CLINICAL SCIENCE

Pharmacogenetics of glucocorticoid replacement could optimize the treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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INTRODUCTION: 21-hydroxylase deficiency is an autosomal recessive disorder that causes glucocorticoid deficiency and increased androgen production. Treatment is based on glucocorticoid replacement; however, interindividual variability in the glucocorticoid dose required to achieve adequate hormonal control has been observed.

OBJECTIVE: The present study aimed to evaluate the association between polymorphic variants involved in glucocorticoid action and/or metabolism and the mean daily glucocorticoid dose in 21-hydroxylase deficiency patients.

METHODS: We evaluated 53 patients with classical forms of 21-hydroxylase deficiency who were receiving cortisone acetate. All patients were between four and six years of age and had normal androgen levels.

RESULTS: The P450 oxidoreductase A503V, HSD11B1 rs12086634, and CYP3A7*1C variants were found in 19%, 11.3% and 3.8% of the patients, respectively. The mean $\pm$ SD glucocorticoid dose in patients with the CYP3A7*1C and wild-type alleles was 13.9 ± 0.8 and 19.5 ± 3.2 mg/m^2/d, respectively. We did not identify an association between the P450 oxidoreductase or HSD11B1 allelic variants and the mean glucocorticoid dose.

CONCLUSION: Patients carrying the CYP3A7*1C variant required a significantly lower mean glucocorticoid dose. Indeed, the CYP3A7*1C allele accounted for 20% of the variability in the cortisone acetate dose. The analysis of genes involved in glucocorticoid metabolism may be useful in the optimization of treatment of 21-hydroxylase deficiency.

KEYWORDS: 21-hydroxylase deficiency; Glucocorticoid replacement therapy; Pharmacogenetics; Polymorphism; CYP3A7*1C allele.

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a common autosomal recessive disorder, caused by mutations in the 21-hydroxylase gene (*CYP21A2*). These mutations result in decreased glucocorticoid (GC) secretion with or without mineralocorticoid deficiency and excess androgens.[1,2]

GC replacement therapy significantly improves the prognosis of CAH. The goal of GC replacement therapy is to restore the levels of the steroid hormones to the normal range and to decrease adrenocorticotrophic stimulation, which would suppress the effects of androgen overproduction. The aims of GC replacement are to avert adrenal crisis and to allow normal growth and puberty development. Similarly, mineralocorticoid replacement restores serum electrolyte levels and water balance without excessive salt retention.

During growth, preference is given to short half-life glucocorticoids (e.g., hydrocortisone acetate) that avoid growth suppression and weight gain, which are commonly observed when long half-life GCs (e.g., prednisone or dexamethasone) are used.[1] Among the GCs with a short half-life, cortisone acetate (CA) is one therapeutic option for the treatment of children with CAH.[3] Cortisone acetate is converted to cortisol by the action of 11β-hydroxysteroid

dehydrogenase type 1 (11βHSD1); however, variability in 11βHSD1 activity has been described.[4]

Despite great advances in our understanding of the pathophysiology of CAH, most studies still report mean final heights in CAH patients under −1.5 Standard Deviation (SD).[5] These shorter heights also reflect the difficulties in treatment compliance and the complexity of GC dose adjustments to balance cortisol insufficiency and androgen overproduction.

Furthermore, the interindividual variability in daily glucocorticoid dose requirements poses an additional challenge in the treatment of CAH[8] and suggests that genetic factors may modulate the glucocorticoid response. In the treatment of Addison's disease, genetic factors play a major role in the differential response to glucocorticoid replacement regimens and/or the incidence of side effects.[9] However, genetic factors have never been evaluated in CAH, which led us to hypothesize that interindividual variability in glucocorticoid metabolism could be involved.

The CYP3A family of enzymes is the most important family of enzymes for drug metabolism in humans because they metabolize the majority of commercially available drugs. Many studies have shown that polymorphisms in CYP3A genes account for interindividual variability in the metabolism of many clinically used drugs.[10] The CYP3A7 gene, which encodes P4503A7, is abundantly expressed in the fetal liver, and its expression declines rapidly after birth. The polymorphic variant CYP3A7*1C, which results from the replacement of an approximately 60-bp sequence within the promoter of CYP3A7 with an analogous region of the CYP3A4 promoter, leads to persistent P4503A7 expression in the adult liver[11] and has been associated with lower serum dehydroepiandrosterone sulfate (DHEAS) and testosterone levels compared with the levels in individuals lacking this variant.[12] Because of the presence of lower androgen levels, we speculated that the CYP3A7*1C allele may decrease the glucocorticoid requirement in CAH patients.

The 11βHSD1 enzyme is involved in glucocorticoid metabolism by converting inactive cortisone into active cortisol.[13] A polymorphism located in intron 3 (rs12086634) has been associated with decreased conversion of cortisone to cortisol and may function as an intronic enhancer.[14] Therefore, CAH patients with this polymorphism could require higher CA doses compared with patients lacking this polymorphism.

Finally, the P450 oxidoreductase (POR) gene encodes a flavoprotein that donates electrons to cytochrome P450 type II enzymes, including P450c21 and P450c17. A frequent POR variant (A503V) has been associated with reduced P450c17 activity and, consequently, with lower androgen secretion.[15] Because of the critical role of this polymorphism in steroid hormone production, we hypothesized that it could reduce the glucocorticoid requirement in CAH patients.

Therefore, the aim of the present study was to investigate whether interindividual variability in glucocorticoid requirements amongst treated CAH patients is influenced by the presence of polymorphisms in CYP3A7, POR or HSD11B1.

SUBJECTS AND METHODS

Subjects

The present study was approved by the Ethic Committee of the Faculdade de Medicina da Universidade de São Paulo, and written consent was obtained from patients and controls.

The medical records of 53 patients with CAH were reviewed from a group of 213 classical CAH patients who underwent glucocorticoid replacement therapy in our hospital. The selection criteria included the use of CA, good compliance and hormonal control between the ages of 4 and 6 years (before starting pubertal development). Adequate control was defined by normal auxological parameters and biochemical assessments according to age during the two years of this study. The assessment comprised eight laboratory measurements.

Testosterone and androstenedione levels were maintained at levels lower than 0.48 nmol/l (14 ng/dl) and 69.9 pmol/L (2 ng/ml), respectively. Bone age was evaluated yearly using the Greulich-Pyle system. No patient presented with adrenal crisis during this period or clinical/hormonal parameters suggestive of overtreatment (e.g., Cushing features, decreased growth rate or 17-hydroxyprogesterone levels less than 36 nmol/l).

Thirty-eight patients (27 females) had the salt-wasting (SW) form, which was defined as the development of dehydration, a sodium level less than 130 mmol/l and high plasmatic renin activity in the neonatal period. Fifteen patients (11 females) had the simple virilizing (SV) form, which was characterized by ambiguous genitalia in girls and signs of postnatal virilization in both sexes without dehydration or salt waste. At diagnosis, all patients had basal 17-hydroxyprogesterone levels that were greater than 50 ng/ml.

Daily CA doses ($mg/m^2/day$) were evaluated retrospectively. The CA doses were administered three times a day, and the highest dose was administered at bedtime. Patients with the SW form also received fludrocortisone 50 ± 25 μg/day in the morning. No patient received gonadotropin-releasing hormone analogs, aromatase inhibitors or anticonvulsants.

Body mass index (BMI) was calculated as the weight (in kilograms) divided by the height (in meters) squared, and the BMI Z-score was determined according to the curves of the Centers for Disease Control and Prevention. Of the 53 patients, 13 achieved final height (FH). The relationship between the achieved FH and target height (TH) is expressed as FH.SDS - TH.SDS, and this value was individually calculated for each patient.

Methods

CYP21A2 mutations were screened by Southern blotting to identify large gene rearrangements and by allele-specific polymerase chain reaction (PCR) to determine the 15 common microconversions.[16] If mutations were not identified in both alleles, *CYP21A2* sequencing was performed. The identified mutations were segregated in parent DNA samples. *CYP21A2* genotypes were divided according to predicted impairment of enzymatic activity as previously described: null and A and B groups, prediciting 0%, 2% and 3–7% residual enzymatic activity, respectively .[1]

Selected regions of the CYP3A7, POR, and HSD11B1 genes were amplified and sequenced as previously described.[11,15,17] One hundred healthy volunteers were selected to determine the allelic frequency of the identified polymorphisms. None of the control subjects had a history of malignancy.

Statistical Analyses

Qualitative variables are listed as frequencies and percentages, whereas quantitative variables are presented as the mean ± SD. Group comparisons based on genotype were

performed using an unpaired Student *t*-test or a Mann-Whitney rank sum test for numerical variables and a Chi-square or Fisher exact test for nominal variables, as appropriate. To assess whether the genetic variants had independent prognostic significance for outcome, we performed single regression analysis followed by multiple regression analysis adjusting for established influential factors. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

The POR A503V, HSD11B1 rs12086634, and CYP3A7*1C polymorphic variants were found in 19%, 11.3%, and 3.8% of the CAH patients and in 26.4%, 17.4%, and 2.3% of the control subjects, respectively. The CYP3A7*1C and POR variants were identified only in heterozygous state in the studied patients. In contrast, 1% of the CAH patients and 6% of the controls were homozygous for the HSD11B1 rs12086634 allele, whereas 10.3% of the CAH patients and 12% of the controls were heterozygous. The frequency of these genetic variants did not differ between children with CAH and the controls ($p>0.05$). All polymorphisms were in Hardy-Weinberg equilibrium.

In patients between 4 and 6 years of age with the SW and SV forms, the mean CA dose was 18.5 ± 3.4 and 20.1 ± 3.3 mg/m^2/day, respectively ($p=0.086$). In SW patients between 4 and 6 years of age, the mean fludrocortisone dose was 50 ± 25 μg/day. There were no differences in the mean CA doses among patients with the null, A or B *CYP21A2* genotypes ($p>0.05$).

In the period of this study, the mean bone age advancement in SW and SV patients was 1.5 ± 0.7 and 1.2 ± 0.6 years, respectively ($p>0.05$). The mean BMI Z-scores at 4 and 6 years of age were 0.8 ± 0.003 and $0.7\pm\pm0.02$, respectively. In addition, the mean 17-hydroxyprogesterone levels during these two years were 194.8 ± 157.6 nmol/l (6,283$\pm$5,063 ng/dl) and 202.7 ± 132.8 nmol/l (6,540$\pm$4,285 ng/dl) in patients with SW and SV forms, respectively. Of the 53 patients, 13 achieved FH, and the corrected FH ranged from −0.87 to +0.86 SD. The clinical data are described in Table 1.

There were no differences in the mean daily CA doses between the patients with the wild-type POR allele and the patients heterozygous for the POR A503V allele or between the patients with the wild-type HSD11B1 allele and the patients heterozygous or homozygous for the HSD11B1 rs12086634 allele (Table 2).

Interestingly, the mean daily CA dose was lower in patients with the CYP3A7*1C allele compared with patients with the wild-type allele: 13.9 ± 0.8 *vs.* 19.5 ± 3.2 mg/m^2/d, respectively (95% confidence interval for difference of means: 2.4 to 8.8 mg/m^2/d; $p=0.001$, power of calculation 96.2%). Moreover, single regression analysis followed by multiple linear regression analysis revealed that the influence of this polymorphism on CA dose did not vary according to clinical form, sex, *CYP21A2* genotype or concomitant use of fludrocortisone. The CYP3A7*1C variant alone accounted for 20% of the CA dose variability ($p=0.001$), and 33% of the observed variability was explained by the presence of CYP3A7*1C in association with the age at diagnosis ($p=0.004$).

DISCUSSION

A short FH and adverse effects have been described during CAH treatment despite the achievement of adequate hormonal control. Several approaches have been proposed to improve treatment, such as the addition of flutamide and testolactone to glucocorticoid replacement therapy[18] and therapy optimization strategies based on *CYP21A2* genotypes.[19] Although a good correlation has been observed between genotype and the clinical form, no such correlation has been identified between genotype and glucocorticoid requirements in classical forms.[19] Similarly, in our cohort, there were no differences in the mean glucocorticoid dose among patients with null, A, or B *CYP21A2* genotypes, which suggests that other factors influence the glucocorticoid requirement.

To investigate these factors, we chose to evaluate the glucocorticoid dose in patients between 4 and 6 years of age, which appears to be a period of steady linear growth. Several studies have shown a tendency to overtreat in the first three years of life to avoid SW crises. During puberty, however, the glucocorticoid requirement generally increases because of alterations in cortisol pharmacokinetics.[20] Despite suppressed androgen levels, we ruled out overtreatment in our cohort because the serum 17-hydroxyprogesterone levels were greater than 36 nmol/l, and the mean BMI Z-score and mean growth rate of all patients were both in the normal range.

Genetic factors have been shown to contribute to inter-individual variability in the response to different drugs.[10] Recently, genetic polymorphisms have been associated with

Table 1 - Clinical, auxological and hormonal data of 53 patients with CAH.

	SW patients	SV patients
Female/Male	27/11	11/4
Age at diagnosis (y)	0.2±0.8	2.9±1.7
Mean bone age (y) at diagnosis	-	5.8±4.5
Basal 17OHP (mmol/L) levels at diagnosis	303±341	199±166
Mean daily CA doses (mg/m^2/day)	18.5±3.4	20.1±3.3
Fludrocortisone (μg/day)	50±25	-
Mean BMI at 4 y (kg/m^2)	17.1±2.1	16.2±1.3
Mean BMI at 6 y (kg/m^2)	16.2±1.6	17.6±2.7
Mean BMI at 4 y (SDU)[1]	1.0±0.9	0.6±0.8
Mean BMI at 6 y (SDU)[1]	1.0±1.1	0.4±0.8
Height SDS for bone age at 4 y	0.02	0.9
Height SDS for bone age at 6 y	0.16	1.3
Final height SDS**	−1.4±1.4	−1.0±0.9
Target height SDS	−0.9±1.0	−0.6±1.2

SW: salt-wasting form, SV: simple virilizing form, CA: cortisone acetate dose used from 4–6 y of age.
Values are presented as the mean±SD.
**13/53 patients achieved final height.
[1]SDU scores are the number of SD units relative to the mean values for normal children of the same age and sex.

Table 2 - Mean±SD daily cortisone acetate doses (mg/m^2/day) according to genetic polymorphisms in patients with classical 21-hydroxylase deficiency.

Allelic Variant	Genotype	CA dose (mg/m^2/d)	*p*-value
HSD11B1	C/C	19.1±3.6	0.852
(rs12086634)	C/T	19.3±2.7	
CYP3A7	Wild type	19.5±3.2	<0.001
(CYP3A7*1C)	CYP3A7*1C	13.9±0.8	
POR	C/C	19.2±3.5	
(A503V)	C/T	18.7±3.4	0.569

CA: cortisone acetate.

variability in glucocorticoid doses and susceptibility to osteoporosis in Addison's disease.[9] Among treated CAH patients, there is great interindividual variability in the daily glucocorticoid dose. To the best of our knowledge, however, there are no data evaluating the effect of allelic variants other than *CYP21A2* gene variants.

The frequency of the POR A503V, HSD11B1 rs12086634, and CYP3A7*1C allelic variants was similar among patients and controls in our study. Although POR A503V is associated with decreased P450c17 activity and decreased androgen synthesis, our data suggest that this enzymatic impairment, at least in the heterozygous individuals seen in our cohort, does not influence the daily CA requirement. Interestingly, the HSD11B1 rs12086634 variant has been shown to reduce gene transcription *in vitro*, which was consistent with reduced cortisol generation and probably higher CA requirements.[14] In our cohort, however, HSD11B1 rs12086634 carriers required a CA dose similar to that required by individuals lacking this variant.

The CYP3A7 gene is predominantly expressed in the fetal liver, and its expression is sharply downregulated after birth.[11] However, the CYP3A7*1C allele contains a modified promoter that causes constitutive CYP3A7 expression in the adult liver.[21] P4503A7 catalyzes the 16α-hydroxylation of DHEA and DHEAS[22], and the CYP3A7*1C allele has been associated with lower DHEAS levels in women with polycystic ovary syndrome.[12] Although the allelic frequency of this genetic variant did not differ between the children with CAH and the controls, the glucocorticoid replacement doses were significantly lower in children with the CYP3A7*1C allele.

Because CAH treatment is designed to normalize androgens, we hypothesized that the CYP3A7*1C allele results in less severe hyperandrogenism and, consequently, decreased glucocorticoid requirements. However, the CYP3A7*1C allele was a rare variant in our Brazilian cohort and probably only affects the treatment of a minority of patients. There is no doubt that other variants in different genes, such as those related to gastrointestinal absorption, could contribute to the interindividual variability in glucocorticoid doses.

We cannot exclude a sample-size effect to explain the absence of a correlation between the POR and HSD11B1 variants and the mean daily glucocorticoid dose. This was a pilot study involving a large series of Brazilian patients followed at a single center, and we were able to select 53 patients who were treated with the same glucocorticoid (i.e., CA) since their diagnosis.

Despite the limitations of this study design, other retrospective studies have also highlighted the influence of pharmacogenetics on individual responses to drugs.[23,24] Indeed, pharmacogenetic testing has become an important tool to guide therapy with drugs such as warfarin, dasatinib and trastuzumab.[25]

The present study is the first to raise the potential advantage of genetic screening to obtain better CAH treatment. Future studies involving both larger cohorts and other candidate genes are certainly warranted to consolidate this approach.

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