CLINICAL STUDY

Genetic, anthropometric and metabolic features of adult Norwegian patients with 21-hydroxylase deficiency

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Abstract

Objective: The aim of this study was to determine the genetic, anthropometric and metabolic features in an unselected population of adult Norwegian patients with 21-hydroxylase deficiency (21OHD).

Patients, methods and design: Sixty-four 21OHD patients participated (23 men and 41 women; median age 38.5 years; range 19–72 years) in a cross-sectional study including DNA sequencing of *CYP21A2*, anthropometric measurements including dual X-ray absorptiometry scanning and biochemical analyses. The results were compared with reference cohorts from the general population.

Results: We identified four novel and plausibly disease-causing *CYP21A2* mutations. Gene deletions/conversions (42.1% of alleles), the splice mutation I2 splice (23.0%) and point mutation I172 N (22.2%) were common. The genotype corresponded to clinical phenotype in 92% of the patients. The prevalence of osteopenia was 48% in males and 34% 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 per cent 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).

Conclusions: In this population-based survey of 21OHD, we identified four novel mutations and high concordance between genotype and phenotype. The patients had increased fat mass, increased diastolic blood pressure, reduced final height and high frequency of osteopenia among males. These results show unfavourable metabolic features in 21OHD patients indicating a need for improvement of treatment and follow-up.

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Introduction

Congenital adrenal hyperplasia (CAH) is caused by mutations in genes encoding steroidogenic enzymes in more than 95% of cases caused by defective *CYP21A2* with ensuing 21-hydroxylase deficiency (21OHD). Classical CAH includes the salt-wasting (SW) form with complete lack of cortisol and aldosterone and the simple virilising (SV) form displaying cortisol depletion; both are accompanied by an ACTH-driven increase in adrenal androgens. There is strong correlation between *CYP21A2* genotype and clinical presentation, although the salt loss typically recovers with age (1), and occasionally aldosterone production is present even in patients with severe mutations (2).

21OHD is treated with replacement of glucocorticoids and mineralocorticoids. The aim is normalisation of

ACTH to avoid androgen overproduction, adrenal hyperplasia, growth of adrenal tumours, and development of testicular adrenal rest tumours (3, 4), resulting in higher glucocorticoid doses than in patients with primary adrenal insufficiency. Excess of glucocorticoids in puberty, in particular long-acting glucocorticoids, add to the reduction in final height, which follows androgen excess (5, 6). Glucocorticoids further increase fat mass, whereas androgens reduce it. Most studies have reported increased BMI in 21OHD patients (7, 8), but not in all (9), and some studies have reported increased fat mass (7, 8, 9). Glucocorticoid therapy may also reduce bone mineral density (BMD), whereas androgens increase BMD in men (10), and studies have shown reduced (11), normal (12) and increased BMD (13) in 21OHD patients. Little is known about the risk of cardiovascular and other metabolic diseases.

A recently published review suggests that adult CAH patients have a cluster of metabolic risk factors, consistent with the metabolic syndrome (14). However, many of the studies on adults with 21OHD included small patient samples and represent single specialist centres, which could imply selection bias. Only a few studies included data from adult men (15, 16). The largest cohort published so far included 199 patients with 21OHD in the UK, of whom 31 had the non-classical form, thus representing only 3–5% of all estimated classical CAH in the UK.

The aim of this study was to determine the genetic, anthropometric and metabolic features in an unselected population of adult Norwegian patients with 21OHD including men and women and to explore the correlations between clinical presentation, genotypes, corticosteroid use and biochemical parameters.

Materials and methods

Subjects and design

Sixty-four Norwegian patients with 21OHD (23 men and 41 women; median age 38.5 (range 19–72) years) were recruited from a previous population-based cross-sectional study that identified 101 adult patients with classical CAH (17); 36% of the patients did not answer or declined to participate. The patients were categorised as SW or SV, as defined by medical history and laboratory assessment (17), and the diagnosis was verified by genotyping of the *CYP21A2* gene. The patients were assessed at four University Hospitals, according to a standardised protocol including measurements of height, weight, waist and hip circumference; supine blood pressure measurements; dual X-ray absorptiometry (DXA) with BMD and body composition. Blood pressure was measured in the supine position, whereas the blood pressure in the normal population had been measured in the sitting position. Change from the supine to the sitting position in normotensive patients usually does not alter the systolic blood pressure, whereas the diastolic blood pressure rises by 5–10% (18). BMI was calculated (kg/m^2). Current treatment was recorded and hydrocortisone equivalent daily dose (mg/day or mg/m^2) estimated as suggested by Rivkees & Crawford (19) (relative potencies: hydrocortisone 1, cortisone acetate 0.8, prednisolone 4 and dexamethasone 70). Body surface area was calculated as the square root of ($\text{height (cm)} \times \text{weight (kg)} / 3600$) (m^2). Blood samples were collected in the morning before breakfast and medication. Blood samples were frozen and all the assays of hormone levels and gene analyses were performed collectively at one centre.

An age-matched control population for evaluations of height, weight, waist–hip measurement, blood pressure, HDL, alanine aminotransferase (ALAT) and calcium

was drawn from the third North-Trøndelag Health Study (HUNT) in 2006–2008, which included clinical investigations of 23 702 women and 12 966 men between 18 and 72 years of age (20). 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 was comparable with the general Norwegian population (21). 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.

Genetic analysis

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 PCR (22, 23, 24). See [Supplementary information](#), see section on [supplementary data](#) given at the end of this article for a detailed method description.

BMD and body composition

BMD at the femoral neck, total hip, lumbar spine (L2–L4) and total body and body composition were measured by dual-energy X-ray absorptiometry (DXA). Lean and fat mass were adjusted for body height (kg/m^2). Three study sites used Lunar scanners and one used a Hologic scanner. Osteopenia was defined as BMD *T*-score ≤ -1 to -2.5 and osteoporosis as BMD *T*-score ≤ -2.5 .

Hormone and biochemical analyses

ACTH, androstenedione, DHEAS, PTH, insulin C-peptide, LH, FSH and testosterone were analysed 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-Hydroxyprogesterone (17OHP) was measured by a RIA method (Siemens). The Gamma Coat [^{125}I] Plasma Renin Activity Radioimmunoassay Kit was used for the quantitative determination of plasma renin activity by the RIA of generated angiotensin I (DiaSorin, Stillwater, MN, USA). Aldosterone was measured by Coat-A-Coat Aldosterone, a solid-phase ^{125}I RIA designed for the quantitative measurements of aldosterone levels in unextracted serum (Siemens, DPC, Los Angeles, CA, USA). Measurements of 17OHP were compared with previously recommended target ranges for patients with CAH (25). Serum 25(OH)-vitamin 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. Sodium, potassium, glucose, HbA1c, cholesterol, HDL, triglycerides and ALAT were measured by the various University Hospital laboratories. The LDL concentration was calculated based on the total cholesterol and HDL measurements.

Statistical analyses

The results are given as mean and s.d., 95% confidence interval (CI) or median and range when appropriate. Concordance between genotype and phenotype is given as the percentage who had a clinical phenotype that corresponded to one of the groups predicted from the gene defect (26). In compound heterozygous cases, the mildest mutation defines the genotype group. Group null has genetically predicted complete enzyme impairment, group A has almost complete enzyme impairment, group B has severe enzyme impairment (<10% residual enzyme activity) and group C has partial impairment (7–75% residual activity). The Mann–Whitney *U* test was used for comparisons between genders and phenotypes, and multiple linear regression was used for analysis of height, BMI, fat mass, blood pressure, vitamin D, androgens and 17OHP levels, with age and glucocorticoid doses as independent variables. Spearman's ρ (*r*) was estimated for the correlations between height, BMI, age and hormone levels. *T*-test for independent samples was used to compare weight, height, waist–hip measurement, blood pressure, HDL, ALAT and calcium measurements with those found in the normal population (HUNT). Other biochemical values were compared with the reference values and considered statistically significant if the CIs of the mean did not overlap with the reference values. BMD is expressed by Z-scores, representing age- and gender-adjusted SDS presented with 95% CI, where CI different from zero indicates statistically significant difference from the reference population. Body composition was compared with the percentage fat distribution given in the Lunar database and differences considered statistically significant if the CI of the mean did not overlap with the Lunar reference values.

Results

Genetic analysis

Altogether, 126 CYP21A2 alleles from 63 patients were studied (Table 1); whole blood from one patient was missing. We identified four novel mutations: L388R,

E140K, V211M and P45L. The phenotype in three patients was reclassified based on their detailed medical history, which had not been revealed in the previous survey. One male in group null was changed from SV to SW; he was diagnosed with 21OHD and commenced treatment with glucocorticoids at 25 years of age, even though his older sister was diagnosed with SW at 3 months of age and two siblings had died as neonates. He was 151 cm tall and had testicular atrophy, had subsisted on massive salt consumption and had experienced periods of profound weakness. The two other patients were reclassified based on new clinical information that corresponded better with the genotype. As shown in Table 1, the genotype grouping corresponded to the revised clinical phenotype in 54 of 59 (92%) of the patients. Of note, one woman in group null and one in group A had typical SV phenotype.

Clinical characteristics, BMD and blood pressure

The characteristics of the patients compared with the general population are shown in Table 2. The mean dose in hydrocortisone equivalents was 28.0 ± 14.0 mg (15.7 ± 7.2 mg/m²) in women and 34.9 ± 13.7 mg (18.4 ± 7.3 mg/m²) in men. The males used significantly higher total glucocorticoid doses than the females ($P=0.013$). The percentages of different glucocorticoid types in men and women, respectively, were prednisolone 26 and 44%, dexamethasone 26 and 27%, cortisone acetate 44 and 24% and hydrocortisone 4 and 7%. Men were more frequently treated with a combination of two glucocorticoids than women (26 vs 7%). Mineralocorticoid replacement with fludrocortisone was given to 42% of the women and 52% of the men. Seventy-two per cent of the patients had renin levels above the normal range, and there was no difference between the patients with or without mineralocorticoid replacement.

The BMI measurements indicate that the patients are moderately overweight, similar to the general population; however, the populations may not be directly comparable as the patients are generally shorter (Table 2). The shortest male patients had the highest BMI ($r=-0.463$ and $P=0.026$) and significantly lower hip circumference and higher waist/hip ratio than the reference population. Both sexes had markedly higher body fat mass than the Lunar reference values (Table 3). Contrary to the reference values, female patients had the highest fat mass at young age (Fig. 1A). In the female patients, fat mass and glucocorticoid doses correlated significantly ($r=0.360$, $P=0.021$). The male patients displayed a trend towards positive correlation between fat mass and age ($r=0.383$, $P=0.078$), similar to that in the reference population (Fig. 1B). There was no significant difference in fat mass between SW and SV.

Table 1 Genotype and phenotype in 63 adult patients with 21OHD.

Genotype ^a	Allele 1	Allele 2	Mutation no.	Phenotype		
				SW	SV	NCC
Group null	del	del	11	11		
	del	Large pseudogene conversion (5')	1	1		
	del	Large pseudogene conversion (5'), P453S	1		1	
Group A	I172N, ClusterE6, V281L, L307Frameshift	I172N, ClusterE6, V281L, L307Frameshift	1	1		
	I2 splice	del	6	6		
	I2 splice, V281L	del	2	2		
	I2 splice	I2 splice, ClusterE6, V281L, R356W	1	1		
	I2 splice	I2 splice, ClusterE6, V281L	1	1		
	I2 splice	R426H	2	2		
	I2 splice	I2 splice	4	4		
	I2 splice, P453P, S ^b , D407D, N ^b	I2 splice, P453P, S ^b , D407D, N ^b	1		1	
Group B	I2 splice	I2 splice, V281L	1	1		
	I172N	Q318x	1		1	
	I172N	del	12		12	
	I172N	I172N, ClusterE6	1		1	
	I172N	I2 splice	1		1	
	I172N	I172N	5		5	
	I172N	G291R	1		1	
	I2 splice heterozygous, I172I, N ^b , V281V, L ^b	I2 splice heterozygous, I172I, N ^b , V281V, L ^b	1		1	
Group C	I2 splice	P30L, promoter conversion	1		1	
	H62L	del	1		1	
	V281L	A434V	1			1
	D407N	P453S	1		1	
	P30L	I172N	1		1	
New mutations ^d	del	<i>L388R</i>	1	1		
	del	<i>E140K</i>	1	1		
	del	<i>V211M</i> , V281L	1		1	
	del	<i>P45L</i>	1		1	
Total (no.)			63	32	30	1

SW, classic salt wasting; SV, simple virilising; NCC, non-classical.

^aGenotype classified according to predicted severity of mutation groups; null, complete enzyme impairment; group A, almost complete enzyme impairment; group B, severe enzyme impairment; group C, partial impairment.^bHaplotype unknown.^cEnzyme activity of G291R is not described in the literature.^dNovel mutations in *italic*.

The male and female patients had significantly higher diastolic blood pressure compared with the general population (Table 2), also when correcting for age in 10-year age strata, and although the supine position would normally underestimate the difference (18). Systolic blood pressure was not different from reference population data. No correlation was found between blood pressure and mutation groups, glucocorticoid doses, BMI or fat mass. Overall, patients with SV had higher diastolic blood pressure than the SW (81 vs 76 mmHg, $P=0.04$), and we found a positive correlation between diastolic blood pressure and aldosterone levels ($r=0.405$, $P=0.002$).

Lumbar spine, femoral and total body BMD Z-scores are shown in Table 3. At the lumbar spine, osteopenia was found in 44% and osteoporosis in 4.3% of the male patients and in 24 and 4.9%, respectively, of the females. The corresponding figures at the femoral neck were 44 and 8.7% in the males and 30 and

7.3% in the females. If all measured locations were merged, the frequencies of osteoporosis and osteopenia were 8.7 and 48% in men and 9.8 and 34% in women respectively. There was no significant correlation between age and Z-scores, neither in the males nor in the females and no significant difference in Z-scores between SW and SV.

Final height was reduced in both genders, and was more pronounced in men (median -11.2 cm, SDS -1.77) than in women (-6.3 cm and -1.07) (Fig. 2). The median height was 168 (range 151–186) cm in males and 159 (144–173) cm in females. The height did not correlate with current glucocorticoid or mineralocorticoid doses. There was a negative correlation between height and age ($r=-0.251$, $P=0.045$), similar to that in the normal population (Fig. 2). There was no significant difference in mean age at diagnosis between the genders (4.8 years in men and 6.4 years in women).

Table 2 Comparison of anthropometric data for Norwegian adults with 21OHD and age-matched controls from HUNT (North-Trøndelag Health Study), mean $\pm$ s.d.

	Female 21OHD SW+SV	Female control	P, female 21OHD vs HUNT	Male 21OHD SW+SV	Male control	P, male 21OHD vs HUNT
Number	41	23.000		23	12.961	
Age, mean (range)	40.3 (19–72)	42.1 (19–72)		39 (20–58)	37.3 (19–58)	
Height (cm)	159.1 $\pm$ 7.1	166.0 $\pm$ 6.0	<0.0001	168.2 $\pm$ 7.6	179.7 $\pm$ 6.5	<0.0001
Weight (kg)	72.7 $\pm$ 19.5	72.6 $\pm$ 14.0	NS	78.2 $\pm$ 12.3	87.2 $\pm$ 13.4	0.002
BMI (kg/m ²)	28.0 $\pm$ 8.6	26.4 $\pm$ 4.9	NS	27.8 $\pm$ 4.9	27.0 $\pm$ 3.9	NS
Waist (cm)	89.5 $\pm$ 16.4	88.5 $\pm$ 12.9	NS	93.0 $\pm$ 13.1	94.5 $\pm$ 10.7	NS
Hip (cm)	103.1 $\pm$ 17.0	103.4 $\pm$ 12.2	NS	96.4 $\pm$ 9.0	103.0 $\pm$ 7.0	<0.0001
Waist/hip ratio	0.870 $\pm$ 0.097	0.852 $\pm$ 0.071	NS	0.964 $\pm$ 0.082	0.909 $\pm$ 0.060	0.004
SBP (mmHg)	23.0 $\pm$ 20.0	121.7 $\pm$ 19.6	NS	124.0 $\pm$ 14.5	128.8 $\pm$ 12.6	NS
DBP (mmHg)	78.1 $\pm$ 10.6	68.8 $\pm$ 9.7	<0.0001	78.5 $\pm$ 8.1	72.2 $\pm$ 9.54	0.02

NS, not significant; NA, not applicable.

Biochemical findings

Forty-eight per cent of the patients had 17OHP levels above the recommended upper target at 36 nmol/l and 33% below the lower target at 12 nmol/l. The high mean values of 649 nmol/l in females and 612 nmol/l in males were largely caused by a subset of patients with extremely high values; the median values were 100 nmol/l in females and 32.7 nmol/l in males. Only 12% of the females had testosterone levels within the normal range, whereas 39% had testosterone levels above, and 49% below the normal range. Twenty-two per cent of the women had androstenedione levels above the normal range. The mean DHEAS level on the other hand was lower than normal (Table 4). In males, 13% had testosterone levels below the normal range and most had testosterone levels in the lower normal reference range (Table 4). There was no correlation between age and androgen levels, including free

testosterone index, in either sex. We found negative correlation in the men between total fat mass and testosterone levels ($r = -0.485$ and $P = 0.022$). The ACTH levels were significantly higher in SW (median 23.1 pmol/l) than in SV (5.6 pmol/l) ($P = 0.029$), whereas serum aldosterone was significantly higher in SV (median 419 pmol/l) than in SW (129 pmol/l) ($P = 0.005$). Aldosterone could be falsely elevated due to interference in the analysis by elevated mineralocorticoid precursors. Otherwise, we found no hormonal difference between the disease categories. There were significant correlations between 17OHP and ACTH ($r = 0.83$, $P < 0.001$), 17OHP and androstenedione ($r = 0.88$, $P < 0.001$), and 17OHP and testosterone in the females ($r = 0.36$, $P = 0.022$) and 17OHP and DHEAS in the men ($r = 0.68$, $P = 0.001$).

Markers of bone and glucose homeostasis, lipids and liver enzymes are shown in Table 4, and all displayed

Table 3 Bone mass density and body composition in adult patients with 21-hydroxylase deficiency (21OHD) compared with Lunar reference data, mean $\pm$ s.d.

	SW	SV	All	CI, All	CI, Lunar	P, 21OHD vs Lunar
Women	<i>n</i> = 19	<i>n</i> = 22	<i>n</i> = 41	<i>n</i> = 41		
BMD						
Z-score lumbar spine (L2–L4)	−0.67 $\pm$ 1.17	−0.005 $\pm$ 1.12	−0.32 $\pm$ 1.18	(−0.69, 0.05)		NS*
Z-score femoral neck	−0.39 $\pm$ 0.92	−0.53 $\pm$ 0.94	−0.47 $\pm$ 0.95	(−0.76, −0.18)		<0.05*
Z-score total body	−0.34 $\pm$ 0.89	0.07 $\pm$ 1.23	−0.13 $\pm$ 1.08	(−0.47, 0.21)		NS*
Body composition						
Body fat (kg)	33.8 $\pm$ 18.4	30.0 $\pm$ 18.4	31.8 $\pm$ 18.3	(29.0, 34.5)	NA	NA
Body fat (kg/m ²)	13.3 $\pm$ 7.5	11.8 $\pm$ 6.8	12.5 $\pm$ 7.1	(10.4, 14.7)	NA	NA
Total fat (%)	40.1 $\pm$ 11.7	37.3 $\pm$ 8.0	38.6 $\pm$ 9.9	(37.0, 40.1)	(31.9, 32.1)	<0.05 ^a
Men	<i>n</i> = 14	<i>n</i> = 9	<i>n</i> = 23			
BMD						
Z-score lumbar spine (L2–L4)	−0.82 $\pm$ 1.09	−0.80 $\pm$ 1.30	−0.81 $\pm$ 1.15	(−1.30, −0.30)		<0.05*
Z-score femoral neck	−0.78 $\pm$ 0.68	−0.84 $\pm$ 0.81	−0.81 $\pm$ 0.72	(−1.10, −0.52)		<0.05*
Z-score total body	0.47 $\pm$ 1.15	−0.57 $\pm$ 1.15	−0.51 $\pm$ 1.11	(−0.96, −0.06)		<0.05*
Body composition						
Body fat (kg)	25.5 $\pm$ 10.9	18.1 $\pm$ 8.5	22.5 $\pm$ 10.4	(19.7, 25.3)	NA	NA
Body fat (kg/m ²)	8.5 $\pm$ 4.8	6.5 $\pm$ 3.2	7.7 $\pm$ 4.3	(6.0, 9.4)	NA	NA
Total fat (%)	31.4 $\pm$ 11.1	24.3 $\pm$ 9.2	28.5 $\pm$ 10.8	(25.2, 31.8)	(22.0, 22.0)	<0.05 ^a

NS, not significant; NA, not available, * $P < 0.05$ against zero.^aComparing 95% CI and considered statistically significant if CI did not overlap.

Figure 1 Total fat per cent in patients with 21OHD compared with the reference population (Lunar), mean and s.d. s.d. is small in the reference population and not indicated.

mean values within the reference ranges. The prevalence of vitamin D deficiency (defined as 25(OH)-vitamin D < 50 nmol/l) was 39% in the women and 26% in the men. Women had higher PTH levels than men ($P=0.047$) (Table 4). There was a negative correlation between 25(OH)-vitamin D level and total fat mass ($r=-0.46$, $P<0.001$). ALAT was lower in 21OHD males than in the men in the HUNT database (25.3 U/l vs 35.9, $P<0.001$), but no such difference was found in the women. The patients had mean total cholesterol, HDL, LDL and triglycerides within the reference range. However, HDL in the female patients showed a trend towards higher values than those of the HUNT women (mean 1.61 (s.d. 0.45) mmol/l vs 1.46 (0.35), $P=0.054$). Sodium levels were significantly lower in SV (mean 139.3 mmol/l (s.d. 1.9)) than in SW (140.6 mmol/l (2.9), $P=0.006$).

Discussion

The particular strengths of this population-based study are that it represents two-thirds of the identified adult 21OHD patient population, including patients with a

wider age range and more men than in most previous publications. The frequencies of the common *CYP21A2* gene defects resembled those of published results from other Western European countries (2, 27). We identified four novel *CYP21A2* mutations that are most likely pathogenic. We found a high correspondence between genotype and clinical phenotype, a correlation that is generally considered robust in 21OHD (28).

High fat mass in the 21OHD patients was a major finding, which was most pronounced in younger women. Most studies have found increased BMI (8, 29, 30), and four studies demonstrated increase in fat mass (7, 8, 31, 32), two of them only in males. Although we did not find higher BMI than the reference population, we found markedly increased body fat mass in both men and women. The high fat mass in younger women is at variance with data from Sweden showing fat mass in female and male 21OHD patients similar to age-matched controls but increased fat mass in male patients older than 30 years (32, 33). One explanation could be the higher hydrocortisone equivalent dose used by the young Norwegian females (38.9 mg/day) compared with the young Swedish females (33.3 mg/day). The glucocorticoid doses were also higher than the hydrocortisone doses of 15–25 mg/day suggested by the Endocrine Society (3). We previously reported that only 47% of the Norwegian

Figure 2 Height in patients with 21OHD compared with the normal population in HUNT (North-Trøndelag Health Study), mean and 95% CI. CI is very small in the normal population and not marked.

Table 4 Biochemical findings in 64 adult patients with 21OHD and corresponding reference values (ref. value), mean and 95% CI.

	Women			Men		
	Patients (n=41)	Ref. value	P*, 21OHD vs ref. value	Patients (n=23)	Ref. value	P*, 21OHD vs ref. value
S-glucose (mmol/l)	4.6 (4.5–4.8)	3.6–5.0	NS	5.0 (4.8–5.2)	3.6–5.0	NS
B-HbA1c (%)	5.5 (5.4–5.6)	4.0–5.8	NS	5.5 (5.4–5.6)	4.0–5.8	NS
S-insulin C-peptide (nmol/l)	761 (678–845)	400–1700	NS	947 (836–1056)	400–1700	NS
S-cholesterol (mmol/l)	5.4 (5.2–5.6)	3.3–6.9	NS	5.1 (4.8–5.4)	3.9–7.8	NS
S-HDL (mmol/l)	1.6 (1.5–1.8)	1.0–2.7	NS	1.3 (1.2–1.3)	0.8–2.1	NS
S-ALAT (U/l)	25.9 (23.8–28.1)	10.0–45.0	NS	25.3 (23.1–27.4)	10.0–70.0	NS
S-17OHP (nmol/l)	649 (399–899)	1.2–9.9	<0.05	612 (351–871)	1.8–7.8	<0.05
P-ACTH (pmol)	25.2 (17.1–33.3)	<11.6	<0.05	49.8 (32.3–67.4)	<11.6	<0.05
S-androstenedione (nmol/l)	12.0 (17.1–33.4)	<11.5	<0.05	13.2 (10.1–16.4)	2.1–10.8	NS
P-renin activity (μg/l/t)	6.9 (5.9–7.8)	0.5–3.4	<0.05	7.1 (5.4–8.8)	0.5–3.4	<0.05
S-aldosterone (pmol/l)	449 (375–523)	80–860	NS	420 (326–515)	80–860	NS
S-free testosterone index ^a	0.53 (0.35–0.71)	0.1–0.6	NS	4.5 (3.69–5.31)	2.3–9.9	NS
	n=31	Patients <50 years		All		
S-DHEAS (μmol/l)	1.3 (1.0–1.5)	2.0–10.5	<0.05	0.9 (0.9–3.0)	2.0–10.5	NS
S-testosterone (mmol/l)	2.8 (2.2–3.5)	0.3–1.9	<0.05	14.5 (13.1–15.9)	8.0–35.0	NS
S-FSH (IE/l)	6.7 (5.4–7.9)	1.2–21	NS	5.2 (4.0–6.3)	0.7–11.1	NS
S-LH (IE/l)	6.8 (5.1–8.6)	1.1–77	NS	3.0 (2.5–3.6)	0.8–7.6	NS
S-PTH (pmol/l)	4.0 (3.7–4.3)	1.3–6.8	NS	3.2 (2.6–3.8)	1.3–6.8	NS
S-25(OH)-vitamin D (nmol/l)	54.4 (49.9–59.0)	43–168	NS	61.8 (55.8–67.7)	43–168	NS
S-osteocalcin (nmol/l)	2.6 (2.4–2.8)	1.4–5.7	NS	3.0 (2.5–3.6)	0.8–7.6	NS
	n=10	Patients ≥50 years				
S-DHEAS (μmol/l)	1.1 (0.4–1.7)	0.3–2.5	NS			
S-testosterone (mmol/l)	2.1 (0.9–3.3)	0.2–1.1	NS			
S-FSH (IE/l)	70.7 (56.3–85.2)	21.7–153	NS			
S-LH (IE/l)	33.5 (26.8–40.2)	11.3–40	NS			
S-osteocalcin (nmol/l)	2.5 (2.0–2.9)	2.1–9.1	NS			

B, full blood; S, serum; P, plasma, *P is considered statistically significant <0.05 if the CI of the mean do not overlap with the reference value.

^aFree testosterone index is calculated as total testosterone/SHBG × 10.

patients use mineralocorticoid (17), which compared with 84% of the Swedish patients (32, 33), which could contribute to the differences in both glucocorticoid doses and body composition.

High blood pressure, including isolated diastolic hypertension, is, like fat mass, a marker of risk of cardiovascular disease (34). In paediatric and adolescent patients, blood pressure has showed a tendency towards elevation in some (35) but not in all studies (14). Some small studies have addressed blood pressure in adult patients, showing no difference in single blood pressure or 24-h blood pressure measurements compared with controls (32, 33). However, Arlt *et al.* (30) recently found increased diastolic blood pressure in 102 female 21OHD patients compared with national data in the UK. Mooij *et al.* (36) found elevated 24-h ambulatory blood pressure in 27 adults of both sexes with 21OHD compared with BMI-matched controls. Thus, our results extend these observations, showing significantly higher diastolic levels found also in men. Interestingly, we found a significant negative correlation between fludrocortisone doses and diastolic blood pressure. Recent guidelines recommend that all classic 21OHD patients be put on fludrocortisone at diagnosis and administered in the first years of life; subsequently, the treatment can be more individualised (3). Although

patients can commonly taper down fludrocortisone doses with age, continued treatment might be prudent (37).

We found significantly reduced BMD in both men and women and higher frequency of osteoporosis and osteopenia compared with a population-based sample of middle-aged Norwegians (21), confirming the results of the majority of other studies (7, 11, 30). Interestingly, we found a much higher proportion of osteopenia in men than in women. One plausible explanation could be that men were using higher glucocorticoid doses and that most of the men had low testosterone levels. In our study, women had lower vitamin D levels and higher PTH than men. Reports on vitamin D status in 21OHD patients are sparse, but Koetz *et al.* (12) also found vitamin D levels in the lower normal range in 41 patients with 21OHD. Our 21OHD patients had lower values of 25(OH)-vitamin D than published data from the general population (mean 56.5 nmol/l (s.d. 27.3) vs 64.4 (28.8), $P \leq 0.05$) (38). One reason for lower than normal vitamin D levels in our study, especially in the women, could be the increased fat mass, as vitamin D levels are lower in obesity probably due to storage of D vitamin in adipose tissue (39).

Another finding in the 21OHD patients was markedly reduced final height, most pronounced in men. Studies

from Sweden also found that height in males were more negatively affected than in females (40), and a recent meta-analysis from 35 eligible studies concluded with reduced final height in both sexes but no significant association with age at diagnosis, sex, type, dose of glucocorticoids and age at onset of puberty (41). They found that mineralocorticoid users had a better height outcome in comparison with the non-users. The meta-analysis suggests improved outcome in height over the last years due to better treatment.

Only 20% of the patients had morning fasting 17OHP levels in the recommended target range (25); for androstenedione, the corresponding figure was 43%. Arlt *et al.* (30) found less elevated 17OHP (11%) and androstenedione (20%) in the UK, but in that study, the blood samples were drawn at median 3.75 h after intake of the last glucocorticoid dose. Biochemical monitoring of 21OHD therapy is controversial and difficult as the serum hormones fluctuate with time of day and intake of glucocorticoid tablets. Thus, a random test of serum hormones is of little value. A recent review article about therapy monitoring in 21OHD recommends clinical parameters such as growth velocity and bone age as gold standards and the use of 24-h urine collection for pregnanetriol (a urinary metabolite of 17OHP) and 17-ketosteroids (a urinary metabolite of androgens). Diurnal 17OHP curves with dried blood spots can also be used to adjust glucocorticoid doses. Unfortunately, target concentrations are not clearly defined (42).

Glucocorticoid excess and elevated androgens in females, and decreased testosterone in males, may lead to insulin resistance (43, 44), and many reports have demonstrated increased insulin resistance in 21OHD (45). We found normal fasting lipids, glucose, HbA1c and insulin levels and a rather favourable lipid profile in the women with elevated levels of HDL. Unfortunately, we do not have an age-matched control population, only the laboratory reference values. The Swedish study showed impaired insulin sensitivity only in patients older than 30 years (32, 33). Falhammar *et al.* (32, 46) further reported increased liver enzymes in adult 21OHD patients and speculated that these findings suggest non-alcoholic fatty liver disease, but we found the opposite results. No information exists on heart disease and mortality in adult 21OHD patients, and most of the studies on cardiovascular risk factors include small cohorts (30).

In conclusion, we have identified four novel mutations and found high concordance between genotype and phenotype. Few of the patients had androgen levels in the recommended target range. Many females had either high or very low testosterone levels and many males had low testosterone levels. The patients had reduced final height, with high frequency of osteopenia in the men, increased fat mass and diastolic blood pressure. These results indicate that therapy and follow-up of patients could be improved.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-12-0196>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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