Congenital Adrenal Hyperplasia in Adults: Epidemiological, Genetic, Clinical and Endocrine Features of CYP21A2 Deficiency in Norway.

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In collaboration with

University of Bergen

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I am grateful to all the patients who have participated in these studies. Hopefully our research will contribute to better knowledge of the disease and ultimately improved care of the patients.

At last I would like to thank friends and family for all support, and especially thanks to my dear and patient husband Bjørn, my children Jostein and Sigrid, my sister Berit and her family and my mother Sigrid, who are always there for me.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Bodily pain</td>
</tr>
<tr>
<td>CAH</td>
<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CSHI</td>
<td>Continuous subcutaneous hydrocortisone infusion</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CYP21A2</td>
<td>Cytochrom P450, Family 21, subfamily A, polypeptide 2 gene</td>
</tr>
<tr>
<td>CYP21A2P</td>
<td>Cytochrom P450, Family 21, subfamily A, polypeptide 1 pseudogene</td>
</tr>
<tr>
<td>DHEAS</td>
<td>Dehydroepiandrosterone</td>
</tr>
<tr>
<td>DSD</td>
<td>Disorders of sexual development</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual X-ray absorptiometry</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GH</td>
<td>General health</td>
</tr>
<tr>
<td>HC</td>
<td>Hydrocortisone</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HRQoL</td>
<td>Health-related quality of life</td>
</tr>
<tr>
<td>HUNT</td>
<td>North-Trøndelag Health Study</td>
</tr>
<tr>
<td>IMT</td>
<td>Intima-media thickness</td>
</tr>
<tr>
<td>I2</td>
<td>Intron 2</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LH</td>
<td>Lutein stimulating hormone</td>
</tr>
<tr>
<td>MH</td>
<td>Mental health</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NC</td>
<td>Non-classical</td>
</tr>
<tr>
<td>17-OHP</td>
<td>17-hydroxyprogesterone</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PF</td>
<td>Physical functioning</td>
</tr>
<tr>
<td>PRA</td>
<td>Plasma renin activity</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>21-OH</td>
<td>21-hydroxylase</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>21-OHD</td>
<td>21-hydroxylase deficiency</td>
</tr>
<tr>
<td>QOLS</td>
<td>Quality of Life Scale</td>
</tr>
<tr>
<td>RE</td>
<td>Role-emotional</td>
</tr>
<tr>
<td>RP</td>
<td>Role-physical</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation score</td>
</tr>
<tr>
<td>SF</td>
<td>Social functioning</td>
</tr>
<tr>
<td>SF-36</td>
<td>Short Form-36</td>
</tr>
<tr>
<td>SV</td>
<td>Simple virilising</td>
</tr>
<tr>
<td>SW</td>
<td>Salt-wasting</td>
</tr>
<tr>
<td>TART</td>
<td>Testicular adrenal rest tumours</td>
</tr>
<tr>
<td>VT</td>
<td>Vitality</td>
</tr>
</tbody>
</table>
SUMMARY

Background
Congenital adrenal hyperplasia (CAH) is an inherited recessive disorder, in which enzymes in the adrenal cortex are mutated. The most common form is caused by alterations in \textit{CYP21A2}, the gene encoding the adrenal steroid 21-hydroxylase enzyme. The consequences are impaired production of cortisol and aldosterone, leading to increased ACTH driven production of adrenal androgens, resulting in virilisation of the female. The patients require life-long treatment with glucocorticoid and mineralocorticoid tablets.

Objective
No published data on CAH in Norway existed when we started our survey. Our aim was to obtain confident epidemiological data from Norway and to describe clinical and genetic aspects in adults with CAH due to 21-hydroxylase deficiency. Specific aims were to describe health-related quality of life and working ability, and the frequency of testicular and adrenal tumours.

Material and methods
Epidemiological data were obtained by scrutiny medical reports at all the University Hospitals in Norway and by contacting endocrinologists throughout the country. First, the patients were invited to a questionnaire survey including medical history, and the Short Form-36 and Quality of Life Scale questionnaire. Second, they were invited to participate in clinical, biochemical and radiological investigations at four University Hospitals, including anthropometric measurements, that is, dual X-ray absorptiometry scanning, adrenal computed tomography, testicular ultrasound, DNA sequencing of \textit{CYP21A2} and hormone analyses.

Results
We showed lower incidence of CAH than expected and especially than that of our neighbouring country Sweden. Fewer males than females with CAH were identified although equal sex ratio was expected. As a group the patients had increased working disability and reduced physical and mental health as compared with the normal population. The women had decreased fertility.

We found a high frequency of adrenal tumours, particularly myelolipomas, and testicular
adrenal rest tumours only associated with salt wasting. The patients had normal BMI but markedly higher fat mass, higher diastolic blood pressure and lower bone mineral density than the general population. We showed higher frequency of osteopenia among the men than the women. Near half of the women had testosterone levels above the normal range and half of the subjects had 17-hydroxyprogesterone (17-OHP) levels above the upper recommended target. We identified four novel and plausibly disease-causing CYP21A2 mutations and, as expected, high correspondence between genotype and clinical phenotype. The frequency of the underlying genetic defects was similar to published results from other Western European countries.

**Conclusion**

Adult men with CAH were missing and perhaps never diagnosed. Androgen levels and 17-OHP-levels were poorly controlled. We found reduced BMD, impaired quality of life, and increased frequency of adrenal tumours, and in females impaired fertility. These findings may reflect inappropriate glucocorticoid and mineralocorticoid therapy and need for improvement of the medical treatment and general care of adults with CAH.
LIST OF PAPERS


BACKGROUND

Congenital adrenal hyperplasia

Definition
Congenital adrenal hyperplasia (CAH) is a spectrum of genetic disorders causing deficiencies in the steroidogenic enzymes in the adrenal cortex. More than 95% of CAH cases are 21-hydroxylase deficiency (21-OHD), with defective 21-hydroxylase (21-OH) enzyme, which is encoded by the CYP21A2 gene (1;2). This enzyme deficiency leads to impaired conversions of progesterone to deoxycortisosterone, a precursor to aldosterone, and of 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol, which is a precursor to cortisol (Figure 1). Patients with 21-OHD therefore have exceedingly high levels of 17-OHP when untreated or undiagnosed. The condition is divided into classical CAH, which includes the salt-wasting (SW) and simple virilising (SV) forms, and non-classical (NC) or “late-onset” form. The SW entails complete lack of cortisol and aldosterone, and the SV displays a degree of cortisol depletion; both are accompanied by adrenocorticotropic hormone (ACTH)-driven increase in adrenal androgens. The NC does not have cortisol or aldosterone deficiency, but has manifestations of hyperandrogenism that typically presents later in childhood or in early adulthood. NC is a common cause of hyperandrogenism in adult woman world-wide (3). The second most common form of CAH is 11-ȫ-hydroxylase (CYP11 B1) deficiency with an incidence of one per 100 000 (4). Deficiency of 11-ȫ- hydroxylase results in decreased conversion of deoxycorticosterone to corticosterone, a precursor to aldosterone, and of 11-deoxycorticosterone to cortisol and is characterised by hyporeninemic, hypokalemic hypertension and hyperandrogenism (4;5). These patients rarely have salt-wasting crises as aldosterone is synthesized by the CYP11B2 enzyme, which is unaffected in this disorder. Other rare disorders of adrenal steroidogenesis are aldosterone synthase deficiency (CYP11B2), 17-α-hydroxylase deficiency (CYP17), 3-ȫ-hydroxysteroid dehydrogenase deficiency (HSD3B2) and lipoid hyperplasia (StAR; Steroid acute regulatory protein), with different clinical representation due to where the adrenal enzymes are blocked (Table 1) (6). In the following CAH refers to 21-OHD.
Figure 1 Adrenal steroidogenesis. Red labelling shows the 21-hydroxylase enzyme and CYP21A2 is the current notation for the mutated gene. From Perrin C. White (6).
Table 1 Characteristics of different forms of congenital adrenal hyperplasia.

<table>
<thead>
<tr>
<th>Disease</th>
<th>21-hydroxylase deficiency</th>
<th>11-β-hydroxylase deficiency</th>
<th>Aldosterone synthase deficiency</th>
<th>17-α-hydroxylase deficiency</th>
<th>3-β hydroxysteroid dehydrogenase deficiency</th>
<th>Lipoid hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defective gene</td>
<td>CYP21A2</td>
<td>CYP11β1</td>
<td>CYP11β2</td>
<td>CYP17</td>
<td>HSD3B2</td>
<td>STAR</td>
</tr>
<tr>
<td>Ambigious Genitalia</td>
<td>+ in females</td>
<td>+ in females</td>
<td>No</td>
<td>+ in males</td>
<td>+ in males</td>
<td>No puberty in females</td>
</tr>
<tr>
<td>Adrenal crises</td>
<td>+</td>
<td>Rare</td>
<td>Salt wasting only</td>
<td>No</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Incidence (general population)</td>
<td>1/15 000</td>
<td>1/100 000</td>
<td>Rare</td>
<td>Rare</td>
<td>Rare</td>
<td>Rare</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>↓</td>
<td>↓</td>
<td>Normal</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>Mineralocorticoids</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Androgens</td>
<td>↑</td>
<td>↑</td>
<td>Normal</td>
<td>▼</td>
<td>▼ in males</td>
<td>↓</td>
</tr>
<tr>
<td>Oestrogens</td>
<td>Relatively ↓ in females</td>
<td>Relatively ↓ in females</td>
<td>Normal</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Elevated metabolites</td>
<td>17-OHP</td>
<td>DOC, 11-deoxycortisol</td>
<td>Corticosterone</td>
<td>DOC corticosterone</td>
<td>DHEA, 17-hydroxy-pregnenolone</td>
<td>None</td>
</tr>
</tbody>
</table>

(17-OHP: 17-hydroxyprogesterone; DOC: deoxycorticosterone; DHEA: dehydroepiandrosterone)
History

In 1631 a famous painting of a virilised woman and her husband was made by Alfonso E.F. Sancick (Figure 2), which could very well be a woman with SV form of CAH.

Figure 2 Painting of a virilised woman from 1631, painted by Alfonso E.F. Sancick (from Google).
The first known patient with CAH was described in 1865 by Luigi De Crecchio, a professor of anatomy in Napoli (7). He was doing an autopsy of a man named Giuseppe Marzo, who died suddenly in his forties. The professor expressed marked surprise at the findings of uterus and fallopian tubes in this man, who also happened to have very large adrenals. The patient had a six centimeter long “penis”, but no testes. At birth he was initially regarded a girl, but at four years of age he was reconsidered a male. As an adult he fell in love with a girl; however, when he proposed to marry her she ran away after realising that his birth certificate carried the name Guiseppina. The story goes that Giuseppe died of a broken heart, but probably he died of a salt-wasting crisis (8). CAH is a common cause of disorders of sexual development (DSD), and Giuseppe’s story is still relevant in 2012. SW CAH was a deadly disease until the glucocorticoids became available in the early 1950’s as patients very often died of salt-wasting crisis in the neonatal period. Some milestones in the modern history of CAH are illustrated in Table 2.

Table 2 Modern history of CAH.

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1920</td>
<td>CAH is an autosomal trait</td>
</tr>
<tr>
<td>1937</td>
<td>T. Reichstein synthesized the first active substance from the adrenal cortex, 11-deoxycortisone (11-DOC) (9)</td>
</tr>
<tr>
<td>1949</td>
<td>E. C. Kendall synthesized cortisone, and the first CAH patients started treatment with cortisone (10)</td>
</tr>
<tr>
<td>1949</td>
<td>17-hydroxyprogesterone was synthesized (11)</td>
</tr>
<tr>
<td>1950</td>
<td>Nobel Prize to Reichstein, Kendall and Hench</td>
</tr>
<tr>
<td>1952</td>
<td>Aldosterone was isolated and synthesized (12)</td>
</tr>
<tr>
<td>1960s</td>
<td>The radioimmunoassays for serum hormone measurement were developed</td>
</tr>
<tr>
<td>1977</td>
<td>The 21-hydroxylase gene was mapped on the short arm of chromosome 6p (13)</td>
</tr>
<tr>
<td>1984</td>
<td>The 21-hydroxylase gene was cloned and the structure of the gene described (14)</td>
</tr>
<tr>
<td>2004</td>
<td>Mutation was found in P450reductase, the obligate electron donor for CYP enzymes (15)</td>
</tr>
</tbody>
</table>
Epidemiology

CAH due to 21-OHD deficiency is considered one of the most common inborn autosomal recessive disorders. The prevalence is lower than the incidence, because of deaths due to salt wasting crises, especially in SW born before the 1950’s. The world-wide incidence of the classical form of CAH, based on neonatal screening programmes with more than 6.5 million neonates included, has been estimated at 1/15 000 (16), but with considerable ethnical and geographical variation. The Yupic Eskimos in Alaska has the highest reported incidence at 1/282 (17), in contrast to Afro-Americans in Texas, who have the lowest known incidence at 1/42 309 (18). In Sweden, incidence at 1/9 800 was recently reported based on neonatal screening programs (19). In Finland, which still does not perform neonatal screening, the reported incidence is 1/15 000 (20) (Table 3). NC CAH is a common cause of hyperandrogenism in adult woman with an incidence of 1-2/1000 in the general Caucasian population and as high as 1-2/100 among inbred populations such as Eastern European (Ashkenazi) Jews (21). However, my personal experience as an endocrinologist is that we rarely diagnose NC in Norway.

Table 3 Incidence of CAH in different countries and populations.

<table>
<thead>
<tr>
<th>Country/population</th>
<th>Incidence</th>
<th>Year</th>
<th>Neonatal screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yupic Eskimos in Alaska (22)</td>
<td>1/282</td>
<td>1993</td>
<td>+</td>
</tr>
<tr>
<td>Réunion in the Indian Ocean (17)</td>
<td>1/2 141</td>
<td>1998</td>
<td>+</td>
</tr>
<tr>
<td>Sweden (23)</td>
<td>1/ 9 800</td>
<td>1994</td>
<td>+</td>
</tr>
<tr>
<td>Finland (24)</td>
<td>1/15 000</td>
<td>1995</td>
<td>-</td>
</tr>
<tr>
<td>The Netherlands (25)</td>
<td>1/11 764</td>
<td>2001</td>
<td>+*</td>
</tr>
<tr>
<td>Australia, states with screening (26)</td>
<td>1/15 488</td>
<td>1997</td>
<td>+</td>
</tr>
<tr>
<td>Mainland France (27)</td>
<td>1/ 15 699</td>
<td>2003</td>
<td>+</td>
</tr>
<tr>
<td>Great Britain (28)</td>
<td>1/18 000</td>
<td>2012</td>
<td>-</td>
</tr>
<tr>
<td>Australia, states without screening (26)</td>
<td>1/18 034</td>
<td>1997</td>
<td>-</td>
</tr>
<tr>
<td>Afro-Americans in USA (Texas) (18)</td>
<td>1/42 309</td>
<td>1998</td>
<td>+</td>
</tr>
</tbody>
</table>

*Pilot screening
Genetics of CYP21A2

The CYP21A2 gene encodes the 21-OH protein, which is a microsomal cytochrome P450 enzyme essential for adrenal steroidogenesis. White et al. cloned the gene and described its protein structure in 1984 (14). CYP21A2 is located on the short arm of chromosome 6 (band 6 p21.3) in the HLA class III gene region, near the genes encoding the fourth component of complement C4A and C4B. The CYP21A2 gene has a 98% homologous inactive pseudogene CYP21A1P, which is located adjacent to the active gene (Figure 3). One 21-OH gene and one C4 gene form one unit; these units are tandemly repeated. The CYP21A2 gene consists of ten exons, whereas the genes for other P450 enzymes contain seven, eight, or nine exons. The enzyme is at most 28% homologous to other cytochrome P450 enzymes that have been studied. The inactive pseudogene has an 8-base deletion in codons 110 through 112, resulting in a frameshift and a stop codon at codon 130; a second frameshift and a nonsense mutation occur farther downstream. The two CYP21 genes have nine introns and are about 3.4 kb long (29). The majority of the disease-causing mutations in CYP21A2 have arisen through interaction with CYP21A1P; the most common are deletions or large gene conversions of the entire CYP21A2 and/or pseudogene-derived point mutations. In a large cohort from The Netherlands analysis of 370 unrelated alleles revealed 31.9% deletion/conversion, 28.1% intron 2 (I2) splice mutation, and 12.4% with the point mutation I172N (30). Mutation frequencies in other Western European countries are similar (31;32).

Correlations between genotype and phenotype

The correlation between genotype and clinical phenotype in CAH deficiency is usually high and robust (33). In compound heterozygous cases, the mildest mutation defines the genotype group. Clinically the three different phenotypes are SW, SV, and NC. Based on genotype four groups are recognized (34) (Table 4). The SW belongs to group Null and A, the SV to group B and sometimes group A or C; the NC to group C. The phenotype can change with age, typically with recovery from salt loss (35). Sometimes patients have residual aldosterone production despite severe mutations (30).
Figure 3  Schematic representation of the CYP21A region and the two actual genes CYP21A2 and the pseudogene CYP21A1P. The several deleterious mutations in the 98% homologous pseudogene can be transferred to CYP21A2 through recombination or gene conversion. The nine most common mutations are marked. RP: telometric RP gene; TNXB: centromeric TNX gene. From Anna Wedell (36) with permission from Karger.
Table 4  Genotypes in CAH grouped according to predicted severity of mutations in CYP21A2, their phenotype and 21-hydroxylase activity in vitro (34).

<table>
<thead>
<tr>
<th>Mutation group</th>
<th>Common Mutation</th>
<th>Clinical severity</th>
<th>Phenotype</th>
<th>Enzyme activity (in vitro) Percentage of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>Deletion, Del 8 bp E3, Cluster E6, L307 ins, Q318X and R356W</td>
<td></td>
<td>SW</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>A</td>
<td>I2 splice</td>
<td>SW/(SV)</td>
<td></td>
<td>&lt;2%</td>
</tr>
<tr>
<td>B</td>
<td>I172N</td>
<td>SV</td>
<td></td>
<td>&lt;10%</td>
</tr>
<tr>
<td>C</td>
<td>P30L V281L and P453S</td>
<td>SV/NC</td>
<td></td>
<td>&lt;75%</td>
</tr>
</tbody>
</table>

Clinical presentation

Classical CAH

One study found that about 70% of those with classical CAH were SW (16). SW is the most severe phenotype, characterised by deficiency of both cortisol and aldosterone, and clinical presentation with life-threatening salt-losing crises early in life. Soon after delivery they develop severe symptoms with vomiting, hypovolemia, hypotension and pronounced electrolyte disturbances characterised by hyperkalemia and hyponatremia. Boys are at increased risk, as they are unlikely to be diagnosed by clinical examination. The increased production of ACTH and adrenal androgens only yield subtle hyperpigmentation and possibly an enlarged penis. A recently published study from UK, a country without neonatal screening, demonstrated that in the first months of life, most girls (77%) present with genital virilisation, whereas most boys (73%) present with salt-wasting crises (28). They found that only 6% of the girls remained undiagnosed by day 14, in contrast to 50% of the boys.

Female neonatals with the SW and SV forms have severe virilisation of external genitalia due to androgen excess in utero, usually less pronounced in SV than in SW. Characteristic findings include an enlarged clitoris, partly fused labia majora and a common urogenital sinus
in the place of a separate urethra and vagina, whereas the internal female organs, the uterus, fallopian tubes, and ovaries, are normal (1). The majority of the SV girls are diagnosed during the neonatal period. However, some girls can be mistaken as boys, because their pronounced clitoris hypertrophy is interpreted as a penis. CAH is a common cause of DSD. Screening for CAH will result in earlier diagnosis of girls and avoid much harm to the girls and the parents.

In older children, untreated or unrecognised CAH may result in premature puberty, accelerated bone maturation, and fertility problems. Untreated boys with the SV form start puberty and virilisation at age two-four years and can become full-grown as early as seven years old. This implies that these patients, and particularly the boys, are taller than their peers as children and lower as adults.

*Non-classical CAH*

The NC or late-onset form of CAH gives symptoms around puberty in females. Affected girls are born with normal clitoris or mild clitoris hypertrophy. The patients have sufficient enzyme activity to produce both cortisol and aldosterone with increased ACTH stimulation. The ensuing adrenal hyperandrogenism is the problem, but to a much lesser extent than in classical CAH. The most common presenting symptom in children is premature pubarche (37;38), severe cystic acne (39), accelerated growth and tall stature in early childhood (40). Women with NC suffer from hirsutism, menstrual disorders including amenorrhea, anovulation, oligomenorrhea (3), and infertility (41). Whether NC is a clinical problem in men is uncertain, and the majority are only diagnosed during family screening (42).

*Diagnosis of CAH*

The diagnosis is made by clinical, hormonal, genetic, and sometimes radiological investigations. It is important to suspect CAH in neonates with ambiguous genitalia. The typical clinical presentation in neonatal is a female with enlarged external genitalia, which could be misinterpreted as a penis. A physical examination is mandatory, that is, to palpate for gonads in the inguinal canals and labia or scrotum and to look for the urethral meatus. Karyotyping should also be done to predict the chromosomal sex. Hyperpigmentation due to increased ACTH could be difficult to discover in the infants if you are not primed to look for it. Two thirds have concomitant salt-wasting crises; however, this rarely occurs before seven
days of life (16). CAH also has to be suspected in neonatal males with salt-wasting crises. The typical electrolyte disorders are hyperkalemia and hyponatremia. To diagnose aldosterone deficiency measurement of aldosterone and renin is required; aldosterone is low and renin compensatorily high and the aldosterone to renin ratio is low (43;44). In the first days of life this is however difficult to interpret as plasma renin activity and aldosterone are elevated in many healthy infants (45).

Basal serum 17-OHP is the diagnostic test; if this is clearly elevated, the diagnosis of 21-OHD is definitive. A three days post partum 17-OHP value above 242 nmol/L is diagnostic of classical 21-OHD in a full-term infant (1); the normal value is less than 3 nmol/L. In countries with neonatal screening most of the patients will be diagnosed shortly after birth (see the next section). If diagnosed later in childhood or in adulthood levels of 17-OHP in the morning below 2.5 mmol/L and 6.0 mmol/L, respectively, effectively rule out CAH (1). Basal or stimulated 17-OHP above 300 nmol/L is typical for classical CAH (2). The cut-off values for NC is basal 17-OHP of 15 nmol/L and/or ACTH-stimulated 17-OHP of 45 mmol/L, measured at any time of the day or any time during the menstrual cycle (1) or basal and/or stimulated 17-OHP level above 6 and 30 mmol/L, respectively, measured in the follicular phase (6) tested with high dose ACTH (250 μg). An ACTH-stimulation test can be done to diagnose NC, to evaluate the cortisol production, and to rule out other enzyme deficiencies. In 11β-hydroxylase deficiency stimulated 17-OHP may be moderately elevated, but rarely above 40 nmol/L. Genetic analysis can be useful to confirm the diagnoses and also provide additional information such as prediction of the clinical course and hopefully prevent serious consequences (46).

**Neonatal screening**

Neonatal screening for CAH implies screening for the classical form of 21-OHD as NC displays too low levels of 17-OHP to be detected. However, occasionally, 11β-hydroxylase deficiency can also be detected by such screening. In many countries, including the USA, neonatal screening for 21-OHD is an approved part of the neonatal screening programme. In 2009, forty-nine states in the USA and at least 16 other countries screened infants for CAH, and at least 13 additional countries had pilot or local screening programs in 2009 (47). In Norway, screening commenced in 2012. The primary goals of neonatal screening for CAH are to prevent neonatal death from salt-wasting crises, inaccurate sex assignment in females
with complete virilisation, and irreversible childhood hyperandrogenism. CAH is well suited for screening as it is fairly common, potentially fatal, and treatable. Initial testing is easy to perform by analysing 17-OHP by immunoassay on dried blood-spots on the filter paper card. However, this assay has low specificity, yielding a positive predictive value of approximately 1% as estimated from data from the entire USA in 2003-2007 (47), and frequent false-positive results. The cut-off levels of 17-OHP is the main issue as too low levels will give many false positive tests; however, too high level will reduce sensitivity and give false negative tests. Most affected neonates have concentrations greater than 105 nmol/L (48). Other factors that limit the accuracy of the test are that the levels of 17-OHP are normally high at birth and decrease rapidly during the first postnatal days, and that premature or sick babies also have high levels. Neonatal girls have lower mean 17-OHP levels than neonatal boys, which slightly reduces the sensitivity of the screening for CAH in girls (49). Many screening programs have established reference ranges that are based upon weight and gestational age to improve the positive predictive values. In The Netherlands this improved the positive predictive value of CAH screening from 4.5% to 16% (25). It is still not well documented that neonatal screening of CAH reduces mortality by identifying infants with severe SW before they develop adrenal crises (50). The documented effects of neonatal screening are earlier diagnosis in affected neonates, increased detection of males, and higher recorded incidence than in countries were diagnosis is based on case reports (16). Since Sweden started neonatal screening in 1986 they have no documented fatal event of salt-wasting crises, the recorded incidence of CAH has increased, particularly for males. One quarter of the CAH girls and three quarters of the CAH boys in the Swedish screening program were diagnosed only by screening and would not have been detected by clinical investigation in the neo-natal period alone (23).

Treatment
The aim of the treatment is to replace the missing glucocorticoid and mineralocorticoid hormones to prevent salt-wasting crises and death, and to normalise androgens to prevent virilisation. Other goals are to optimise growth and development and to protect against co-morbidities.

Glucocorticoids
Since the aim of glucocorticoid treatment in CAH is not only to replenish glucocorticoids and
mineralocorticoids, but also to suppress ACTH driven androgen production, the doses needed are often higher than in patients with primary adrenal insufficiency. Neither of the conventional glucocorticoid therapies satisfactorily mimics the normal diurnal cortisol profile, and new modes of glucocorticoid delivery have been proposed. Modified-release hydrocortisone (HC) tablets have been produced, which better restore the normal rhythm and hence might yield suppression of adrenal androgens production with lower glucocorticoid doses (51). Recently, cases with poorly treated CAH have been successfully controlled with continuous subcutaneous HC infusion (CSHI) (52-54) a treatment previously introduced for patients with Addison’s disease (55).

Suppression of ACTH is important in both women and men, as high ACTH levels stimulate growth of both testicular adrenal rest tumours (TART) and the adrenals themselves, sometimes with the development of myelolipomas (56;57). Adrenal myelolipomas are benign tumours composed of adipose and myeloid tissue. Whether high adrenal androgens are harmful for adult males is not known, but when the adrenal source of androgens dominates, the hypothalamo-pituitary-testicular axis is suppressed, resulting in sterility and infertility.

Adults are often treated with long-acting glucocorticoids or a combination of long-acting and short-acting glucocorticoids. The most recent guidelines suggest that adult patients with classical CAH should be treated with HC or long-acting glucocorticoids (2), which is a very unspecific advice. In practice, adults are often treated with prednisolone (58;59); however, there are no studies on long-term follow-up of different modes of treatment of adults, and practice varies.

Physiological cortisol secretion rates are about 6 mg/m² daily (60;61) and most patients have satisfactory control of androgen production with HC doses of 12-18 mg/m² daily divided into two or three administrations (1). The latest Endocrine Society Clinical Practice Guidelines recommend 15-25 mg HC daily, alternatively prednisolone 4-6 mg, or dexamethasone 0.25-0.5 mg daily (2). This approach is a trade-off between overtreating the patients, with side effects of glucocorticoids, or undertreating, with complications due to hyperandrogenism and hypocortisolism (62). In children there are more established guidelines for management recommending the use of short-acting glucocorticoids such as HC 10-15 mg/m²/day divided into three doses, to optimize growth and final adult height (63). The treatment in children also deals with gender assignment, genital surgery and pubertal development. In Norway
cortisone acetate is the drug of choice in both children and adults, as HC is only available on registration exemption. Cortisone acetate has to be converted to cortisol for biological activity and this conversion can be impaired due to low activity of hepatic 11β-hydroxysteroid dehydrogenase type 1 and therefore perceived a less favourable alternative (64). However, dysfunction of this enzyme is not known to pose a problem in clinical practice. The latest Endocrine Society guidelines recommend against the use of stress doses in mental and emotional stress, minor illness, and before physical exercise (2). However many of our patients, perceive that extra doses in these situations are helpful and Reisch et al. have also recommended this (62).

As in patients with Addison’s disease (primary adrenal failure) it is important to educate the patient to increase the glucocorticoid doses under stress such as febrile illness and surgery to avoid life threatening adrenal crises. Only the study by Reisch et al. has focused on this important issue in adult patients with CAH (62) in which few patients were equipped with emergency cards. The Reisch et al. recommendations to prevent adrenal crises are summarized in Table 5.

The optimal dose and administration schedule of hydrocortisone in critically ill CAH patients is unknown, but 100 mg bolus doses of HC i.v. four times daily is often used (2). However Charmadari et al. measured plasma cortisol concentrations after i.v. HC in children with CAH and demonstrated very high peak levels within ten minutes after the bolus was given, followed by a rapid fall thereafter (65). They concluded that critically ill patients with classical CAH would be best managed with a single intravenous HC bolus followed by a constant infusion.

Mineralocorticoids
Fludrocortisone is the only drug available as mineralocorticoid replacement. It is recommended that all classical CAH patients be treated with mineralocorticoids at diagnosis in the neonatal and early infancy period. The need for mineralocorticoids often decreases with age for a number of reasons. One is that serum aldosterone is high in healthy subjects at birth, and this could be due to low renal mineralocorticoid receptor activity (45). Another reason could be extra-adrenal 21-hydroxylation (35;66) being able to generate enough aldosterone to decrease salt loss. Another argument for using fludrocortisone are studies
Table 5  Practical guidelines for CAH patients to prevent Adrenal Crises (Adapted from Reisch et al. (62).

<table>
<thead>
<tr>
<th></th>
<th>Practical guidelines for CAH patients to prevent Adrenal Crises</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Always carry your steroid emergency card with you.</td>
</tr>
<tr>
<td>2</td>
<td>Situations that require glucocorticoid dose adjustment by yourself (triple dose of glucocorticoid immediately):</td>
</tr>
<tr>
<td></td>
<td>a) Nausea with vomiting and diarrhoea: if no i.v. or i.m. injection is available, repeatedly take three times the oral dose of hydrocortisone despite vomiting, as some absorption of hydrocortisone takes place very quickly. See a physician without delay.</td>
</tr>
<tr>
<td></td>
<td>b) Intercurrent illness with fever &gt;38.5°C</td>
</tr>
<tr>
<td>3</td>
<td>Situations where you may benefit from glucocorticoid adjustment:</td>
</tr>
<tr>
<td></td>
<td>a) Sustained psychological distress</td>
</tr>
<tr>
<td></td>
<td>b) Extensive physical exercise: be cautious with glucocorticoid dose adjustment, as this has not been shown to be beneficial, but increase intake of sugar/carbohydrates (67)</td>
</tr>
<tr>
<td>4</td>
<td>Other situations: extreme heat- increase water, and in particular salt intake</td>
</tr>
<tr>
<td>5</td>
<td>Mineralocorticoid dose can be continued as usual, no increase of dose necessary</td>
</tr>
<tr>
<td>6</td>
<td>Situations that require glucocorticoid dose adjustment by a physician:</td>
</tr>
<tr>
<td></td>
<td>a) Surgical intervention</td>
</tr>
<tr>
<td></td>
<td>b) Shock, severe trauma, coma, and emergency surgery</td>
</tr>
<tr>
<td></td>
<td>c) Continued vomiting and diarrhoea</td>
</tr>
</tbody>
</table>

indicating that all forms of 21-OHD have some degree of aldosterone deficiency (44;68) and that patients requiring mineralocorticoids have better height outcome compared with non-SW patients (69). Fludrocortisone replacement even in mild aldosterone deficiency results in lower glucocorticoid dose requirement and better height outcome (70). The usual daily dose of fludrocortisone ranges from 0.05-0.2 mg/day in adults independent of body size administered in the morning. Recovery from salt-wasting has been described and sensitivity to mineralocorticoids may vary over time. Therefore the need for continuing mineralocorticoid and/or salt supplementation should be reassessed regularly based on blood pressure, plasma renin activity (PRA), and perhaps the aldosterone to PRA ratio (44;66).

Many patients have a large salt consumption, which could be an indication of need for more fludrocortisone. Additional salt supplements are given on a routine basis to maintain plasma sodium concentrations in the normal range in the first 6-12 months of life (1-2 g daily) (71).
Later additional salt intake may be needed with exposure to hot weather and patients should be encouraged to use salt freely to satisfy salt cravings (72) and be told that sodium salt-intake is important for them and not harmful.

**Monitoring**

The glucocorticoid doses are monitored with clinical and biochemical parameters. Biochemical monitoring of therapy in CAH is however controversial and difficult as the serum hormones fluctuate with time of day and intake of glucocorticoid tablets. Hence, unfortunately, target concentrations are not clearly defined. The three most commonly used adrenal steroids for monitoring treatment are 17-OHP, androstendione and testosterone. Levels of 17-OHP may be elevated at night and suppressed after the morning glucocorticoid doses 17-OHP, and displays a circadian rhythm resembling that of cortisol with nadir at midnight and peak in the early morning (73). However, although a random test of serum hormones has limited value, the therapeutic efficacy has until now been assessed by measurement of 17-OHP in the mornings before intake of glucocorticoid tablets (6;63;74). The level of 17-OHP should not be normalized because of risk of iatrogenic Cushing’s syndrome (signs and symptoms associated with prolonged exposure to inappropriately high levels of cortisol). Thus, the recommended target range in the morning is from 12-36 nmol/L (1). Androstendione levels reflect the levels of adrenal androgens and should be within a range appropriate for the patient’s age and sex (74). Testosterone can also be a useful parameter, especially in women. In men, the measured testosterone levels mainly derives from the testicles; it may be reduced due to high glucocorticoid doses or pronounced TART (75). Alternative measurements for adjusting the glucocorticoid doses include 24h urine collection for pregnantriol, a urinary metabolite of 17-OHP, and 17-ketosteroids, which are urinary metabolites of androgens, or by 17-OHP time series with dried blood spots or salivary 17-OHP day profiles (76-78).

The mineralocorticoid doses should be adjusted to maintain plasma renin activity in mid-normal range. Nimkarn et al. suggested that the aldosterone to PRA ratio is a better marker of mineralocorticoid requirement (44). The clinical assessment is as important as or more important than the biochemical parameters. In children growth velocity and bone age are important. In adults weight, blood pressure, bone mineral density (BMD), amenorrhoea and
signs of both iatrogenic Cushing’s syndrome and undertreatment of glucocorticoids with fatigue and adrenal crises should be assessed (2;62).

**Outcome**

Studies of clinical outcome in adult CAH patients over 30 years of age, and especially men, are sparse and the cohorts often small, with the exception of two larger studies published recently (59;62). The studies often originate from single treatment centres. No studies on mortality or serious outcomes as cardiovascular disease have been published for this patient group. No clear consensus exists for the medical management of adults with CAH although the Endocrine Society Guidelines appeared in 2010 (2). During the research period several actual studies appeared with the outcomes we were looking for, and published either before or after our papers. These references are marked with *italic*.

**Health-Related Quality of Life (HRQoL)**

Data on HRQoL in CAH are diverging and before the start of this study; less than five surveys included adult CAH, and only one included men. Some studies revealed better and some worse HRQoL than reference populations. The CAH population is heterogeneous, ranging from patients with a traumatic childhood with intersex issues, girls with severe virilisation who have never been on a public beach, to the patients who were diagnosed prenatally and treated in a specialist centre with excellent results.

Another issue is that many studies have shown that girls with CAH have a masculinized behaviour, that is, tomboy play behaviour, aggressive manners and altered spatial perception (79). Gender-atypical behaviour is also seen in adult age, such as homosexuality, choice of typical ‘male’ professions and leisure activities (80). Behavioural masculinisation in girls with CAH is believed to result from the exposure of the brain to high levels of androgens during fetal development (81). It may also be assumed that the younger CAH population has received better treatment than the older population and that introduction of neonatal screening in many countries contributes to improvement in HRQoL. Two recent larger studies of younger men and women with classical CAH from UK and Germany showed significantly impaired HRQoL (59;82). Several smaller studies including only women support these findings (83-85). No difference from controls was seen in two other reports in women.
In contrast, a Finnish study with men and women reported significantly better HRQoL in the CAH patients than in the normative controls (88).

**Fertility**

*Female fertility*

Low pregnancy rates among females with CAH have been reported, especially among women with the SW form (89-92). Many factors could contribute to this, for instance congenital genital malformations with masculinization of the external genitalia and an inadequate introitus (89). Other causes may be directly hormonal under conditions of undertreatment; high adrenal androgens result in virilisation and suppression of gonadotropin secretion from the pituitary, leading to oligo- and amenorrhoea and anovulation (90). Moreover progesterone hypersecretion has a negative effect on endometrial receptivity. Overtreatment with glucocorticoid also suppress gonadotropins (93). Higher frequencies of polycystic ovaries are also reported in CAH, which might inhibit ovulation (94).

Homosexuality and single status is more common among CAH women than in the background population. Homosexuality is reported at 20% in two different studies vs. 1.6-5.7% in the control groups (80;95). In the study by Frisén et al. homosexuality was most common in the patients with severe CYP21A2 mutations, 50% in the null genotype group (N=14), 30% in the I2 splice group (N= 15), and 5% in I172N (N=25) in contrast to 2% in their controls (80). Four studies reported pregnancy or live birth rates at 20-26% as opposed to 65-76% in the normal population (90;92;95;96). The reported pregnancy or live birth rates among women with SW were very low at 1/9 (91) and 1/40 (89). However, normal pregnancy rates were reported in a recent study including 106 women with classical CAH (81 SW and 25 SV), when pregnancy rates were calculated as successfully attempted conceptions (97). Twenty-five women (23.6%) considered motherhood; nine were SW. Twenty-three had actively tried conception of whom 21 (91.3%) achieved 34 pregnancies; hence, the authors stated that the pregnancy rates were similar to the normal population in those planning pregnancy (95%). They found similar pregnancy rates in SW (89%) and SV (93%). Thus, a pertinent question seems to be why CAH women do not want to become pregnant?

In the literature only a total of 150 pregnancies or live births have been reported in women with classical CAH and the health of the children are considered good (90;95;97-99). Prenatal treatment with dexamethasone is effective in reducing virilization in CAH-affected girls and
is considered safe by many groups (100). Dexamethasone is not inactivated by placental 11β-hydroxysteroid dehydrogenase type 2 and traverses placenta; however, current Clinical Practice Guidelines classifies this treatment as experimental and states that larger studies are needed to evaluate its risks and benefits (2). In Sweden, a study is ongoing on prenatal dexamethasone treatment in pregnancy, which has included 30 women over the last ten years (101).

Male fertility

Data on fertility in men with CAH are conflicting, and few data exists on paternity. Urban et al. found normal fertility in 18 of 20 patients, as evaluated by paternity and/or sperm count (102) whereas Jääskeläinen et al. found significantly reduced male birth rate in 29 patients compared with age-matched controls (child rate was 0.07 vs. 0.34 (p<0.001)) (103). TART, as described above, may result in oligoazooospermia or Ledyig cell failure, and have been associated with reduced male fertility (104). Other factors could be overtreatment with glucocorticoids, hereditary or psychological factors. Stikkelbroeck et al. also reported an association between low gonadotropines and high levels of adrenal androgens (104). Reisch et al. found that all the 22 males studied (15 SW and 7 SV) had pathological semen analysis and in several men, hormonal control parameters suggested hypogonadism, with glucocorticoid overtreatment as probable cause of poor semen quality (105). Other factors, such as social status or sexual preference have not been investigated in adult males, with the exception of Falhammar et al.’s recently published study (106), which showed reduced fertility compared with national data (0.9 ± 1.3 vs. 1.8± 0.5 children/father ratio) in a cohort of 30 CAH males, aged 19-67 years. There were no major differences in social and sexual factors between patients and controls, apart from more fecundity problems, particularly in the I172N group vs. null and I2splice groups. Fecundity problems were defined as attempting to become a father for more than one year.

Tumours

Adrenal tumours and adrenal size

It is well known that CAH results in large adrenals (as the name implies), caused by chronic increased ACTH stimulation of the adrenal cortex. Whether ACTH hypersecretion also cause tumour growth is uncertain. Few studies have determined the prevalence and size of adrenal hyperplasia in adult CAH patients or looked for adrenal tumours. Reisch et al. recently found
significantly higher combined adrenal volume in male CAH patients (median 9.3 ml (range 3.2-124.5)) than in controls (7.4 ml (5.5-10.8 ml)), and documented an association between total adrenal volume and hormonal control. They found adrenal nodules in 19 of 26 (73%) males with CAH in contrast to 3 of 26 (11%) controls (56). Jaresch et al. demonstrated increased frequency of adrenal incidentalomas in heterozygous CYP21 mutation carriers (nine of 20 (45%)), and in SV (18 of 22 (83%)) (107). Conversely, series of adrenal incidentalomas have been tested for germline CYP21 mutations, showing diverging results (108-110). In summary, adrenal tumours seems to be common in CAH, but that CAH is not a common cause of adrenal tumours.

Testicular adrenal rest tumours (TART)
The presence of TART in CAH was first described in 1940 (111). TARTs are well-defined hypoechoic lesions in the testicle near the rete testis. They are benign and often bilateral, and arise from adrenal-like cells that migrate into the testis in foetal life. These aberrant adrenal cells are most likely stimulated by ACTH (112) and probably disappear if not stimulated (113). TART cells express CYP11B1 and CYP21 activities and have receptors for both ACTH and angiotensin II (112). Thus, undertreatment with glucocorticoids and mineralocorticoids can stimulate tumour growth (112). Long standing TART can lead to obstructive azoospermia and irreversible damage of the testicular parenchyma with fibrosis and peritubular hyalinization, ultimately leading to gonadal dysfunction and infertility (75). TART may also have paracrine effects via steroids produced by the tumour cells (114) which could be toxic to the germ cells (104;115). High doses of glucocorticoids could possibly reduce tumour size and increase fertility (116). The prevalence of TART in CAH males is reported between 0 and 94% (102;104); confounding factors being methods of tumour detection, experience of the investigator, and patient selection. The true prevalence of TART in an unselected CAH population is unknown. The localisation of the tumours within the rete testis renders them difficult to identify by clinical examination and only tumours larger than two cm are detectable by palpation (115). Avila et al. diagnosed TART in 12 of 42 (29%) patients, of whom 10 had no palpable testicular abnormalities (117). Ultrasound or MRI is therefore required to detect TART (115). Whether this should be a routine investigation in CAH males is arguable as the consequence of detecting a small TART is uncertain. Ultrasound and MRI are considered equal modalities for detecting TART (118;119), however
no gold standard exist for diagnosis. Ultrasound is often preferable as it is easily available, quick and inexpensive.

Ovarian tumours
As males with CAH could develop TART the women could perhaps develop ovarian adrenal rest tumours. However, these are exceedingly rare. Only ten cases have been reported; five of them had CAH due to 21-OHD, three with other adrenal enzyme deficiencies and two cases in women with Nelson’s syndrome (120), which is characterised by enlargement of an ACTH-producing pituitary adenoma after adrenalectomy. Stikkelbroeck et al. searched for ovarian adrenal rest tumours in 13 female CAH patients with both ultrasonography and magnetic resonance imaging (MRI), but did not detect any (121).

Clinical Characteristics
Height
Patients with CAH often reach a final adult height significantly below their parentally determined target height and they are shorter than the normal population (59;122;123). The UK study showed that the CAH men were 14 cm shorter than the men in the general population, 162 vs. 176 cm; corresponding figures for women were 10 cm shorter, 152 vs. 162 cm. Excess of androgens results in rapid linear growth with premature epiphyseal fusion and reduced final height. Overtreatment with glucocorticoids also suppresses growth, especially if potent longer-acting glucocorticoids such as prednisone or dexamethasone are used (123). Bonfig et al. recommend that the dose of HC should not exceed 17 mg/m²/day to give optimal pubertal growth (124). A recent review and meta-analysis from 35 eligible studies concluded with reduced final height in both sexes at -1.38 SDS. The SD score (SDS) is a normalised SD that expresses height differences in terms of the SD for height in the reference population. They found no correlation between height and age at diagnosis, gender, type and dose of glucocorticoids, nor age at onset of puberty. Mineralocorticoid users had a better height outcome in comparison with the non-users (69). The meta-analysis also suggests improved outcome in height over the last years due to better treatment. An older meta-analysis from 2001 showed more reduced final height in males and females compared to mid-parental height at -1.57 SDS and -1.24 SDS, respectively (125). These findings indicates that appropriate treatment with glucocorticoids and mineralocorticoids is important to achieve
optimal adult height in patients with CAH. Furthermore, newer reports have shown improved height outcome with additional treatment with growth hormone alone or growth hormone in combination with LH releasing hormone, but this is not an established therapy (126).

**Body mass index (BMI) and body composition**

Glucocorticoids increase fat mass and reduce lean body mass (127), whereas androgens have the opposite effects (128). Overtreatment with glucocorticoids might even induce serious side-effect such as Cushing’s syndrome with weight gain, striae, buffalo hump, facial plethora and proximal myopathy. Many studies have reported increased BMI in children, adolescents and adults with CAH (59;129;130). The largest study from the UK including 62 men and 103 women showed that 37% of the men were obese (BMI ≥ 30.0 kg/m²) vs. 22% in the general population; in women the corresponding numbers were 52% vs. 27% (59). The authors proposed that both glucocorticoid overtreatment and mineralocorticoid undertreatment could explain these findings. Völk et al.’s report on overweight in children and adolescents with CAH indicated that glucocorticoid dosage, chronologic age, premature bone age maturation, and parental obesity contributed to high BMI, whereas birth weight and length, serum leptin levels, glucocorticoid used, and fludrocortisone dosage did not (129). It has been debated whether fat mass is a better indicator of body fat in CAH than BMI, which is systematically skewed due to short height. Furthermore, increased muscle mass due to androgen excess in women can overestimate body fat if BMI is used (58). Five studies have investigated body fat in adults, two in both sexes (131;132), two in women (58;133), and one in men (134). The two studies in men and women showed increased fat mass in males but not in females with CAH (131;132), of which one showed no significant difference in BMI (131). Two Swedish studies that included only females showed diverging results. The first, which included 13 females, showed significantly higher fat mass than the controls, 24.9 vs. 16.2 kg (mean). The mean age in these women was 23.9 years (range 20-29). The other study in 55 females did not show any differences in fat mass or BMI compared with controls. These patients were slightly older, with a median age of 30 years (range 18-63). Finally the male-only study subgrouped the participants below and above 30 years. The older had significant higher fat mass than the controls, 8.2 vs. 5.9 kg/m² (mean), whereas the younger group were not different from controls.
Bone mineral density (BMD) and fractures

Glucocorticoid therapy may reduce BMD in CAH, whereas androgens could increase BMD in men (128). Whether higher adrenal androgens in females increase BMD is uncertain, but one small study supports this (135). Glucocorticoids accelerate reduction in bone mass by inhibition of osteoblast activity, stimulation of osteoclast activity, inhibition of intestinal vitamin D dependent calcium absorption, and increase in the renal excretion of calcium (136;137). Chronic treatment with glucocorticoid for various disorders induces osteoporosis, which typically occurs within the first six months of treatment, followed by slower but steady loss of bone with continued use (136). The incidence of osteoporosis and fractures is related to the doses and duration of glucocorticoid therapy (136). Prolonged glucocorticoid therapy, even in replacement doses, may lead to reduced BMD, but it is uncertain whether glucocorticoid replacement therapy affects bone mass in patients with CAH. Previous reports in adult patients revealed variable results. The majority of studies showed reduced BMD (59;118;138-142); others showed no impairment (130;131;143;144). Certainly, other factors such as genetic predisposition, premature puberty, elevated adrenal androgens and hypogonadism also impact on BMD. To our knowledge only one study addressed fractures; among 61 CAH women; 31 fractures occurred in 18 individuals as opposed to two fractures in two of the 61 controls (138).

Cardiovascular risk factors

As mentioned above, clinical reports in CAH patients above 30 years are few and therefore no reports exist on prevalence of coronary or cerebral vascular disease.

Dyslipidaemia

Lipid profiles in adult CAH patients have only been measured in a few studies and the majority did not show any difference from controls (58;134;145;146). Mooij et al. investigated 12 men and 15 women (30.2± 8.0years, mean± SD) with CAH and found equal levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides, but significantly higher levels of high-density lipoprotein (HDL) cholesterol than in 27 age, sex and BMI matched controls (146). Falhammar et al. investigated women and men separately, and found no difference between CAH and controls, however the women above 30 years of age had higher HDL to LDL ratios and a tendency towards higher HDL-cholesterol than
healthy controls (58;134). The larger UK study showed that many adult men and women with CAH had dyslipidemia, but lack of controls renders the interpretation difficult (59).

**Insulin resistance**

In general, glucocorticoid excess can cause insulin resistance and increase in both fasting and postprandial glucose levels in patients without pre-existing diabetes mellitus; however, the development of diabetes mellitus in a patient with initially normal glucose tolerance is uncommon (147). No report exists on the prevalence of diabetes in adults with CAH except Swedish observations of increased prevalence of gestational diabetes (90). Three of 14 with CAH (21%) compared with none of 41 controls, had a history of gestational diabetes (P=0.026). The reported frequencies of gestational diabetes from the general population were 1.4 - 4%. Two studies found increased insulin resistance in classical CAH (145), but not in NC females without glucocorticoid treatment (148).

**Blood pressure**

Studies of blood pressure in adult CAH patients show diverging results. Two studies by Falhammar et al. measuring either single blood pressures (58) or 24h ambulatory blood pressures (134) found values similar to controls. In contrast, Mooij et al. found elevated systolic and diastolic 24h ambulatory blood pressure in 27 adults with 21-OHD of both sexes compared with BMI matched controls (146). Arlt et al. recently found significantly increased diastolic blood pressure in 102 female CAH patients compared with national data in the UK, but no difference in systolic blood pressure. The 61 male CAH patients in that study had slightly but significantly lower systolic blood pressure than the population-based norm (59).

**Intima-media thickness**

Increased intima-media thickness (IMT) has been described a risk factor of cardiovascular and cerebrovascular disease (149). Sartorato et al. investigated 19 CAH patients (ten females, nine males; 28±3.5 years) and reported increased IMT in CAH patients (145). No correlations between IMT and cumulative glucocorticoid doses or androgen levels were observed.
**Vocal pathology**
The Swedish group demonstrated voice problems in females with CAH (150), who rated higher agreement than controls to the statement “my voice is a problem in my daily life”. The women speak with lower mean, lower minimum and lower maximum frequencies than controls.

**Mortality**
Only data on increased perinatal mortality exists. Before 1950 when the glucocorticoid tablets became available, neonates with SW died in the neonatal period due to lack of glucocorticoids and mineralocorticoids, which indicates that the oldest living patients with SW are approximately 63 years. There is no data on mortality in adults CAH patients.
AIMS OF THE STUDY

When we initiated our studies in 2006 no publications or information about CAH in Norway existed. World-wide, only a few population-based studies had been published on adult CAH, and hardly any including males. However, over the project period some larger studies have been published from the UK (59), Germany (82), and Sweden (58;106;134;138;151).

Our main goal was to collect and describe the burden of CAH in adult life in Norway. The specific aims were to describe:

- Epidemiology of CAH in Norway (Paper II)
- Genetics and clinical characteristics (Paper IV)
- HRQoL and working ability (Paper II)
- Testicular and adrenal tumours in CAH (Paper III)
METHODS

Subjects

Identification

To identify all patients in Norway over 18 years of age with classical CAH we searched in electronic diagnosis registries at the six university hospitals in Norway that have adult endocrine units. The diagnosis registries had start dates ranging from 1972 to 1999. The search criteria were age 18 years or more on 1.1.2007, and the International Classification of Diseases (ICD) codes 255 (ICD 8 and 9) and codes E25 and E27.9 (ICD 10), that is, adrenogenital syndromes and unspecific adrenal diseases. Furthermore, endocrinologists at all the regional hospitals were contacted and requested to report CAH patients. All practitioners in the country were approached in an advertisement in The Journal of the Norwegian Medical Association, on the web pages of the Norwegian Society for Endocrinology, and in the Journal of the Norwegian Addison’s Association, which organizes patients with CAH.

The CAH diagnosis was verified by endocrinologists by scrutiny of original medical records in all the patients, including data on genital examination, symptoms at presentation (hypotension, nausea and electrolyte abnormalities), levels of adrenal steroids, and, if investigated, genetic analyses. We identified 115 patients uniformly distributed throughout Norway. One hundred and four had 21-OHD; of these, 101 were alive. Five patients were registered with 17-\(\alpha\)-hydroxylase deficiency, three with 11-\(\beta\)-hydroxylase deficiency, and two with 3-\(\beta\)-dehydrogenase deficiency and one unknown.

Most likely we identified nearly all adult CAH patients who have been provided specialist endocrine care. Of the 104 identified patients with 21-OHD, 65 were women and 39 were men and nine of them were identified via endocrinologists at regional hospitals. All the registered live patients with 21-OHD were invited to participate in a questionnaire survey. Altogether, 72 of 101 patients agreed to participate (47 females), yielding a response rate of 72% (one patient was not competent to answer questionnaires). The subjects had a median age of 38 years (range 18-72) (paper II). The patients were classified clinically as SW if they had signs of severe mineralocorticoid deficiency during the first three years of life, that is, hypovolemic shock, vomiting, low serum sodium, or high serum potassium, and if the term...
salt-losing was used; in girls with the presence of virilisation of external genitalia. SV was diagnosed in females with virilisation of external genitalia, and in men with signs of peripheral precocious puberty before age six years, and no signs of mineralocorticoid deficiency.

In the clinical studies (paper III and IV), which were conducted in 2008 - 2010, six patients dropped out and two women were withdrawn as we could not find any mutation in the CYP21A2 gene and in retrospect the 21-OHD diagnoses were uncertain. In the X-ray study (paper III) 62 patients participated, but two more patients were withdrawn, one due to claustrophobia and one was excluded due to previous adrenal surgery. In the last study, paper IV, 64 patients participated.

**Data collection**
Each participant completed a registration form covering medical history, diagnosis, treatment and working ability. The form also included questions about marital status, number of children, and symptoms and interventions related to sex hormone disturbances. Further, they completed the Short Form 36 (SF-36) and Quality of Life Scale (QOLS) questionnaires (see next page). Non-responders were reminded with a second letter.

In the clinical studies, the patients were examined at four University Hospitals, according to a standardized protocol including measurements of height, weight, waist and hip circumference, and supine blood pressure measurements, DXA with BMD and body composition, ultrasound of the testicles, adrenal CT and blood samples. Blood samples were collected in the morning after an overnight medication fast. Blood samples were frozen and all the hormonal analyses were collectively performed at the Hormone Laboratory at Haukeland University Hospital. The radiological and DXA investigations were performed at four University Hospitals.

**Controls**
We did not collect our own control group, and instead chose to use normative national data as comparison for the HRQoL data (152;153). Data concerning fertility and social parameters were compared with data from Statistics Norway (http://www.ssb.no). An age-matched control population for evaluations of height, weight, waist-hip measurement, blood-pressure,
HDL-cholesterol, ALAT and calcium was drawn from the third North-Trøndelag Health Study (HUNT) in 2006-2008, which included clinical investigations of 23702 women and 12966 men between 18-72 years of age (154). The Lunar database was used as reference for BMD and fat mass measurements; a previous study demonstrated that BMD in the femur and total body of the subjects in this database were comparable with the general Norwegian population (155). The biochemical analyses were compared with established reference values.

The Regional Ethics Committee of Western Norway and the Data Inspectorate of Norway approved the study. The study was performed according to the Helsinki Declaration.

Health-Related Quality of Life
We assessed HRQoL with the Short Form-36 (SF-36 form) (156), which is known and validated worldwide, and used in many different clinical settings. Normative data is available from the general Norwegian population (153). The SF 36-questionnaire comprises 36 items; the responses are transformed into eight subscales, namely, perception of physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE) and mental health (MH) (156). PF, RP and BP primarily measures physical health, whereas GH and VT are sensitive to both physical and mental health (157). Higher scores are favourable.

No disease-specific questionnaire for CAH is available. We chose to include the Quality of Life Scale (QOLS) in the survey (158). This questionnaire is a 16-item, domain-specific instrument, which measures an individual’s overall satisfaction with life in different areas not usually included in health-related QOL instruments, such as independence, material comfort, work satisfaction, recreation, etc. The QOLS has been used in both cross-sectional and randomised controlled studies of different patient groups with chronic diseases (158), tested for validity and reliability (159), and normative reference ranges for the general Norwegian population exist (152). The response is scored by adding up the items to obtain a total index (min 16–max 112); higher scores are favourable.
Fertility
The participants reported number of childbirths, if they had been investigated or treated for infertility and in women, menstrual disorders. Expected birth rates for the women were estimated from the birth rates of the population cohorts of each of the patients (Statistics Norway: http://www.ssb.no). The standardised incidence ratio (SIR) for birth was calculated as the ratio between the number of observed and expected children in the patient group.

Clinical chemistry
We measured sodium, potassium, glucose, HbA1c, cholesterol, HDL, triglycerides and alanine aminotransferase (ALAT) by the various University Hospital laboratories. The LDL concentration was calculated based on total and HDL cholesterol measurements.

Hormone assays and bone markers
All the hormonal and bone markers analyses were collectively performed at the Hormone Laboratory at Haukeland University Hospital. ACTH, androstenedione, dehydroepiandrostenedione (DHEAS), parathyroid hormone (PTH), insulin C-peptide, LH, FSH and testosterone were analyzed with chemiluminescent immunometric assays by Immulite 2000 (Siemens, Surrey, UK). The ACTH, Insulin C-peptide, LH, FSH and PTH assays are solid phase two-site assays, whereas competitive assays were used for androstenedione, DHEAS and testosterone. 17-OHP was measured by a RIA-method (Siemens). The Gamma Coat [125 I] Plasma Renin Activity Radioimmunassay Kit was used for the quantitative determination of plasma renin activity by the radioimmunassay of generated angiotensin I (DiaSorin, Minnesota, USA). Aldosterone was measured by Coat-A-Coat Aldosterone, a solid phase 125 I radioimmunassay designed for the quantitative measurements of aldosterone levels in unextracted serum (Siemens, DPC, Los Angeles, USA). Serum 25-hydroxyvitamin D was analysed by an isotope dilution tandem mass spectrometry method (Applied Biosystems/MDS Sciex, Foster City, CA, USA). Osteocalcin (intact and N-terminal fragment) was analysed with an ELISA assay from Nordic Bioscience Diagnostics (Herlev, Denmark). Interassay variation was below 11.2% for all the hormone analyses, and well below 10% for most of the analyses.

CYP21A2 genotyping
The gene analyses were performed by one laboratory technician and researcher (IB). DNA
isolated from peripheral blood lymphocytes was used for the genetic analysis. Mutations in the CYP21A2 gene were identified by direct DNA sequencing and deletions were determined by real time polymerase chain reaction (PCR). Primers used for the PCR and sequencing reactions have been described previously (160-162). The CYP21A2 gene was amplified in two different fragments, with the first primer pair selected in the promotor region and in the exon 6 cluster region (fragment 1), and the second primer pair in the exon 3, 8bp deletion region and 170 bp posterior to exon 10 (fragment 2). These specific amplifications were done to distinguish the pseudogene CYP21P from the active gene CYP21A2. Sequencing data were aligned to the CYP21A2 sequence (http://www.ncbi.nlm.nih.gov/nuccore/M12792).

Copy number of the CYP21A2 gene was determined by an assay adapted from a method by Parajes et al. (162). A 132 bp fragment extending from exon 3 to intron 3 of the CYP21A2 gene was amplified by duplex Taqman real-time PCR, and DSP (Desmoplakin) was used as a reference gene (163).

**Ultrasound of the testicles**

Testicular ultrasonography was performed or evaluated by four experienced radiologists in 22 of the 23 male 21-OHD patients using high-resolution 12 mHz or 8 mHz transducers. One patient had had his testicles removed and ultrasound was not applicable. Measurements of testicular size were made from frozen images on the local ultrasound machines, and copied to CDs, which were sent to the central unit and read by one observer (JR). Testicular and tumour volumes were calculated by US using the ellipse formula: \( V = L \times W \times D \times 0.52 \), where \( V \) is the volume (ml), \( L \) maximal length (cm), \( W \) maximal width (cm), and \( D \) maximal depth (cm), according to Martinez-Aguayo et al. (164). Testicular size was also estimated with an orchidectometer for comparison with published normative data (165).

**Adrenal CT**

Somatom (Siemens, Erlangen, Germany), Brilliance 64 (Philips, Cleveland, OH, USA) and Aquillon (Toshiba, Tochigi, Japan) CT machines were used. The CT-protocol included three series; before intravenous contrast media and 60 sec and 15 min after intravenous contrast media was given. The following imaging parameters were used: collimation 6.4 mm, section thickness 3 mm, pitch 1.4, 120 kVp and 180 mA. Two different contrast media, iodixanol (Visipaque 320 mg/ml) or iohexol (Omnipaque 350 mg/ml) were used. The amount of
contrast (80-160 ml) was calculated on basis of the patient’s weight. All CT series covered the region of both adrenal glands, starting cranially. The images were saved at the local radiological department, copied to CDs and sent to a central reading unit where one observer (JR) with 20 years’ experience in adrenal imaging, assessed all the CT-examinations. Images were stratified as normal adrenals (limb junction <8 mm and limbs <5 mm), hyperplasia (limb junction >8 mm and limbs >5 mm), and hypoplasia (limbs ≤2 mm), all measured in axial plane according to Vincent et al. (166). Myelolipomas were diagnosed when a tumour had focal areas of negative attenuation values <-30HU signifying fat (167).

**Body composition, BMI and BMD**

Total and regional body fat, lean mass, total body, femoral neck and lumbar spine (L2-L4) were measured by DXA in 64 patients. Three study sites used Lunar scanners and one used a Hologic scanner. An earlier Norwegian study did not find significant difference between the Lunar compared with the Hologic scanner tested with phantom measurement (168). Lean and fat mass were also adjusted for body height (kg/m²) to compare with the Swedish data. BMD is expressed by Z-scores, representing age and gender-adjusted SDS presented with 95% confidence interval (CI). Osteopenia was defined as BMD T-score ≤ -1 to -2.5 and osteoporosis as BMD T-score ≤ -2.5, WHO criteria from 1994. BMI was calculated (kg/m²).

**Blood pressure**

Blood pressure was measured in the supine position, whereas the blood pressure in the normal population (HUNT) had been measured in the sitting position. Change from the supine to the sitting position in normotensive patients usually do not alter the systolic blood pressure, whereas the diastolic blood pressure rises by 5-10% (169).

**Statistics**

*Descriptive statistics*

The results are given as mean and standard deviation (SD), or with 95% confidence interval (CI) when comparing with published control data (papers II, and IV); the parameters were considered statistically significantly different if the CIs of the patients and the control group did not overlap. Descriptive data or data not considered normally distributed are presented as median and range (paper III).
Hypothesis testing

The majority of the statistical analyses involve comparison between patients and a control population. The null hypothesis is that the difference in the parameter of interest is zero. The null hypothesis is rejected if the probability of the actual finding is small, that is, by convention P<0.05. In paper II we have a cohort of 72 patients and in paper IV 64 patients and we used two sample T-test for the descriptive data included SF-36 results for comparison between two groups. The Mann-Whitney U-test was used to compare two groups when variables were not normally distributed, such as comparing adrenal and testicular size between the 21-OHD forms and sex (paper III) and comparing fat mass, blood pressure and biochemical values between sex and phenotypes (paper IV). QOLS and adrenal size were compared with published CIs in the general population (153;166). In paper IV biochemical values were compared with the reference values and body composition was compared with the percentage fat distribution given in the Lunar database. Fischer’s exact test was used to test the frequencies of adrenal tumours and TART in the SW and SV groups.

Correlations and regression

Another common statistical analysis is to study the relation between two variables within a group of subjects. We used the non-parametric Spearman’s rho (R) for the correlations between adrenal and testicular size and hormone levels (paper III), and height, BMI, age and hormone levels (paper IV). Multiple linear regression analysis was used for SF-36 scores to investigate age and gender as independent variables; and for analysis of height, BMI, fat mass, blood pressure, vitamin D, androgens and 17-OHP levels, with age and glucocorticoid doses as independent variable.
SUMMARY OF THE RESULTS

Paper I
Paper I is a case report of two adult men with untreated 21-OHD, who both had incidentally discovered large adrenal myelolipomas. We postulated that prolonged excessive ACTH stimulation might contribute to the growth of adrenal tumours. These two cases sparked our research interest in CAH and the current research project.

Paper II
Paper II is an epidemiological questionnaire-based survey of classical CAH due to 21-OHD in Norway, including subjective health status, working ability and fertility. We identified 104 adult patients (101 alive) with 21-OHD, of whom 63% were female. This yielded an overall incidence at 1/20 000 live births, and an estimated incidence for girls at 1/16 000. Seventy-two patients (72%) agreed to participate in the questionnaire survey (median age 38 years (range 18-72); 47 women and 25 men), of whom 40 classified as SV and 32 as SW. All the SF-36 scales were significantly impaired, most pronounced for general health and vitality perception. Subgroup analysis did neither show differences between the sexes, nor between SW and SV. Nineteen percent of the patients reported that they received disablement benefits, as opposed to ten% in the general population. The disabled patients had significantly lower scores in all the SF 36 items compared with the non-disabled patients. The female patients were more often single than the general female population, and they had only 21% of the expected number of children; fewest children were observed in the SW group. The male patients had significantly more children than the females and we found that the childless fraction of CAH males over 45 was roughly the same as in the general population, that is, about 20%.

Paper III
Paper III is a study to determine adrenal size and the frequency of adrenal tumours in adults with 21-OHD and the frequency of TART in the males, and to investigate the correlation between such tumours and sex, disease categories, and hormone levels. Nine adrenal tumours were detected in seven of 62 patients (11%) (bilateral in two); four were myelolipomas and one a phaeochromocytoma. Seventeen (27%) had normal adrenal size, whereas 36 (58%) had persisting hyperplasia, and seven (11%) adrenal hypoplasia. Abnormal adrenals were more
common in SW than in SV. TART occurred exclusively in SW, and was present in eight (57%) of these 14 men. Testicular volumes in the males were smaller than normative data. Morning ACTH and 17-OHP levels correlated positively with adrenal dimensions and frequency of TART.

**Paper IV**

Paper IV summarises the genetic, anthropometric and metabolic features in 64 Norwegian adult patients with 21-OHD, who were assessed by one of the investigators at one of the University Hospitals. We identified four novel and plausibly disease-causing CYP21A2 mutations. Gene deletions/conversions (42% of alleles), the splice mutation I2 splice (23%) and point mutation I172 N (22%) were common. The genotype corresponded to clinical phenotype in 92% of the patients. The prevalence of osteopenia was 44% in males and 29% in females. Both men and women had normal BMI, but markedly increased fat mass compared with the normal population. Diastolic blood pressure was higher than normal. Thirty-nine percent of the women had testosterone levels above the normal range; 13% of the men had testosterone levels below normal. Reduced final height was more pronounced in men (median –11.2 cm, -1.77 SDS) than in women (-6.3 cm, -1.07 SDS).
GENERAL DISCUSSION AND FUTURE PERSPECTIVES

My interest in this field started after a consultation with a 55 years old CAH male with a large adrenal tumour considered to be a myleolipoma, who had refused treatment with glucocorticoids since puberty. As I am writing this thesis, a colleague just diagnosed SV in a 70 years old man, who was hospitalised due to abdominal pain. CT of the abdomen showed a three cm large adrenal tumour and the surgeon asked for an endocrinological evaluation. Paper I illustrates the importance of medical case reports as a gateway to clinical research. Case reports may expand the general medical knowledge as they permit discovery of new diseases, collection of rare diseases and unexpected effects. They can provide new ideas and hence initiate original research in medicine.

Epidemiology and subjects
We found 104 adult patients with 21-OHD distributed across Norway after our intensive effort to identify all the adult CAH patients in Norway. We believe that we have identified the majority of those diagnosed before 1990. We expected to find 170 patients based on an estimated incidence of 1/15 000 (16). However, the prevalence and incidence in Norway was unknown until now. Accurate figures will only be available when the results from the neonatal screening programme for CAH becomes available in the years ahead.

We assume that our study participants are representative for the identified CAH patients as the distribution of sex, age, disease category and geographic distribution were almost similar in these population sets. Another important aspect is that our study participants are recruited from an unselected population and not a single specialist centre. In comparision the recent UK study identified only 5% of the expected CAH population and only 54% of them participated. Our study population is also older than many previous studies on CAH patients. In our first study (paper II) ten women and seven men were 50 years or older.

We found remarkably fewer males than females. An autosomal recessive inheritance dictates equal sex distribution. Suprisingly, the differences in sex distribution was greater for SV than SW. We can only speculate if this is due to undiagnosed SV in adult men or increased neonatal mortality due to undiagnosed adrenal crises in the neonatal period, or other reasons. Paper I and the history above illustrate that SV in men can go undiagnosed or be diagnosed
late in life by chance. Fewer males with CAH are also reported from England (170) and in Sweden before the neonatal screening started (19;23).

**Treatment**

Nearly all the patients received glucocorticoid therapy, and prednisolone was the mostly used glucocorticoid. Cortisone acetate came second. Fewer of the Norwegian than the Swedish and English patients use fludrocortisone, 46% in Norway vs. 82% and 72% in Sweden and UK, respectively. This disparity is due to differences in the treatment of SV with mineralocorticoids. The fludrocortisone dose was similar in Norway and Sweden, but higher in the UK (58;59;134). Another difference is that we are using higher doses of glucocorticoids and more dexamethasone than the other two countries. Four of our patients had also undergone either unilateral, partial or total (one patient) adrenalectomy. This is not established treatment and is only likely to be used in patients with severe 21-OHD (null mutations) refractory to standard medical treatment. More studies are needed to find the optimal treatment for adult patients with CAH and to determine whether novel treatment such as modified-released HC tablets (51) or CSHI (52) will improve health outcome. We also need better guidelines for treatment and follow-up.

**HRQoL**

HRQoL is a broad term, which is difficult both to define and evaluate. Many of the definitions of HRQoL is based on WHO’s expanded definition of health from 1946: “A state of complete physical, mental and social well-being and not merely the absence of disease or infirmity”. Our findings of reduced HRQoL were supported by others, published a few months after our Paper II in 2010 (59). This implies that the majority of studies in CAH now show impaired HRQoL. The cause of impaired HRQoL is complex, including hormonal disturbances, genotype, steroid treatment, and psychological issues. Hopefully, systematic studies and increased knowledge about the disease can help the clinicians influence these result in a favourable direction. We compared the SF-36 results with other patient groups in Norway, showing that the CAH patients have similar HRQoL perception as patients with Addison’s disease, who also had pronounced impairment of general health and vitality (171). The scores were lower than in patients with renal transplant and Turner’s syndrome (172;173). Patients with rheumatoid arthritis had definitively the lowest score in all subscales when comparing SF-36 results (174) (Figure 4).
Measuring HRQoL is difficult and the validity of SF-36 or other questionnaires for evaluation of this disease has limitations. It is well accepted that SF-36, which is a generic instrument, contains dimensions that are important for HRQoL. Generic forms make comparison with other diseases groups possible, and might be the best option, as no specific HRQoL questionnaire for CAH exists. A new disease-specific questionnaire has been...
produced for Addison’s patients, AddiQoL (177;178), which could possibly be appropriate for HRQoL assessment in CAH patients.

Another question is whether the patients estimate their HRQoL appropriately. In empirical studies the patients tend to assess their own health as better than considered by their healthy peers (179). Recall bias is often present in retrospective studies, where one group, usually the cases, recall events better than the references. Response bias occurs when respondents answer questions in the way they think the questioner wants them to answer rather than according to their true perception. Scale bias implies that subjects have different perceptions of what the scale of a phenomenon essentially measures.

**Working disability**

To our knowledge we are the only group to publish data on working ability in adult CAH patients. The disabled patients had significantly impaired SF-36 scales compared to the working patients, and we can only speculate if they have low scores due to the disability or vice versa. We did not find any special factors that correlated with the proportion of disability, be it glucocorticoid doses, hormone levels, sex differences, mutation groups, age at diagnosis or other parameters. However it may be assumed that patients with a chronic disease more easily obtain working disability benefits. A Norwegian study in patients with Addison’s disease showed similar results on working disability (171).

**Fertility in women**

Our results support the results from other studies showing low pregnancy rates among CAH women, especially in the SW (89-92). Several factors contribute. We found that many patients had irregular menstruations and long periods of amenorrhoea, but had never consulted a gynaecologist as adults. Regular gynaecological investigations are recommended in international literature (72). Forty-five percent of the Norwegian female patients had irregular menses compared to 28% of the Swedish. One main difference between these countries is the use of mineralocorticoids. A beneficial effect of adding fludrocortisone is the lowering of 17-OHP, which could favour ovulation (99). On the other hand, investigators have found normal fertility rates in those attempting conception, even in the SW group (97;99). Whether this observation can be generalised to all female patients remains to be shown, as these results were based on studies of few patients.
Concerning sex hormones in our female patients, the levels of FSH and LH were in the reference range in our patients below 50 years of age, and not increased as can be seen with polycystic ovary syndrome. On the other hand, high adrenal androgen levels and the glucocorticoid treatment might suppress the gonadotropins. Interestingly, all the seven women above 50 years had normal post-menopausal FSH and LH levels.

We think that the low birth rate and high single status in female with CAH, specially the SW, affects both the general quality of life and HRQoL, and must be considered a major problem for these patients. These issues should be given special attention by the clinicians treating them.

**Male fertility and TART**

We found TART only in the men with the SW form (57%), that is, the men with severe $CYP21A2$ mutations. The findings by Mouritsen et al. support these results. They looked specifically for the association between TART and $CYP21A2$ mutations, and found TART only in the men with the most severe mutations (groups Null and A); 73 % had TART in these groups and none in the more mildly affected groups B and C (180). Our results support Mouritsen’s theory that development of TART is determined by the mutations in the $CYP21A2$ gene.

One accepted reason for development of TART is the elevation and stimulation of ACTH (112), and in line with this we found significantly higher levels of ACTH, 17-OHP and androstenedione in the men with TART than in men without. It has been shown that TART may regress when glucocorticoid therapy is given or intensified (116;181). However, TART is also reported in men with good hormonal control (104).

We found that the men in our study had testosterone levels in the lower normal range without tendency of increments in FSH and LH values as could be expected if a primary gonadal dysfunction due to TART was present. However LH and FSH levels are difficult to interpret in CAH because the gonadotropines may be suppressed by elevated adrenal androgens and glucocorticoids (182). Another issue is that we are measuring the hormone values in plasma or serum and we know little about the intracellular concentrations of steroids. Androstenedione may be converted in peripheral tissues to testosterone and oestrogens. One
study in healthy young men showed that after a 300 mg oral dose of androstenedione the serum levels of both oestrogens and testosterone increased. After 100 mg androstenedione only oestrogens increased. The serum levels of oestrogens and testosterone varied widely between the individuals (183).

Only a few studies in men with CAH have examined paternity, with diverging results from normal to impaired (102;103). We found no overall reduction in fertility in the men and there was no difference in the number of children between the SW and the SV groups, even if 57% in the SW group had TART. However, we had no age-matched control group with which to compare fertility. This finding is in contrast to findings by Falhammar et al. in 30 CAH males that showed impaired fertility compared with age-matched national data (0.9± 1.3 vs. 1.8± 0.5 children/father ratio). Several studies in men with CAH show pathological semen analyses (104;105) indicating reduced fertility. We have not investigated semen quality in our patients.

Adrenal tumours and adrenal size

CT has until now been the gold standard for visualising the adrenal and to discover adrenal tumours. CT is also a more available modality than MR, so we decided to use CT even if we exposed the patients to radioactivity. Only two other studies have been performed to visualise the adrenals in CAH. The first study from Jaresch et al. in 1992 looked for adrenal tumours in 22 CAH patients (107). The recent study from Reisch et al. evaluated adrenal size and adrenal tumours in 26 adult male with CAH (56). Both showed high frequencies of adrenal tumours at 82% and 73%, respectively, whereas we found only 11% in our study. However, the other studies did not characterise the tumours any further. One explanation for the large difference could be the way adrenal incidentalomas were defined; if nodules in macronodular hyperplasia are counted as solitary tumours the prevalence of adrenal incidentalomas will be high. Jaresch et al. intervened surgically in three patients with tumour size > six cm. Histological examination revealed one nodular hyperplasia, one adrenal adenoma, and one myelolipoma.

It has been speculated if chronic elevation of ACTH may induce adrenal tumour growth in CAH. We found a positive correlation between morning ACTH and 17-OHP levels and adrenal dimensions, and significantly higher levels of ACTH in the patients with adrenals tumours than in those without. These results support the ACTH stimulation hypothesis.
Reisch et al. found a positive correlation between adrenal volume, tumour size and levels of andostenedione and 17-OHP, but not ACTH. However, they measured the hormones after intake of the glucocorticoid morning dose which would significantly lower several of these parameters.

Reisch et al. did not report any myelolipomas and Jaresch et al. diagnosed one myelolipoma histologically after surgical intervention, whereas we detected six large myelolipomas radiologically in four (6.5%) of our patients. This is high compared with the normal population, where myelolipomas are reported in 1.5-8% of adrenal incidentalomas (184;185). To our knowledge, this is the first demonstration of high myelolipoma frequency in a large group of unselected patients with CAH. Myelolipomas have previously been associated with CAH in single cases, mostly after surgical intervention (57;186). Reisch et al. used MRI of the adrenals in their study, but the applied technique did not include fat suppression imaging that could have discriminated myelolipomas from adenomas. We believe the frequency of myelolipoma in patients with 21-OHD has been underestimated in previous studies. It is important that endocrinologist and radiologist are aware that these tumours may occur in CAH as myelolipomas are benign and asymptomatic tumours that usually need not be removed. Surgical excision is only to be considered in large myelolipomas above ten cm because of a possible risk of bleeding (109). Moreover, it is important to rule out CAH if an adrenal myelolipoma or adenoma is discovered. As early as 1950 Selye and Stone elegantly transformed adrenal tissue in rats into adipose and bone marrow elements by injecting them with an extract derived from the anterior pituitary gland along with methyltestosterone, and postulated that ACTH elevation could induce transformation of adrenal tissue into myeloid tissue (187). Sometimes the clue to the diagnosis in adults is a random CT finding of bilateral adrenal hyperplasia or myelolipoma (57;188).

Reisch et al. and we both found normal adrenals in less than one third of the patients. Two thirds of our patients had hyperplasia. Interestingly, we also demonstrated adrenal hypoplasia in 11% of the patients, which may indicate overtreatment with glucocorticoids.

**Genetics**

Whether genotyping in CAH should be mandatory is debatable. The majority of our patients had not been genotyped before the inclusion in our study. In two cases, both of whom had
received glucocorticoid treatment for years, we did not find mutations in \( CYP21A2 \) and scrutiny of their files lead to the conclusion that they were misdiagnosed. They were excluded from paper III and IV. One clear reason for genotyping is to verify the diagnoses especially in NCCAH. The Endocrine Society Clinical Practice Guidelines also recommend genotyping in adults with CAH and/or their partners before planning children, and genetic counselling is recommended for parents of a child with CAH and to adolescents at transition to adult care (2). Falhammar and Thorén recommend genotyping as their experience is that the clinical course can be predicted more precisely and serious consequences be prevented (46). They exemplify this with females with the Null genotype who are more severely affected with reduced fertility, impaired social functioning and psychosexual issues than other genotype groups. These patients need extra support and follow-up. Another issue is the importance of genotyping in research to discover new mechanism in CAH as i.e. P450 oxidoreductase deficiency, which is due to mutations affecting P450 oxidoreductase that serves as mandatory electron donor enzyme to all microsomal cytochrome p450 enzymes.

The results of our mutation analyses show similar frequencies of the common \( CYP21A2 \) gene defects as those published from other Western European countries (30;31). We also confirm the earlier reported high genotype-phenotype correlations. Interesting a 54 year old male with CAH was diagnosed as SV and started treatment with glucocorticoids at 25 years of age. His older sister was diagnosed with SW at three months of age and two siblings had died as neonates. The genetic analyses showed deletion/large pseudogen conversion (5') consistent with genotype group null. He had large adrenals, small testicles and a height of 1.5 meters. We started treatment with mineralocorticoids due to large salt craving. This case is an unusual example of increasing aldosterone and cortisol deficiency with age and we have no reasonable explanation for this; or simply an example of late diagnosis and incorrect treatment in youth.

We identified four novel \( CYP21A2 \) point mutations that are most likely pathogenic, as indicated by the following phenotypes: del/L388R and del/E140K were SW, whereas del/V211M, V281L and del/P45L were SV (novel mutations in italic). Ongoing functional studies of the mutated enzymes revealed no or reduced enzyme activities (Brønstad,
Clinical characteristics, BMD and blood pressure

One striking result of our survey is the increased fat mass in CAH patients, especially in the young women. Increased fat mass is a major health problem in the population at large, and is a known risk factor for cardiovascular disease, diabetes and cancer. Obesity also increases the risk of infertility and could be a contributing factor to infertility in CAH women. In the transition from childhood to adulthood many CAH patients are switched to long acting synthetic glucocorticoids, which are much more potent and prone to give side-effects than the short-acting natural glucocorticoids. We believe it is important to avoid high doses and perhaps a short-acting glucocorticoid should also be recommended in adults. Mineralocorticoids should be used in addition when possible, as this could reduce the glucocorticoid doses. Measurement of body composition is not usually performed in general practice and BMI only rarely in adult CAH patients (personal experience), but these parameters might be important to optimise the treatment.

The studies in BMD in adult patients with CAH are conflicting, and few studies have included men more than 30 years old (59;143). We found reduced BMD both in men and women and a high frequency of osteopenia, especially in the male patients. This could be due both to high glucocorticoid doses and lack of testosterone and vitamin D. We recommend regular monitoring of BMD in both men and women with CAH, which unfortunately was not discussed in the latest clinical guidelines (2).

Surprisingly, the patients have higher diastolic blood pressure than the normal population, despite that the glucocorticoid or mineralocorticoid doses in the morning were not taken before the measurement. Arlt et al. reported the same finding, however only for females (59). Elevated diastolic blood pressure has also been reported in Addison patients and the mechanism for this was speculated to be high doses of mineralocorticoid (189).

Biochemical findings

Few of the female patients had testosterone levels in the normal range and many had suppressed androgens, indicating overtreatment with glucocorticoids. Arlt et al. and Falhammar et al. reported similar findings (58;59), demonstrating that optimal glucocorticoid
treatment in CAH is difficult. Half of our patients had 17-OHP levels above the upper recommended target range; again the same phenomenon has been seen in other populations (59). The value of a single measurement of 17-OH is debatable, but gives some information. We found larger adrenals and higher frequency of TART in patients with high morning levels of 17-OHP, suggesting that single measurement of 17-OHP can be informative. We did not show any differences from reference values in lipids, lever enzymes or glucose metabolism in our patients. The CAH patients, especially the women, had low vitamin D levels and one contributing factor to this could be the increased fat mass. Lower concentrations of serum 25-hydroxyvitamin D levels in obese subjects may be explained by enhanced uptake by adipose tissue and increased metabolic clearance. A sedentary lifestyle with less exposure to sunlight could also contribute (190). We recommend regularly monitoring of vitamin D and replacement of low levels.

**METHODOLOGICAL ASPECTS/LIMITATIONS**

The principal limitation to the interpretation of our data is that we did not have age and sex matched control populations. Some analyses utilised matched control data drawn from the previous HUNT 3 study. The HRQoL data were compared with a Norwegian reference population published ten years ago, and other data were compared with the reference data given by the manufactures of DXA machines or laboratories. Concerning fertility, semen-analyses in the men and analysis of inhibin B and oestradiol in both women and the men would have added value to the study. Small cohorts limit the certainty of the analysis of frequencies of adrenal tumours, TART, and might contribute to low statistical power to detect significant associations, for instance between hormonal control and tumour size or between mutation groups or other parameters. Larger multicentre studies are needed to obtain further knowledge about this patient group.
CONCLUSIONS

The studies of adult Norwegian patients with CAH have shown that

- The recorded incidence of CAH due to 21-OHD of one in 20 000 in Norway is lower than the expected incidence of one in 15 000, with a lower incidence in men than in women. Untimely, deaths and insufficient treatment may be avoided by screening of neonates.
- Gene deletions/conversions, I2 splice and the point mutation I172N were as frequent as expected, and four novel disease-causing CYP21A2 mutations were identified.
- Patients with CAH, as a group, have reduced physical and mental health perception and especially reduced scores for general health and vitality perception; working disability is increased.
- Women with CAH have impaired fertility, most pronounced in the SW.
- CAH patients have an increased frequency of adrenal tumours, particularly myelolipomas.
- BMI, fat mass and diastolic blood pressure is increased in CAH patients. The findings may indicate increased risk for cardiovascular disease.
- CAH patients have high frequencies of osteopenia and osteoporosis, but whether fracture rates are increased is not known.
- Hormonal parameters were poorly controlled. There is a need for a more systematic approach to therapy and more studies on replacement therapy.

Taken together, our findings most likely reflect inappropriate glucocorticoid and mineralocorticoid replacement. Improvement in the clinical management of adults with CAH is required, and we suggest that these patients be followed by endocrinologists, and that international guidelines are followed. More research is needed to fill the gap in knowledge in areas such as replacement therapy, fertility, and the understanding of psychoendocrinological factors involved in subjective health status and working ability.
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Case Report

Two Adults with Adrenal Myelolipoma and 21-Hydroxylase Deficiency

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We present incidentally discovered adrenal myelolipomas in two adult males with untreated congenital adrenal hyperplasia (CAH). The patients had simple virilizing form of CAH due to mutations in the CYP21 gene coding for 21-hydroxylase; one was heterozygous for the I172N mutation and the other compound heterozygous for the I172N and I2splic mutations. The masses were not removed since myelolipomas are considered benign tumors, and the tumor size did not increase during four- and nine-year observation periods. An adrenal myelolipoma is an important exception to the rule that large tumours should be removed. Untreated CAH with prolonged excessive ACTH stimulation might contribute to the growth of adrenal masses. CAH should be considered as a differential diagnosis of patients with adrenal masses or adrenal myelolipomas.

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1. Introduction

Adrenal incidentalomas are common; the challenge is to rule out malignancy or excess hormone secretion [1]. Myelolipomas are benign endocrine inactive tumors composed of adrenal, adipose, and myeloid tissue, representing 1.5%–9% of adrenal incidentalomas; the radiological characteristic is round, sharp-demarcated lesion with negative attenuation values because of its high fat content [2–5]. Large adrenal masses should usually be removed, but myelolipomas usually do not need resection. However, many of the reported cases were diagnosed after adrenalectomy. Rarely, myelolipomas have been associated with hormone excess syndromes, such as congenital adrenal hyperplasia (CAH) [6–10]. We here report two cases, which illustrate that large adrenal myelolipomas need not to be surgically removed and that CAH must be considered in patients with adrenal incidentalomas.

2. Case Report 1

In 1999 a 50-year-old man presented with a right adrenal mass 5.7 × 5.1 × 1.9 cm. Medical history revealed precocious puberty at age 4, and he was diagnosed with “Morbus Glandulae Suprarenalis” when he was 9. Urinary 17-ketosteroids were found elevated, but suppressible with dexamethasone. He was treated with cortisone acetate 12.5 mg thrice daily from age 9, but at 18 he stopped the medication, after which he felt that his general wellbeing is improving. After this time he was lost to follow-up. At the age of 37 he underwent radioiodine treatment for thyrotoxicosis. He was otherwise healthy, working full time, and was the father of one child.

In spite of radioiodine treatment he felt he had persistent goitre, and his general practitioner referred him to a cervical CT, which due to his short stature included the upper abdomen. Pronounced bilateral adrenal hyperplasia and a 5.7 cm in diameter tumor described as a typical adrenal
myelolipoma (capsule, heterogeneous stroma, and fat in the adrenal mass) was found (Figure 1).

Further investigation revealed height 164 cm, weight 76 kg, and blood pressure 120/80 mmHg. On palpation the patient had no enlargement of the thyroid gland. Genital examination showed normal penile size and subnormal testicular volume of 12 mL bilaterally. Biochemical investigation revealed low basal cortisol, which did not increase after ACTH stimulation (Table 1). He had a high basal level of 17-OH-progesterone, typical of 21-hydroxylase deficiency, which was suppressed after a conventional dexamethasone test (Table 1). DNA sequencing of the \textit{CYP21} (steroid 21-hydroxylase) gene revealed the c. 515T \textgreater A point mutation, which results in the predicted amino acid change I172N. The mutation was present in hemi- or homozygous form (i.e., the genotype was I172N/deletion or I172N/I172N). The adrenal mass caused no symptoms and was considered highly likely benign and therefore left untreated. The tumor and hyperplasia did not change radiologically over a nine-year subsequent observation period. The patient continues to do well without steroid medication, but is instructed to take steroids during stress.

3. Case Report 2

A previously healthy 68-year-old man, father of one child, presented in 1994 with fatigue and nausea. For many years he had suffered arthralgic pain and had been treated with antiflogistics. The last months he had experienced problems with nocturia and impotence. He was referred

### Table 1: Myelolipoma: baseline measurements and endocrinological test results.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH, pmol/L</td>
<td>6–32.8*</td>
<td>10.9–94.2</td>
<td>(2–13)</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>210</td>
<td>387</td>
<td>(250–750)</td>
</tr>
<tr>
<td>17 OH-progesterone, nmol/L</td>
<td>&gt;605</td>
<td>445</td>
<td>(&lt;6)</td>
</tr>
<tr>
<td>DHEAS, μmol/L</td>
<td>14.4</td>
<td>1.8</td>
<td>(6–12)</td>
</tr>
<tr>
<td>Androstendione, nmol/L</td>
<td>43.8</td>
<td>10.9</td>
<td>(&lt;5.6)</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>11.7</td>
<td>10.1</td>
<td>(10–40)</td>
</tr>
</tbody>
</table>

**ACTH stimulation**: Cortisol 30 minutes, nmol/L: 211, 409; Cortisol 60 minutes, nmol/L: 231, 412; 17 OH-progesterone 30 minutes, nmol/L: >605, 445; 17 OH-progesterone 60 minutes, nmol/L: >605, 560; Dexamethasone suppression**: Cortisol, nmol/L: 22, 45; 17 OH-progesterone, nmol/L: 14.3, 18.8

* Most values in the higher range. **Corticotropin (250 ug i.v.). ***1 mg overnight test in Patient 2 and 0.5 mg × 4 for 2 days in Patient 1.
to ultrasonography of the abdomen, on which a tumor in the right adrenal was detected. An adrenal CT scan showed a 4 × 4 × 2.5 cm tumor in the right adrenal with high fat content, which was radiologically considered to be a myelolipoma. The left adrenal was evaluated as normal.

Further clinical investigation showed height 165 cm, blood pressure 140/80 mmHg, and normal male genitalia with testicular tenderness bilaterally. Biochemical investigation showed high basal level of 17-OH-progesterone, which was suppressible with dexamethasone, and an impaired cortisol response to ACTH stimulation (Table 1). Adrenal iodomethyl-19-norcholesterol scintigraphy showed high uptake in the right adrenal, which however was suppressed by dexamethasone; uptake was normal or low in the left adrenal. DNA sequencing of CYP21 revealed compound heterozygosity for the I172N mutation (c. 515T>C) and the I2 splice mutation (g.655A>C, I172N/I2splice). The myelolipoma was not removed and the tumor size did not increase during a four-year observation period. The patient was started on treatment with 5 mg prednisolone daily for one year, later cortisone acetate 12.5 mg twice a day whereupon his condition improved. The patient died suddenly, 73 years old, in his home; the cause of death remains unknown.

4. Discussion

We here report two male patients who presented late in life with large adrenal myelolipomas. One was previously treated for CAH, whereas one was diagnosed with 21-hydroxylase deficiency after being diagnosed with the adrenal tumor at 68 years of age. Both tumors were left untreated and did not grow over five- and nine-year observation periods.

An adrenal mass normally requires surgical removal if the largest diameter exceeds 3 cm [1]. Undiagnosed CAH must always be considered as a differential diagnosis to tumor, particularly with bilateral adrenal masses. Adrenal myelolipomas are benign and usually asymptomatic tumors that usually need not to be resected [2, 6, 7, 11]. Many of the cases that have been reported in literature were diagnosed after adrenalectomy [2, 6, 7, 11]. The imaging characteristics of myelolipomas usually allow presumptive diagnosis, but which may be confirmed by percutaneous needle biopsy in cases of extra-adrenal myelolipomas [5].

However, large myelolipomas could be dangerous if they start bleeding and give symptoms with pain, nausea, vomiting, and hypotension [2]. Because of mass effects and the risk of haemorrhage, elective removal of myelolipomas that exceed 10 cm in diameter might be considered.

Increased prevalence of adrenal tumors has been reported in CAH patients and in heterozygote mutation carriers, possibly due to increased levels of ACTH which acts as a growth factor [12–15]. It has been suggested that inadequate glucocorticoid coverage in early life or frequent episodes of infection, both resulting in chronic ACTH increase, contribute to the development of adrenal tumors later in life [15, 16]. The aetiology is unknown, but several theories have been proposed, such as development from residual embryonic mesenchymal tissue in the adrenal glands, or metaplasia of reticuloendothelial cells as a result of chronic stress [2].

It was postulated early in the 1950s that ACTH elevation might induce transformation of adrenal tissue into myeloid tissue [17], and myelolipomas could thus be particularly associated with CAH. Hagiwara et al. investigated ACTH receptors and androgen receptor in a resected giant myelolipoma in a woman with CAH. They did not find that the receptors were overexpressed and suggest a limited direct role for these hormones in the development of the myelolipoma [18]. However this conclusion is debatable.

To our knowledge less than 20 cases of adrenal myelolipomas have been reported in patients with CAH to date [7–10, 16, 18–20]. Jaresch et al. found adrenal masses in 83% of CAH patients and 45% of heterozygous CYP21 mutation carriers, which is high compared to the general population [13]. However, their study included only 22 homozygous CAH patients and 20 heterozygous siblings. Conversely, series of adrenal incidentalomas have been tested for germline CYP21 mutations, showing inconsistent results which could be due to small samples [12, 13, 15]. Consequently, it appears that adrenal masses are common in CAH, but that CAH is not a common cause of adrenal masses.

The diagnosis of CAH in a patient with an incidental adrenal mass is important as the patient is at risk of adrenal crises [21]. Such patients may benefit from glucocorticoid therapy and should at least be equipped with emergency medication and a medical alert card. Patient 1 had a blunted cortisol response to ACTH stimulation, but he had managed well for 30 years without steroids, working full-time and did not want to take steroids because of the psychological side effects he experienced during adolescence. He was nonetheless advised to take steroids under stressful situations. Patient 2 had higher basal level of cortisol, but suboptimal cortisol response to ACTH stimulation. He had symptoms of cortisol deficiency, and his condition improved considerably after treatment with prednisolone was commenced. He had a low normal testosterone level and tenderness of the testicles. This could be the result of testicular adrenal rest tumors but was not investigated further. Whether glucocorticoid suppression therapy in adult age halts further growth of the tumor is uncertain. In our patients the tumors did not grow over four to nine year observation periods despite high ACTH levels. Conversely, Raiput et al. described progressive evolution of an adrenal myelolipoma in a woman with CAH who was treated with prednisolone, although taken irregularly, rendering the level of 17-OH-progesterone persistently elevated [19].

In conclusion, congenital adrenal hyperplasia, per se, should be included in the differential diagnosis of adrenal masses. If suspected clinically, assay of cortisol, ACTH, and 17OH-progesterone should be performed. Adrenal tumors are common in CAH and might require surgical removal, but myelolipomas are benign tumors that usually can be left untreated. Whether, treatment of CAH halts myelolipoma growth is uncertain.
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