginning of the study was 6.8 years (range 5.0–9.7 years). All patients were given hydrocortisone thrice and fludrocortisone once daily. The main clinical characteristics of the patients are given in Table 5.

The study was approved by the Tartu University's Ethics Committee. Informed consent was obtained from all parents of the patients.

Methods

All patients received the hydrocortisone regimen with the higher dose in the morning (regimen A) followed by the regimen with the higher dose in the evening (regimen B). Each regimen lasted at least 4 months (Table 5). There was no change in hydrocrortisone or fludrocortisone dose during the 4 months before the study or during the study. Patients were admitted to the hospital between 17:00–19:00 for 24-hour monitoring after each regimen. Height was measured by Harpenden stadiometer and weight by electronic scales to 0.1 kg. Height SD scores (SDS) were calculated from Estonian reference data (Grünberg *et al.* 1998) and BMI according to Cole's international standardisation of childhood obesity and overweight (Cole *et al.* 2000). Hydrocortisone was given at 07:00, 13:00 and 19:00. Blood samples for the 24-hour cortisol profile were taken from 20:00 at 2-hour intervals. 17-OHP level was measured three times a day (08:00, 14:00 and 20:00) and plasma renin activity at 08:00 hours. Blood pressure was measured with the Tonoport V (Ce-0482, Firmware version 1.4, GE Medical Systems Information Technologies) oscillometric blood pressure monitoring system using an appropriately sized cuff. The patients pursued their normal daily activities, but during the measurement were instructed to rest their arm. Readings were taken at 20-minute intervals over the 24-hour period except from midnight to 06:00 when 30-minute intervals were used. If a reading was taken incorrectly then the measurement was repeated

after 3 minutes. The blood pressure values were also calculated into age and sex related SD scores using German reference data (Wühl *et al.* 2002). The following blood pressure characteristics were measured: mean 24-hour systolic and diastolic, daytime systolic and diastolic, nighttime systolic and diastolic blood pressure.

Statistical analysis

Kolmogorov-Smirnov criterion was used for the assessment of normality. The one sample T-test was used to compare SDS with population standards. The paired two sample T-test was used to compare mean 24-hour systolic and diastolic blood pressure SDS values between the two regimens. The two sample T-test was used to compare single blood pressure values during the 24-hour period in each subject between the two regimens. P-values < 0.05 were considered statistically significant. All statistical analysis was done with SAS Version 8.2) and Minitab Version 15.0. Nocturnal systolic blood pressure drop was calculated: [(mean daytime systolic blood pressure – mean nighttime systolic blood pressure) / mean daytime systolic blood pressure] x 100%. Physiological nocturnal drop was defined as a drop of $\geq 10\%$ in mean nighttime blood pressure compared to the mean daytime blood pressure (Nathwani *et al.* 2000).

5. RESULTS

5.1. Incidence of classical 21-OHD in Estonia

Altogether, 20 patients with classical forms of CAH were identified in Estonia. This makes the incidence of classical 21-OHD in Estonia over the 27-year period (1978–2004) about 1 in 25,500 live births. However, the incidence over the last 13-year period (1992–2004), after Estonia regained independence in 1991, was 1:16,100 per live birth. Fourteen patients (70%) were suffering from the SW form (7 males and 7 females) and 6 patients from the SV form (1 male, 5 females). Thirteen patients (65%) were Estonians, and the remaining 7 patients (35%) non-Estonians. This corresponds to the average proportion of nationalities in Estonia. When we looked at the distribution of clinical forms separately, we found the SW form more common in Estonians (12 out of 14) and the SV form in non-Estonians (5 out of 6). However, the number of patients was too small for a comparative analysis of different subgroups. There were two families with more than one affected sibling. In the first family (non-Estonian), both a brother and sister were affected by the SV forms, and in the second family (Estonian) a brother and 2 sisters were affected by the SW forms (Table 2).

The median age at diagnosis of the SW form was 30 days in males and 2 days in females. One boy with the SW form was diagnosed at the age of one year: during the first year of life he had several long lasting and severe infections, some of them with hypoglycaemic episodes indicative of an Addisonian crisis. Four females and all 7 males with the SW form presented with signs of adrenal crisis (hyperkalaemia and hyponatraemia). All females with SW had clitoromegaly (Table 2). The median age at diagnosis of the SV form in females was one year. The only boy with the SV form was diagnosed at the age of 7 years with signs of precocious pseudopuberty and severe acne**.** All 5 females with the SV from had clitoromegaly. The other symptoms were hirsutism and acne (Table 2).

5.2. CYP21A2 mutations in Estonian patients

Seven different *CYP21A2* mutations were found in 34 alleles of all 17 unrelated patients (Table 2). The most frequent mutation was a deletion/conversion (chimeric) occurring in 7 alleles, 4 of them in unrelated Estonians. P.Ile172Asn was the most common point mutation occurring in 5 alleles, 4 of them in unrelated non-Estonian patients. The intron 2 splice-mutation (g.655A/C>G), 8 base-pair deletion (g.707_714delGAGACTAC), p.Arg356Trp and p.Gln318X occurred each in 3 alleles. Two mutations were small conversions involving

Table 2. Age at diagnosis, clinical picture and maximum serum 17-OHP values at diagnosis (or later), phenotype, genotype and genotype group by Wedell (1994) of 20 patients.

No:	Sex	Age at diagnosis	Phenotype	Clinical picture at diagnosis	Max.17-OHP	Genotype	Group
1[a]	F	14 days	SW	adrenal crisis, clitoromegaly	199 nmol/l[e]	Chimeric [f]/p.Arg356Trp	0
2[a]	F	2 days	SW	clitoromegaly	217 nmol/l	small conversion [g]/p.Gln318X	0
3[a]	M[c]	1,5 months	SW	adrenal crisis	149 nmol/l	p.Arg356Trp/ [h]	0
4[a]	F[c]	7 days	SW	clitoromegaly,hyperpigmentation of genitalia	108 nmol/l	p.Arg356Trp/ [h]	0
5[a]	F[c]	2 days	SW	clitoromegaly	169 nmol/l	p.Arg356Trp /[h]	0
6[b]	M	1 month	SW	adrenal crisis, hyperpigmentation of genitalia	118 nmol/l	Chimeric /[h]	0
7[a]	M	17 days	SW	adrenal crisis	93 nmol/l[e]	8bp del [i] / [h]	0
8[a]	F	2 days	SW	clitoromegaly, adrenal crises	141 nmol/l	8bp del/ [h]	0
9[a]	F	5 days	SW	adrenal crisis, clitoromegaly	132 nmol/l	chimeric/p.Gln318X	0
10[a]	M	1 month	SW	adrenal crisis	112 nmol/l	Chimeric/ [h]	0
11[a]	M	1 year	SW	adrenal crisis	222 nmol/l	In 2 splice [j]/ [h]	A
12[a]	M	10 days	SW	adrenal crisis	128 nmol/l	In 2 splice/chimeric	A
13[a]	M	1,5 months	SW	adrenal crisis	102 nmo/l	In 2splice/p.Arg356Trp	A
14[b]	F[d]	3 years	SV	clitoromegaly, hirsutism, acne	11 nmol/l[e]	p.Ile172Asn/ [h]	B
15[b]	M[d]	7 years	SV	acne, precocious pseudo puberty	63 nmol/l[e]	p.Ile172Asn/ [h]	B
16[b]	F	1 year	SV	clitoromegaly	41 nmol/l[e]	p.Ile172Asn/chimeric	B
17[b]	F	2 days	SV	clitoromegaly	106 nmol/l[e]	p.Ile172Asn/chimeric	B
18[b]	F	2 days	SW	adrenal crisis, clitoromegaly	99 nmol/l	p.Ile172Asn/8bp del	B
19[a]	F	14 days	SV	clitoromegaly	84 nmol/l	small conversion/ p.Ile172Asn	B
20[b]	F	3 years	SV	clitoromegaly,hirsutism	31 nmol/l	p.Gln318X/ [k]	–

[a] Estonians; [b] Russians; [c,d] Siblings; [e] Maximum serum 17 OHP levels measured later during treatment; [f] Chimeric = common deletion/conversion of 5' *CYP21A2*; [g] Small conversion = conversion of 3' section of *CYP21A2* gene involving sequential point mutations p.Ile172Asn, p.Gln318X, p.Val281Leu, p.Arg356Trp; [h] Hemi/homozygous genotype i.e other large deletion or 2 copies of the mutation.; [i] 8bp del = 8 base pair deletion in exon 3; ; [J] In 2 splice = g.655 A/C>G mutation; [k] Heterozygous for p.Gln318X with no other detected mutations.

four sequential point mutations (p.Ile172Asn, p.Gln318X, p.Val281Leu, p.Arg356Trp) in the 3' end of the gene. In the 7 unrelated patients we were not able to distinguish whether the second allele has the same mutation as on the first allele (homozygosity) or if it carries a deletion/conversion (hemizygosity) which is not detected by our test (Table 3). Patient No 20, who was heterozygous for p. Gln318X, had some other mutation not included in the panel on her second allele. The mutational spectrum of 34 unrelated alleles is given in Table 3.

Table 3. The mutational spectrum of *CYP21A2* among the patients with a 21-hydroxylase deficiency in Estonia. The frequency is calculated from all unrelated alleles (n=34).

Mutation	Alleles found	Frequency (%)
Del/conv[a]	7	20.6
Small conversion	2	5.9
p.Ile172Asn	5	14.7
p.Arg356Trp	3	8.8
p.Gln318X	3	8.8
Intron 2 splice	3	8.8
8bp. deletion	3	8.8
Uncertain alleles:	8	23.5
Del/conv[a,b]	2	
p.Ile172Asn[b]	1	
p.Arg356Trp[b]	1	
Intron 2 splice[b]	1	
8bp. Deletion[b]	2	
Other mutation	1	

[a] – Only a chimeric form of a deletion or a large gene conversion was detected
[b] – Homozygosity was not distinguished from hemizygosity due to a lack of parental samples. The uncertain allele may involve the same mutation OR a deletion/conversion mutation not detected by our test.

There were 10 patients in group 0, six patients in group B and 3 in group A. We were unable to determine the genotype group of patient No.20 (Table 2). As the patient has not been fully characterized, one cannot exclude that she is heterozygous for a milder mutation.

5.3. Short-term growth

The auxiological and clinical data of study group patients is given in Table 4. Original measurements and estimated regression curves for height of patient 1 and height velocity for all five patients are given in Figure 4. Four patients had a biphasic growth pattern characterised by growth peaks and periods of very slow growth or stasis. The only child who did not have any stasis (No 4) was the shortest (height SDS –2.08) and had the highest mean 17-OHP level over the study period (mean 67 nmol/l). In spite of the relatively high hydrocortisone dose the biochemical control expressed as a mean serum 17-OHP value over the study period in 3 patients was not good. However, growth over the study period in these 3 patients was normal (mean Δ height SDS –0.02). In the other 2 patients with normal 17-OHP values, despite high hydrocortisone doses, their Δ BA/year was positive i.e. BA maturation was quicker than chronological age development and also their height gain over the study period was increased, implying some degree of non-compliance.

Patients with CAH spent significantly less time in stasis than normal children (mean 5 ± 4.8% vs. 11.4 ± 7.2% of study period; $p < 0.05$). The mean length of height velocity peaks was significantly longer (110.4 ± 28.3 days vs. 54.0 ± 13.1 days; $p < 0.05$) and the mean amplitude of spurt was significantly lower (0.022 ± 0.008 cm/day vs. 0.037 ± 0.001 cm/day; $p < 0.01$) than those in normal children. Height gain over the study period (Δ height SDS) was positively correlated to the mean amplitude of growth spurts ($r = 0.9$, $p < 0.05$) (Figure 5).

Table 4. Auxological and clinical data of 5 children with CAH. Normal values in table are from 43 healthy children (Tilmann *et al.* 1998)

Subject	1	2	3	4	5	Mean	Normal
Age	5.9	3.9	4.3	5.2	9.7	5.8	5.7 – 7.7
Sex	F	F	M	F	F		
Time of the study (days)	255	466	267	284	304	315	
Height1 (cm)	107.1	100.6	96.2	100	140.6	108.9	
Height2 (cm)	110.9	114.6	103.2	106.6	144.4	115.9	
ΔHeight (cm)	3.9	14.0	7.0	6.6	3.8	7.06	
Height SDS1	–2.0	–0.15	–1.9	–2.11	–0.42	–1.15	0.0 (–2.9 – +2.3)
Height SDS2	–1.78	0.75	–1.52	–2.08	0.15	–0.89	0.2 (–2.7 – +2.5)
ΔHeight SDS	0.22	0.9	0.45	0.03	–0.27	0.26	0.2 (–0.2 – +0.7)
Target-height SDS	–0.65	–0.47	0.76	1.33	1.33	0.46	
Δ BA/year	–0.5	+1.4	+0.7	–0.1	–0.5	+1.0	
Hydrocortison (mg/m2/day)	15.9	23.9	24.6	21.9	22.9	21.8	
Fludrocortison daily (mg/day)	0.05	0.1	0.175	0.1	0.1	0.1	
Mean 17-OHP (nmol/l)	56	7	0.2	67	63	39	
Mean amplitude of growth spurt (cm/day)	0.016	0.032	0.028	0.023	0.013	0.022 [a]	0.037(0.025–0.09)
Mean length of growth spurt (days)	74	144	128	116	90	110.4 [b]	54(37–96)
Time in stasis (%)	10	6	1	0	10	5.4 [b]	11(6–16)
No. of stasis	2	1	1	0	2		
No. of spurts	3	3	2	3	3		

[a] – Statistically different from the controls ($p < 0.01$)
[b] – Statistically different from the controls ($p < 0.05$)

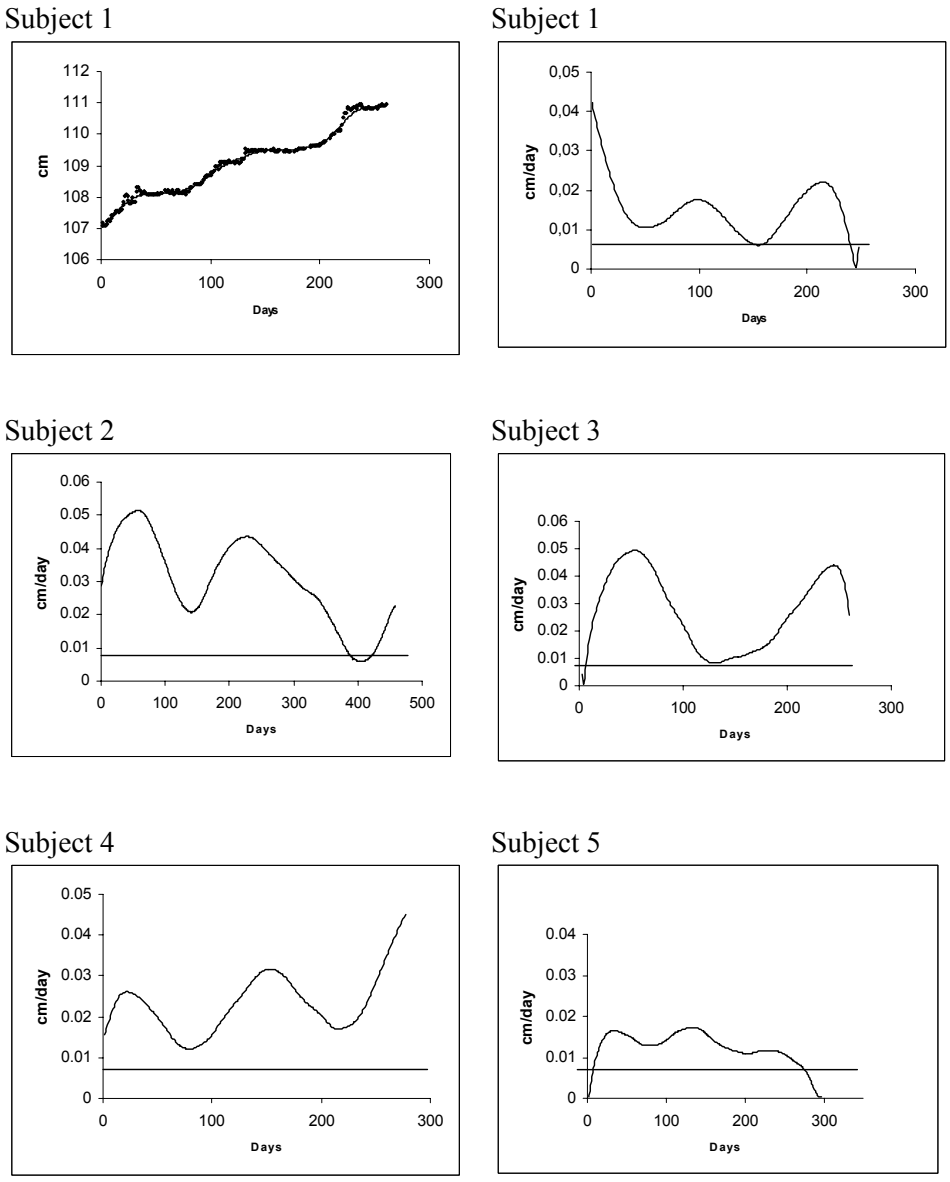

Figure 4. Original height measurements (dots) and estimated height and height velocity curves (lines) in subject 1 and estimated height velocity curves (lines) in subjects 2, 3, 4 and 5. Growth stasis was defined as any period in which the height velocity curve fell below 0.007 cm/day.

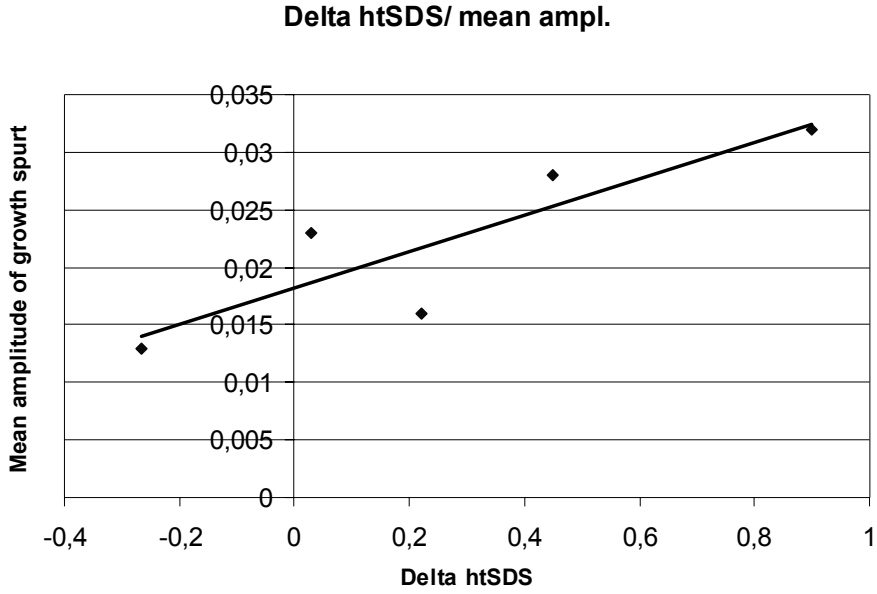

Figure 5. Height gain over the study period was positively correlated to the mean amplitude of growth spurts ($r=0.9$, $p<0.05$)

5.4. 24-hour blood pressure profiles on two different hydrocortisone treatment regimens

Patient clinical characteristics are shown in Tables 5 and 6. According to the Cole BMI standards one child was obese, in other children BMI remained below the cut-off line for overweight i.e. were in normal range. Despite the relatively high daily hydrocortisone dose (15.9–24.3 mg/m^2) the biochemical control over the disease in 4 patients was not satisfactory as seen by their elevated serum 17 OHP levels (Table 5). The mean 24-hour serum cortisol profiles on both treatment regimens are shown in Figure 6. The 24-hour cortisol profile measured as area under the curve (AUC) on regimen A (6597 nmol/l) was not significantly different from that seen on regimen B (5654 nmol/l). Mean 17-OHP levels between the 2 regimens (Table 5) were not significantly different.

Table 5. Clinical characteristics of 6 patients with CAH. Serum 17-OHP concentration is given as a mean of the 3 measurements in a day in both regimens. HC – hydrocortisone, FC – fludrocortisone, sBP – systolic blood pressure, dBP – diastolic blood pressure.

Patients	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**
Age (years)	6.0	5.4	9.0	5.5	9.7	5.0	6.8
Sex	F	F	M	F	F	M	
Height SDS	–1.80	0.29	1.80	–2.00	–0.12	–1.73	–0.59
BMI (kg/m^2)	17.2	15.9	18.6	20.9	19.9	18.1	18.4
HC dosage (mg/m^2/d)	15.9	23.5	16.0	21.5	22.9	24.3	20.7
HC dosage daily (A)	5/5/2.5	12.5/5/2.5	10/5/5	10/5/2.5	15/10/5	10/5/2.5	
HC dosage daily (B)	2.5/5/5	2.5/5/12.5	5/5/10	2.5/5/10	5/10/15	2.5/5/10	
FC dosage (mg/day)	0.05	0.1	0.1	0.1	0.1	0.175	0.10
17-OHPA (nmol/l)	57	13	43	81.3	31.6	0.1	37.7
17-OHPB (nmol/l)	55	0,3	81	54	36.1	0.3	37.8
PRA (ng/ml/h)	3.8	1.6	0.35	3.7	0.2	0.85	1.75
24-h sBP SDS (A)	–0.01	–0.4	0.8	–0.9	1.6	–1.9	–0.13
24-h sBP SDS (B)	0.1	1.7	1.1	0.01	2.8	–0.3	0.92
24-h dBP SDS (A)	0.5	–1.2	–1.1	–0.9	0.6	–2.1	–0.72
24-h dBP SDS (B)	0.2	0.9	–0.3	–0.6	2.5	–1.0	0.28
Nocturnal drop A (%)	5.1	11.8	10.1	14.8	6.5	4.2	8.75
Nocturnal drop B (%)	11.2	3.9	8.4	10.8	3.6	10.4	8.05

Table 6. Mean individual blood pressure values (mmHg) on 2 treatment regimens (A and B). Significant differences of the same blood pressure characteristics from Regimen B:* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. sBP – systolic blood pressure, dBP – diastolic blood pressure.

Patient	24h sBP(A)	24h sBP(B)	24h dBP(A)	24h dBP(B)	Day sBP(A)	Day sBP(B)	Day dBP(A)	Day dBP(B)	Night sBP(A)	Night sBP(B)	Night dBP(A)	Night dBP(B)
1	104.1	105.2	68.2	66.4	106.1	109.1	70.4	69.2	100.6	96.9	63.2	58.8
2	99.9***	115.4	59.4***	70.4	104.0***	116.8	63.4**	73.1	91.6***	112.1	52.3***	64.1
3	114.0	116.8	60.8	65.2	119.0	120.1	64.1	69.8	106.9	110.0	56.1	55.9
4	96.3*	103.5	60.7	62.4	101.5	107.5	65.6	65.6	86.5*	95.8	51.2	56.2
5	121.9***	131.4	69.9***	79.6	124.1***	132.4	72.6**	80.1	116.0**	127.6	62.8***	77.8
6	94.5**	103.1	55.9	60.4	97.1**	108.8	55.5	64.1	93.0	97.3	56.4	56.7

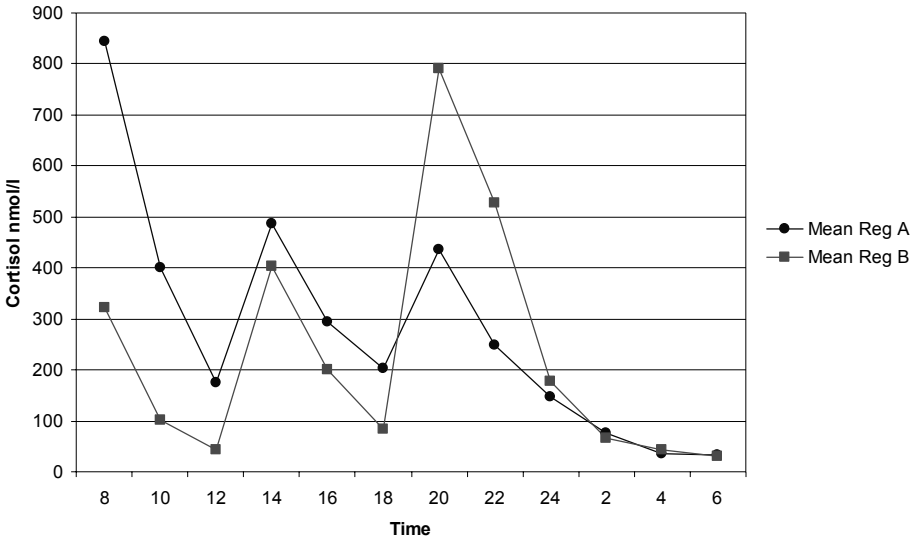

Figure 6. The mean diurnal serum cortisol profiles on regimens A and B.

The mean systolic and diastolic blood pressures of regimen A were below the normal population (–0.13 and –0.72 SDS accordingly). In spite of the slightly lower cortisol profile of regimen B, the mean 24-hour systolic blood pressure on regimen B was more than 1 SDS higher than on regimen A (0.92 ± 1.17 SDS vs –0.13 SDS ± 1.23 SDS, $p < 0.05$). This difference was seen both at nighttime (1.19 ± 1.2 SDS on regimen B and 0.28 ± 1.13 SDS on regimen A) as well as during daytime (0.47 ± 1.0 SDS on regimen B and –0.35 ± 1.2 SDS on regimen A). The mean 24-hour diastolic blood pressure was significantly lower on regimen A compared to regimen B (–0.72 ± 1.0 SDS vs. 0.28 ± 1.3 SDS, $p < 0.05$). Despite of the lower values in mmHg scale, the mean diastolic blood pressure SDS was higher at nighttime (0.20 ± 0.82 SDS on regimen A and 0.80 ± 1.14 SDS on regimen B) compared to daytime (–1.24 ± 1.0 SDS on regimen A and –0.41 ± 1.0 SDS on regimen B).

When we compared respective single blood pressure values in mmHg measured over the 24-hour period in each subject on regimens A and B, four patients (Nr 2,4,5,6) increased significantly heir mean systolic blood pressure on regimen B and 2 of them (Nr 2,5) also their diastolic blood pressure (Table 6). The mean drop in nighttime systolic blood pressure was 8.8% on regimen A and 8% on regimen B. In one patient (Nr 4) the drop in nighttime systolic blood pressure was more than 10% on both regimens (Table 5).

6. DISCUSSION

6.1. Epidemiology

This is the first study describing the incidence, phenotype and genotype of classical forms of CAH in Estonia. All patients with a diagnosis of the classical form of CAH agreed to participate in the study. Thus, the incidence, calculated on the number of clinically diagnosed cases, reflects the real situation in the country. Brosnan *et al.* (1999) showed no significant difference in the incidence based either on clinically diagnosed cases (1:17,396), or in the results of the screening programme (1:15,974) in three states of the USA. The biggest survey from the 6.5 million newborn infants screened in 13 countries gave an overall incidence of the classical form of CAH of 1 in 15,000 live births (Pang *et al.* 1993, Therell *et al.* 1998), which is higher than the 1: 25,500 we found over the 27-year period (1978–2004), but very similar to the 1:16,100 found in the last 13 years. One of the reasons for the discrepancy between these 2 periods may be the fact that after regaining our independence in 1991, about 200,000 people emigrated from Estonia in the following years and therefore some patients with CAH might also have left. According to the data from the Statistics Estonia Death Register there have been no lethal cases from adrenal disorders since 1997, but computerised data from previous years are not available. Thus, it is possible that there might have been some lethal cases of the SW form in previous years. Third, and not least important, is the improvement of general knowledge of the disease and our diagnostic standards, especially after serum 17-OHP assays became routinely available in Estonia in 1989. The skewed female-male ratio in the SV group (5 females and 1 male) indicates that there could be some undiagnosed male cases of the SV form in Estonia, particularly from the late 70-s and 80-s when the SV form was diagnosed in 3 girls, but not in any boys. Therefore, it is likely that the incidence of CAH over the last 13 years (1:16,100) reflects more accurately the real situation in Estonia. This is slightly smaller than the figure from Sweden, where the incidence of CAH based on clinically diagnosed cases was 1:11,500 (Thilen *et al.*1998).

6.2. Clinical picture

In our study the boys received their diagnosis at the median age of 30 days, which is later than the 21 days reported in Sweden before the introduction of a newborn screening programme (Thilen *et al.* 1998). However, in countries with neonatal screening programmes for CAH, the median time of diagnosis is 9–14 days (Cutfield *et al.* 1995, Thilen *et al.* 1998, Therell *et al.*1998). All girls with the SV form presented with clitoromegaly, unfortunately not all case reports of older patients included Prader genital stages and therefore it is difficult to

estimate the severity of clitoromegaly in those girls. The majority of them (9 out of 12) have been operated on for their clitoromegaly. The only boy with the SV form presented with precocious pseudopuberty and accelerated growth rate. The relatively late age at diagnosis in SW males and the skewed female-male ratio in SV patients supports the need for a newborn screening programme for early detection of male patients with CAH.

6.3. CYP21A2 mutations and genotype phenotype correlations

All patients in our study were screened for 6 common point mutations, an eight base pair deletion in exon 3 and a large deletion/conversion resulting in the formation of a ”chimeric” mutation. Our screening protocol is estimated to detect at least 70% of all *CYP21A2* mutations. However, due to insufficient DNA quality and/or quantity in 7 unrelated patients, we could not perform the Southern blot analysis to determine whether the second allele has the same mutation as on the first allele (homozygosity) or if it carries a deletion/ conversion (hemizygosity) which is not detected by our test. One patient (No 20; Table 2) who was heterozygous for p. Gln318X, had some other mutation not included in the panel, on her second allele. In this way, we identified mutations in 26 out of 34 unrelated alleles. A large deletion/conversion (chimeric) was the most common mutation of all unrelated alleles and occurred with similar frequency in both Estonian and non-Estonian patients. Although its frequency (20.6 %) is lower than the 29% in Russia (Kalintchenko *et al.* 2002), 32.2% in Sweden (Wedell 1998) or 31.9% in Netherlands (Stikkelbroeck *et al.* 2003), the real percentage may be much higher because some of the 7 patients from the uncertain allele group may have deletion or conversion in their second allele. We found 2 patients with a contiguous run of four point mutations (p.Ile172Asn, p.Val281Leu, p.Gln318X and p.Arg356Trp), which must have been transferred as a small conversion. One of them (patient No 2 in Table 2) has the SW phenotype and the other (patient No19) the SV phenotype. The first patient was homozygous for the p.Gln318X mutation and the second for the p.Ile172Asn mutation. This explains the phenotypic difference between these two mutations. In comparison with other countries (Wedell 1998, Kalintchenko *et al.*2002, Stikkelbroeck *et al.* 2003), we had a lower frequency of intron 2 splice mutations. Again, the actual frequency of 8bp deletion, p.Ile172Asn, p.Arg356Trp and intron 2 splice mutations may be slightly higher due to their possible presence in the uncertain allele group.

We were surprised to find differences in clinical forms and mutational spectrum between Estonians and non-Estonians. The Estonian population has been influenced by different waves of migration from Europe (Germany, Sweden, Denmark) and Russia. The comparison of mitochondrial DNA in Estonians has shown similarity with Western-European, most of the Scandinavian

countries (Villems *et al.* 1998, DeBakker *et al.* 2006) and also with the South – Western Russian population [personal communication with Prof. A. Metspalu]. The studies of other genetic diseases (cystic fibrosis, phenylketonuria) in Estonia have shown that mutation frequency in Estonian and Russian patients is quite similar (Lilleväli *et al.* 1996, Teder *et al.* 2000). The spectrum of *CYP21A2* gene defects in Russian patients was similar to those reported in other Caucasian populations (Kalintchenko *et al.* 2002). The ratio between the SW and the SV forms in non-Estonian unrelated patients (2:4) is very unusual and may be just due to the small number of patients in this group. It's important to underline that childhood mortality and morbidity in Estonia does not differ significantly between Estonians and non-Estonians. Therefore it is unlikely that we have missed the SW forms only in non-Estonian children. Thus, the differences in phenotype and genotype between Estonians and non-Estonians are most likely due to the sample size rather than true distributions.

The genotype-phenotype correlation in our study was good. In groups 0 and A, there was no phenotypic variance as expected. In group B, where the predicted residual enzyme activity is about 2%, genotype-phenotype correlation is variable (Wilson *et al.* 1995, Jääkelainen *et al.* 1997, Speiser and White 2003). Most commonly, group B is associated with the SV form of CAH (Wilson *et al.* 1995, Wedell 1998). In our study there were five patients with the SV form and one with the SW form, which were classified in Group B. This SW patient (No18 in Table 2) had the p.Ile172Asn/8bp del genotype. The detection of a wild-type allele at position g.999, as well as the mutated allele (i.e. p.Ile172Asn), was indicative of a possible duplication of *CYP21A2*. The presence of the 8bp deletion in trans (i.e. on the other chromosome) to the point mutation would normally destroy a primer binding site for the ARMS assay on the chromosome carrying the 8bp deletion. This would not lead to the corresponding wild-type amplimer from the p.ILe172Asn ARMS test. Clinically she had clitoromegaly at birth and during the second week of life she developed adrenal crisis with hyponatraemia and hyperkalaemia. A similar mutation (p.Ile172Asn/8bp del) in SW patients has also been reported by Krone *et al.* (2000). They also described a marked divergence in group B, where 26% of the patients had the SW form.

6.4. Short-term growth in children with CAH

This is the first study describing short-term growth in children with CAH. As in our previous studies (Tillmann *et al.* 1998, Tillmann *et al.* 2002), we used a non-parametric technique to generate growth curves to avoid imposing a particular form to the growth process, which would result from the use of a linear, polynomial or step function. However such curves do not adequately define the dynamic growth process: the smoothing component tends to mask abrupt changes in height. Therefore we constructed height velocity curves to

describe the characteristics (eg. length, amplitude) of the growth process. Curve characteristics were compared with data from 43 normal prepubertal children (Tillmann *et al.* 1998) constructed using the same methodology and with the same bandwidth. There is no reason to consider that the essential character of non-linear growth is different in children in Estonia compared with those living in Manchester, UK. This is further supported by the observation that two Estonian boys measured daily over a year (Tillmann and Clayton 2001) showed similar growth pattern to those seen in the UK study (Thalange *et al.* 1996). Measurement error calculated by the same methodology in 2 observers who did the measurements in control children was similar (0.13–0.15 cm) to that seen in this study where growth was measured by parents. Thus, the comparison to that control group is valid.

All 5 patients with CAH had growth spurts (Figure 4); 4 had 1 or 2 periods of slow growth or stasis, but one subject, with poor control had no discernible period of stasis. The mean amplitude of growth spurt in all 5 patients was significantly lower than in normal children, and in 3 it was below the lowest value seen in normal children (<0.025cm/day). The mean amplitude of the other 2 subjects was below the mean for normal children. The mean length of growth spurts was twice that seen in normal children (110 days versus 54 days). In healthy children it is known that the length and not the amplitude is a significant determinant of child's height (Tillmann *et al.* 1998). Thus, these long-lasting, low-amplitude growth spurts may ultimately result in impaired final height. The two patients with the longest growth spurts (No 2 and 3 in Table 4) showed rapid growth and bone age maturation despite high hydrocortisone doses, implying non-compliance with treatment, and are consequently at high risk of impaired final height. We were surprised that children with CAH spent less time in stasis, in contrast to children with growth hormone deficiency and Turner syndrome, who spent more time in stasis (Tillmann *et al.* 2002). Thus, children with CAH grow with a relatively steady growth rate, with only small fluctuations in their growth velocity. Our patients (except patient No 2; Table 4) with CAH grew with normal growth rate, over the period of the study (mean Δ height SDS +0.26), similar to that seen in normal children (Δ height SDS +0.20) (Tillmann *et al.*1998). In that group growth through the year was positively influenced by the mean amplitude of height velocity peaks. Similar correlation was also seen in this study. Growth over the study period (Δ height SDS) was positively correlated to the mean amplitude of growth spurts ($r = 0.9$, $p = 0.037$) (Figure 6). This indicates that children who grow faster have higher amplitude growth spurts.

The weakness of the study is a small number of patients. However, it was enough big to detect statistically significant differences in 3 curve characteristics, one of them (mean amplitude of growth spurt) with high probability ($p < 0.01$). Thus, this small group was sufficient to describe short term growth pattern in children with CAH by comparing the curve characteristics to those seen in normal children. However, the number of patients is too small to assess

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the impact of biochemical control or hydrocortisone dose on that growth pattern. Further studies of a larger group of patients are needed to confirm the growth pattern detected and to define the relative contribution of good and poor control of the disease.

The observed pattern of growth in children with CAH is similar to shorter children within the cohort of normal controls, who also exhibited longer growth spurts (Tillmann *et al.* 1998). The physiological basis for this alteration in normal growth is unknown. One explanation may be that children with CAH compared to normal children have a more regular pattern of growth hormone (GH) secretion (Charmandary *et al.* 2002), known to be associated with poorer growth (Gill *et al.* 2001), which in turn is likely to be consequent of hydrocortisone treatment (Charmandary *et al.* 2002). Altered circadian rhythmicity of GH secretion which is glucocorticoid-independent, may be another possible explanation (Barkan *et al.* 2000).

Our study has also a clinical implication. Growth monitoring, at 3–4 monthly intervals, is an important part of the follow-up of children with CAH. Small fluctuations in growth rate are interpretable either as a consequence of over- or under-treatment, or the underlying non-linear growth process. Our data indicates that the normal short-term growth pattern of children with CAH is "damped", with reduced amplitude growth spurts of increased length, and consequently that significant variations in growth rate are more likely to reflect treatment effects, than to be a consequence of non-linear growth itself.

6.5. 24-hour blood pressure in 2 different hydrocortisone regimens

This small study describes the 24-hour blood pressure profiles in 6 patients with the SW form of CAH using different treatment regimens. To our knowledge this is the first study examining the effect of two different hydrocortisone regimens on 24-hour blood pressure profiles and biochemical control in children with CAH. As our patients were shorter (mean height SDS = –0.59), we used blood pressure standards adjusted to chronological age only. It has been suggested that blood pressure standards adjusted for height must be interpreted with care (Merke *et al.* 2000, Völkl *et al.* 2006-b). Mean systolic and diastolic blood pressure values of regimen A were very close to the population mean, but increased both about 1 SD on regimen B. Our children with CAH had elevated systolic blood pressure with regimen B (0.92 SDS), particularly during the night and slightly elevated mean diastolic blood pressure (0.28 SDS) compared with population standards. Ambulatory blood pressure monitoring is accurate and well tolerated in children and it also may avoid the "white coat hypertension" (Khan *et al*. 2000, Roche *et al.* 2003). Although the reference data was not collected from hospitalized patients, we believe that the "white coat effect" did not influence the results of our study. The conditions were similar on both

regimens and it's not plausible that "white coat effect" influenced the results only on regimen B, when BP values were significantly higher. The nocturnal drop of systolic blood pressure over the two study periods in these patients was only 8.3% from daily systolic blood pressure. Five patients out of six dropped their nocturnal systolic blood pressure to less than 10% of the daytime mean (4 at different and 1 at both regimens). Higher blood pressure at night together with obesity has shown to increase the risk for cardiovascular diseases in adulthood in patients with CAH (Cornean *et al.* 1998, Charmandari *et al.* 2002-a).

When two different hydrocortisone regimens were compared, all 6 blood pressure characteristics were at least 0.6 SDS higher on regimen B compared to regimen A. It is not easy to explain this difference since mean serum 17-OHP and 24-hour cortisol concentrations were similar; the latter was even slightly lower on regimen B. Previous studies have shown that conventional hydrocortisone replacement therapy leaves patients' cortisol levels too low in the early hours of the morning, so treatment cannot reproduce the normal cortisol circadian rhythm (Charmandari *et al.* 2001-a, Merza *et al.* 2006). Our data shows that no matter how hydrocortisone was administrated, early morning (4–6 am.) cortisol levels remained very low and the mean 17-OHP levels were highest in the morning (data not shown). This is similar to the study by Winterer *et al.* (1985) who also found that different hydrocortisone dose regimes did not cause any differences in the mean 24-hour 17-OHP concentrations. Increased blood pressure is seen in patients who receive too much fludrocortisone which can be detected by suppressed plasma renin activity (PRA). All our patients had a mean PRA above the minimum of normal range (0.2 ng/ml/h).

The exact mechanism for increased blood pressure in patients with CAH is unclear. It is well known that obesity is a major risk factor for hypertension. Elevated 24-h ambulatory blood pressure was associated with a raised BMI in children with CAH, particularly for girls (Roche *et al.* 2003). However, only one child was obese in our study. Obesity-associated hypertension has been linked to hyperleptinaemia and hyperinsulinaemia (Rahmouni *et al.* 2005), both of which are also found in CAH (Charmandari *et al.* 2002-a). However, Völkl *et al.* (2006-a) showed that after adjustment for age, sex and BMI, the serum leptin levels in CAH did not differ from controls. The other cause for elevated blood pressure may be excessive steroid replacement therapy. Glucocorticoids have also mineralocorticoid effects, probably through saturating the 11β-HSD2 enzyme (Dötch *et al.* 2001, Chemaitilly *et al.* 2003,), which normally converts cortisol to cortisone. The mean daily hydrocortisone dose in our patients was quite high, but in spite of that, the mean daily 17-OHP levels remained still high in 4 patients. However, the higher hydrocortisone dose does not explain why the increased blood pressure occurred only in regimen B as the total daily HC dose was similar in both regimens. Neither Roche *et al.* (2003) nor Völkl *et al.* (2006-b) found any correlation between blood pressure and glucocorticoid or mineralocorticoid replacement dosage. Considering all these factors the exact

mechanism why the blood pressure was higher in our study when hydrocortisone was administrated in the evening still remains unclear.

One of the weaknesses of the study is the small number of subjects. In spite of the small number of patients, this was sufficient to establish statistically significant differences in blood pressure SDS values between the two treatment regimens. Another weakness is that the same order of treatment regimens was used for all subjects i.e. regimen A followed by regimen B. This could theoretically influence the results, but not the main results of our study. All patients had received hormone replacement therapy since the diagnosis within 1.5 months after birth. There was no increment in hydrocortisone or fludrocortisone dose during 4-months prior to the study. In our opinion the only way how non-randomisation could theoretically influence the results is through the child's growth. Within 4 months, children should be taller and heavier and thus, the dose per body surface area slightly smaller. This also explains why the mean 24-hour cortisol profile (measured as AUC) was slightly smaller on regimen B than on regimen A, although this was not statistically significant. Thus, in the case of randomisation we should expect that the dose per body surface area and therefore also the possible influence of cortisol profile on blood pressure on regimen B would be smaller than in the current order. However, the opposite tendency was observed – blood pressure was higher on regimen B. Therefore it is very unlikely that the non-randomisation in our study could have influenced the main results of our study.

Further studies with larger number of patients with CAH are needed to confirm the differences in arterial blood pressure and to clarify the possible influence of the different treatment regimens on biochemical disease control.

7. CONCLUSIONS

1. The incidence of classical 21-OHD in Estonia in 1992–2004 was 1:16,100.
2. The mutational spectrum of the *CYP21A2* gene in Estonia was similar to that in other Caucasian populations, with the deletion/conversion being the most frequent mutation. The correlation between genotype and phenotype was good. The relatively late age at diagnosis and the skewed female-male ratio supports the need for newborn screening for 21-OHD.
3. Short-term growth in children with CAH compared to normal controls is characterised by long-duration low-amplitude growth spurts with reduced periods of growth stasis. Better growth over the study period was correlated to the amplitude of growth spurts. The relatively smooth short-term growth in children with CAH suggests that significant variations in growth rate are more likely to be a consequence of under- or over-treatment rather than non-linear growth itself.
4. The hydrocortisone treatment regimen with a higher dose in the evening did not improve the biochemical control in children with CAH, but it did increase their 24-hour blood pressure profiles.

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ESTONIAN SUMMARY

21-hüdroksülaasi puudulikkusest tingitud kaasasündinud adrenogenitaalse sündroomi klassikaline vorm Eestis: esinemissagedus, genotüüp ja fenotüüp, haiguse mõju vererõhule ja lühiajalisele kasvamisele

Kirjanduse ülevaade

Kaasasündinud adrenogenitaalne sündroom (AGS) on autosoom-retsessiivselt päranduv haiguste grupp, mille puhul on häiritud viiest ühe kortisooli tootmiseks vajaliku ensüümi sünteesimine. 90–95% juhtudest on see tingitud 21-hüdroksülaasi defitsiidist (Speiser ja White 2003). Selle ensüümi aktiivsuse langus põhjustab kortisooli, soolakaotusvormi korral ka aldosterooni, sünteesi häire, millele järgneb AKTH tõus, mis viib androgeenide üleproduktsioonile ja neerupealiste hüperplaasia tekkeni. Haiguse kliinilise pildi järgi eristatakse klassikalist vormi, mis jaguneb soolakaotuse ja lihtsaks viriliseeruvaks vormiks, ja mitteklassikalist vormi. Kliiniline pilt klassikalise vormiga tütarlastel avaldub virilisatsioonis, kergematel juhtudel kliitori suurenemises. Raskematel juhtudel on tegemist ulatusliku kliitori, häbememokkade ja ureetra transformatsiooniga, kus sünnijärgselt on lapse soo määramine raske. Vastsündinud poeglastel on üleviriliseerumise tunnuseid raske märgata ja neil avaldub haigus esimesel elukuul adrenaalse kriisina (hüponatreemia, hüperkaleemia, metaboolne atsidoos).

Kirjanduse andmetel on AGS klassikalise vormi esinemissagedus 6,5 miljoni skriinitud vastsündinu põhjal 1:15000 elussünni kohta (Pang *jt.* 1993, Therrell *jt.* 2001). Harvem esineb AGS mustanahalistel ja asiaatidel, samas on see mõningates regioonides jällegi ülikõrge (Alaska Yupic eskimod 1:280) (Pang *jt.* 1988). Seniajani puudusid andmed AGS esinemissageduse kohta Eestis. Mitteklassikaline vorm avaldub reeglina puberteedieas või hiljem. Seda vormi iseloomustavad kiirenenud kasv lapseeas, virilisatsioon, menstruatsioonihäired ja fertiilsuse probleemid.

Haigus on päritav autosoom-retsessiivsel teel ja seda põhjustav geen *CYP21A2* asub kuuendas kromosoomis (6p21.3). Enamus mutatsioone kantakse üle geeni konversioonina *CYP21A2* geeni kõrval asetsevalt pseudogeenilt (*CYP21A1P*). Tänaseks on teada üle 100 erineva mutatsiooni, milledest 8–10 põhjustavad 80–90% kõigist AGS juhtudest. Paljud autorid on kirjeldanud tugevat seost haiguse genotüübi ja fenotüübi vahel (Speiser *jt.* 1992, Jääskelainen *jt.*1997-a, Speiser *jt.* 2001), kuid mõned tööd on toonud sellest seosest välja ka erandlikke juhtusid (Wilson *jt.* 1995, Wedell *jt.* 1998).

Varasemalt on näidatud, et lapse lühiajaline kasv, s.t. kasv vähem kui 1 kalendriaasta jooksul on mittelineaarne protsess, mida iseloomustavad kasvuspurdid ja staasid. Kasvuspurdid kestavad keskmiselt 7–8 nädalat ja nende vahele jäävad mõõnaperioodid ehk staasid keskmise pikkusega 2 nädalat (Till-

mann *jt.* 1998). Kasvuhäiretega laste kasv on samuti kahefaasiline, kuid neil esinevad muutused kasvuspurtide amplituudis (vähenenud näiteks kasvuhormooni puudulikkuse korral) või on pikenenud staasides oldud aeg (näiteks Turneri sündroomi korral) (Tillmann *jt.* 2002).Vaatamata adekvaatsele biokeemilisele kontrollile ei saavuta enamus AGS-iga lapsi oma geneetilist täiskasvanu kehapikkust ja jäävad keskmiselt 1,2–2 standardhälbe võrra lühemaks kui terved eakaaslased (Pinto *jt.* 2003, New *jt.* 2001). Seetõttu on AGS-i ravis hakatud kasutama kasvuhormooni eesmärgiga parandada lõplikku kehapikkust (Quintos *jt.* 2001, Lin-Su *jt.* 2005). Seoses sellega on oluline teada, milline on lühiajalise kasvamise iseloom AGS-i korral: kas on vähenenud kasvuspurtide amplituud, suurenenud aeg staasides või kombinatsioon neist mõlemaist. Antud hetkel kirjanduses selle kohta andmed puuduvad.

AGS patsiente ravitakse soolakaotusvormi korral lisaks glükokortikoididele ka mineralokortikoididega. Ebaadekvaatne ravi või üleravimine võib põhjustada erinevaid komplikatsioone nagu elektrolüütide taseme häire, adrenaalne kriis, lühike täiskasvanuea kehapikkus, ülekaalulisus. On näidatud, et AGS patsientidel esineb kõrgenenud arteriaalset vererõhku (Roche *jt.* 2003, Völkl *jt.* 2006–b).

Erinevad autorid on uurinud ka glükokortikoidravi erinevate režiimide mõju 17-hüdroksüprogesterooni ja 24-tunni kortisooli profiilidele (Winterer *jt.* 1985, Plat *jt.* 1999, Charmandari *jt.* 2001-a). Enamuse uurijate arvates tagab hommikuse suurema glükokortikoidi annuse manustamine haiguse parema kontrolli , samas kui teiste arvates on parem kontroll saavutatud õhtuse suurema annusega (Van der Kamp *jt.* 2002, Rosenfeld 2002, Ross *jt.* 2005). Glükokortikoidravi üheks eesmärgiks on tagada kortisooli puudusest tingitud AKTH üleproduktsiooni mahasurumine. Hüdrokortisooni lühikese poolestusaja tõttu on seda aga väga raske saavutada, kuna AKTH ja kortisooli füsioloogiline kõrgtase tekib kella 4–8 vahel hommikul. Varasemad uuringud ei ole kirjeldanud seost arteriaalse vererõhu ja erinevate hüdrokortisooni manustamisviiside vahel.

Uuringu eesmärgid

1. Teha kindlaks 21-OHD klassikalise vormi esinemissagedus Eestis.
2. Kirjeldada 21-OHD klassikalise vormiga patsientide feno- ja genotüüpi.
3. Kirjeldada 21-OHD klassikalise vormiga laste lühiajalist kasvamist ja võrrelda seda tervete laste omaga.
4. Hinnata hüdrokortisooni manustamise erinevate režiimide mõju klassikalise vormiga AGS patsientide 24-tunni vererõhule ja biokeemilisele kontrollile.

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Patsiendid ja meetodid

Epidemioloogia, genotüüp ja fenotüüp

Töö esimeses osas küsitlesime kõiki Eesti Endokrinoloogia Seltsi liikmeid neile teada olevate AGS patsientide kohta. Selgus, et antud sündroomiga patsiente on Eestis aastatel 1978–2004 diagnoositud 20 juhul. Kõigilt 20-lt patsiendilt ja/või nende vanematelt saadi nõusolek uuringus osalemiseks, Vereanalüüsid saadeti geeniuuringuks Suurbritanniasse St-Mary haigla Rahvusliku Geneetika Referentslaboratooriumisse Manchesteris. Seal eraldati DNA ja uuriti kõigepealt *CYP21A2* kuue sagedasima punktmutatsiooni suhtes [p.Pro30Leu, g.655A/C>G (intron2 splice site), p.Ile172Asn, p.Val281Leu, p.Gln318X ja p.Arg356Trp] kasutades PCR ARMS teste. Lisaks teostati skriining kahe sagedase mutatsiooni suhtes: 8. aluspaari deletsioon 3. eksonis (g.707_714del-GAGACTAC) ja suur deletsioon, mis avaldub „kimäärina“ 5`pseudogeeni ja 3´funktsionaalse geeni kokkusulamisel. Kuna DNA kvaliteet ja/või kvantiteet ei olnud mõningatel juhtudel piisav, siis ei saadud teha *Southern Blot* teste, eristamaks kas teises alleelis olev mutatsioon on sama, mis esimeses (homosügootsus) või on seal deletsioon/konversioon (hemisügootsus).

Et selgitada, milline on meie patsientidel genotüübi ja fenotüübi vaheline seos, jagati patsiendid genotüübi järgi kolme rühma. Grupi 0 moodustasid patsiendid, kellel mutatsioon ei jäta mingit 21-hüdroksülaasi aktiivsust (näiteks: deletsioon, konversioon, 8bp. del, p.Arg356Trp, p.Gln318X). Grupi A patsientidel esines mutatsioon, mis jätab väga madala, kuid siiski mõõdetava ensüümi aktiivsuse (näiteks: intron 2 splice mutatatsioon). Grupi B moodustasid patsiendid, kellel esines mutatsioon, mille korral on säilunud 1–2% ensüümi aktiivsusest (näiteks: p.Ile172Asn).

Lühiajaline kasvamine

Uuringugrupi moodustasid 6 AGS soolakaotusvormiga last (4 tütar- ja 2 poeglast) vanuses 3,9–9,7 aastat. Kuna ühe poeglapse mõõtmistulemustest puudus üks kolmandik, siis tema andmed jäeti lõplikust analüüsist välja. Kõik patsiendid said raviks hüdrokortisooni kolm korda ja fludrokortisooni üks kord päevas. Nende diagnoos kinnitus kliinilise pildi, tõusnud 17-OHP ja geenianalüüsidega. Uuringu ajal jälgisime 17-OHP taset kolmekuulise intervalliga, samuti hindasime luulist vanust enne ja pärast uuringu algust, et jälgida luulise küpsemise mõju kasvamisele.

Mõõtmiseks kasutasime Raveni minimeetreid, mis paigaldati patsientide kodudesse. Mõõtmine teostati lapsevanema poolt igapäevaselt enne magamaminekut. Uuringule eelnes kahenädalane treeningperiood mõõtmistehnika õppimiseks. Mõõtmisviga lapsevanemale arvutati 9 „pimeda“ kolmekordse mõõtmise standardhälbena, mis meie uuringus oli vahemikus 0,08–0,14 cm. Kokku saime 5 patsiendi kohta 4728 mõõtmistulemust. Saadud tulemusi analüüsiti

Kerneli regressiooni meetodil, kasutades nn. liikuvat „20-päeva keskmist“ kehapikkuse ja „60-päeva keskmist“ kasvukiiruse iseloomustamiseks. Toetudes varasemale uuringule defineerisime staasi kui perioodi, mil kasvukiirus jäi alla 0,007 cm/päevas. Kasvukiiruse graafikutelt hindasime järgmisi tunnuseid: kasvuspurtide ja staaside arv, spurtide keskmine kestus ja amplituud ning staasis veedetud aeg protsendina uuringuperioodi ajast.

Saadud tulemusi võrdlesime 43 terve lapse analoogsete tunnustega. Nende laste keskmine vanus oli vahemikus 5,5–7,7 aastat. Uuringu ja kontrollgrupi tulemuste võrdlemiseks kasutasime *Two Sample T-Testi.*

24-tunni vererõhk

Uuringus osalesid kõik Tartu Ülikooli Lastekliinikus jälgimisel olevat 6 last (2 poeg- ja 4 tütarlast) AGS soolakaotusvormiga. Nende vanus oli vahemikus 5,0–9,7 aastat. Kõik lapsed said varasemalt raviks hüdrokortisooni 3 korda päevas, suurema doosiga hommikul, ja fludrokortisooni 1 kord päevas. Patsiendid hospitaliseeriti 24 tunniks kahel korral. Esimesel korral said nad suurema doosi hommikul (režiim A). Haiglas oleku ajal määrati neil 17-OHP kolmel korral (kell 8.00, 14.00 ja 20.00) ning seerumi kortisool kahe tunni tagant. Vererõhu monitoorimisel kasutasime 24-tunni monitori Tonoport V. See väike kaasaskantav monitor võimaldab lapsel vabalt liikuda ja sooritada oma tavapäraseid päevaseid tegevusi. Aparaat oli programmeeritud mõõtma rõhku päeval iga 20 minuti ja öösel iga 30 minuti järel . Mõõtmisvea korral toimus kordusmõõtmine automaatselt 3 minuti pärast. Vererõhu väärtused arvutasime vanust ja sugu arvestades standardhälve punktideks (SDS), kasutades Saksamaa referentsväärtusi (Wühl *jt.* 2002). Pärast esimese uuringutsükli lõppu muutsime ravirežiimi: patsient võttis järgmised 4 kuud suurema hüdrokortisooni annuse õhtul (režiim B). Samal ajal jäid hüdrokortisooni ja fludrokortisooni päevased annused samaks. Seejärel toimus teine hospitaliseerimine, kus kordasime täpselt esimesel korral tehtud uuringuid. Statistiliseks analüüsiks kasutasime Minitab versiooni 15.0 *Two sample T-Testi.*

Uuringu peamised tulemused

1. Aastatel 1978–2004 oli AGS klassikalise vormi esinemissagedus Eestis 1:25 500 elussünni kohta ja uurimisperioodi viimase 13 aasta (1992–2004) jooksul 1:16 100.
2. Neljateistkümnel patsiendil (70%) esines kliiniliselt AGS soolakaotusvorm (7 mees- ja 7 naissoost) ja kuuel lihtne viriliseeruv vorm (1 mees- ja 5 naissoost).
3. Soolakaotusvormiga patsientide keskmine vanus diagnoosimisel oli 2 päeva tüdrukutel ja 30 päeva poistel. Nendest neljal tüdrukul ja kõigil seitsmel poisil avaldus haigus adrenaalse kriisina (hüponatreemia ja hüperkaleemia). Kõigil selle vormiga tütarlastel esines lisaks veel ka kliitori hüperplaasia erinevates raskusastmetes.

4. Lihtsa viriliseeruva vormiga tüdrukute keskmine vanus diagnoosimisel oli 1 aasta ja ainus poiss diagnoositi 7 aasta vanusena. Kliiniliselt avaldus haigus tal enneaegse pseudopuberteedi ja raske aknena, samas kui kõigil viiel tüdrukul esines kliitori hüperplaasia.
5. Kahekümnest patsiendist olid 17 mittesugulased ning nende 34 alleelil leiti 7 erinevat *CYP21A2* mutatsiooni. Sagedaim mutatsioon oli deletsioon/ konversioon („kimäär"), mis esines 7 alleelil (neist 4 eestlastel). P-Ile172Asn oli sagedaim punktmutatsioon, mis esines viiel alleelil (4 mitte-eestlastel). Intron 2 splice mutatsioon, 8bp. Del, p.Arg356Trp ja p.Gln318X esinesid kõik kolmel alleelil. Kaks mutatsiooni olid väikesed konversioonid, mis haarasid endas nelja meie poolt määratud järjestikust punktmutatsiooni (p.Ile172Asn, p.Arg356Trp, p.Val281Leu ja p.Gln318X). Seitsmel mittesuguluses oleval patsiendil ei saanud me määrata, kas teises alleelis oli esimese kordus (homosügoot) või kandis see deletsiooni/ konversiooni, mida ei määratud meie testiga (hemisügoot). Ühel lihtsa viriliseeruva vormiga patsiendil, kes oli heterosügootne mutatsioonile p.Gln318X, oli teises alleelis mutatsioon, mis ei olnud meie paneeliga määratav.
6. Genotüübi gruppidesse jaotusid patsiendid järgmiselt: Grupp 0–10 patsienti, Grupp A–3 patsienti ja Grupp B–6 patsienti. Eelmises punktis mainitud lihtsa viriseeruva vormiga heterosügootsest patsienti ei saanud grupeerida Seos genotüübi ja fenotüübi vahel oli hea.
7. Neljal AGS lapsel viiest oli bifaasiline lühiajalise kasvu muster spurtide ja staasidega.
8. Kasvukiiruse graafikute analüüsil selgus, et AGS lapsed veetsid statistiliselt oluliselt vähem aega staasides kui terved lapsed (5 ± 4,8% vs. 11,4 ± 7,2% uuringuperioodist; $p < 0,05$). Nende kasvuspurdi amplituud oli tervetest lastest oluliselt madalam (0,022 ± 0,008 cm/päevas vs. 0,037 ± 0,001 cm/päevas; $p < 0,01$) ning kasvuspurdi kestus oluliselt pikem (110,4 ± 28,3 päeva vs. 54,0 ± 13,1 päeva; $p < 0,05$). Lisaks leidsime, et pikkuse juurdekasv uuringuperioodi jooksul oli positiivselt seotud kasvuspurdi amplituudiga ($r = 0,9$, $p < 0,05$).
9. Režiimi A korral olid nii süstoolsed kui diastoolsed vererõhu väärtused madalamad kui populatsiooni keskmised (vastavalt –0,13 ja –0,72 SDS). Süstoolne rõhk tõusis tunduvalt režiimis B (0,92 SDS), olles üle 1 SDS kõrgem kui režiimis A. Kõrgemat süstoolset rõhku režiimis B võrreldes režiimiga A täheldasime nii öösel (1,11 vs 0,18 SDS) kui ka päeval (0,47 vs –0,35 SDS). 24-tunni keskmine diastoolne rõhk režiimis A oli madalam kui režiimis B (–0,7 vs 0,28 SDS). Mõlemas režiimis oli diastoolne rõhk öösel kõrgem (0,2 ja 0,8 SDS) kui päeval (–1,24 ja –0,41 SDS).
10. Neljal patsiendil tõusis režiimis B statistiliselt olulisel määral süstoolne rõhk ja kahel diastoolne rõhk. Keskmine öine vererõhu langus võrreldes päevasega oli 8,8% režiimis A ja 8% režiimis B. Ainult ühel patsiendil oli öise vererõhu langus mõlemas režiimis rohkem kui 10% päevasest.

11. Uuritud kuuest vererõhu näitajast (süstoolsed ja diastoolsed rõhud 24 tunni jooksul, eraldi nii päeval või öösel) olid kõik vähemalt 0,6 SDS kõrgemad režiimis B.

Järeldused

1. Aastatel 1992–2004 oli 21-hüdroksülaasi puudulikkuse klassikalise vormi esinemissagedus Eestis 1:16 000 elussünni kohta.
2. *CYP21A2* geeni mutatsioonide spekter Eestis oli sarnane teiste Euroopa populatsioonidega. Sagedaim mutatsioon oli deletsioon/konversioon. Seos genotüübi ja fenotüübi vahel oli hea. Suhteliselt hiline diagnoosimise aeg ja ebavõrdne mees ja naissoost patsientide suhe viitab vastsündinute 21-OHD skriiningu vajadusele Eestis.
3. Võrreldes tervete lastega iseloomustavad AGS laste lühiajalist kasvamist kestvad lühikese amplituudiga kasvuspurdid koos vähenenud ajaga staasides. Uuringuperioodi kehapikkuse juurdekasv oli korrelatsioonis kasvuspurdi amplituudiga. Suhteliselt stabiilne lühiajaline kasvamine AGS lastel viitab, et kui neil lastel tekib märkimisväärne kasvukiiruse tõus või aeglustumine, on see tingitud pigem üle- või alaravimisest, ja mitte mittelineaarsest kasvust enesest.
4. Õhtuse suurema hüdrokortisooni annuse manustamine ei parandanud AGS laste biokeemilist kontrolli, kuid tõstis nende 24-tunni vererõhku.

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The subjects of my research activities have been congenital adrenal hyperplasia in Estonia, its incidence, clinical picture and diseases influence on children's short-term growth and blood pressure. Total: 3 publications, 3 oral presentations at international conferences (including ESPE meeting 2007 in Helsinki) and 2 poster presentations.

List of publications:

I Liivak K, Tobi S, Schlecht H, Tillmann V. Incidence of classical 21-hydroxylase deficiency and distribution of CYP21A2 mutations in Estonia. Horm Res 2008;69:227–232.

II Liivak K, Foster PJ, Thalange N, Tillmann V. Short-Term Growth in Children with Congenital Adrenal Hyperplasia. Horm Res 2009;71:142–147.

III Liivak K, Tillmann V. 24-hour blood pressure profiles in children with congenital adrenal hyperplasia on two different hydrocortisone treatment regimens. Accepted for publications in Journal of Pediatric Endocrinology and Metabolism on 09.11.2008

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Teadustöö

Peamiseks uurimisvaldkonnaks on kaasasündinud adrenogenitaalse sündroomi esinemissagedus Eestis, selle kliiniline pilt ja haiguse mõju laste lühiajalisele kasvamisele ja vererõhule. Ilmunud on 3 publikatsiooni, 3 suulist ettekannet rahvusvahelistel konverentsidel (nendest 1 ESPE 2007 Helsingis) ja 2 poster-ettekannet.

Publikatsioonide loetelu:

I Liivak K, Tobi S, Schlecht H, Tillmann V. Incidence of classical 21-hydroxylase deficiency and distribution of CYP21A2 mutations in Estonia. Horm Res 2008;69:227–232.

II Liivak K, Foster PJ, Thalange N, Tillmann V. Short-Term Growth in Children with Congenital Adrenal Hyperplasia. Horm Res 2009;71:142–147.

III Liivak K, Tillmann V. 24-hour blood pressure profiles in children with congenital adrenal hyperplasia on two different hydrocortisone treatment regimens. 09.11.2008 publitseerimiseks vastu võetud ajakirjas Journal of Pediatric Endocrinology and Metabolism.

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