Software-assisted Analysis of the Urinary Steroid Metabolome in treated prepubertal Children with classic Congenital Adrenal Hyperplasia

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Background

Treatment of children with classic congenital adrenal hyperplasia (CAH) is a difficult balance between hypercortisolism and hyperandrogenism. Biochemical monitoring of treatment is not well defined.

Objective and hypotheses

Retrospective software-based analysis of the urinary steroid metabolome obtained by gas chromatography-mass spectrometry (GC-MS) for treatment monitoring of children with CAH.

Methods

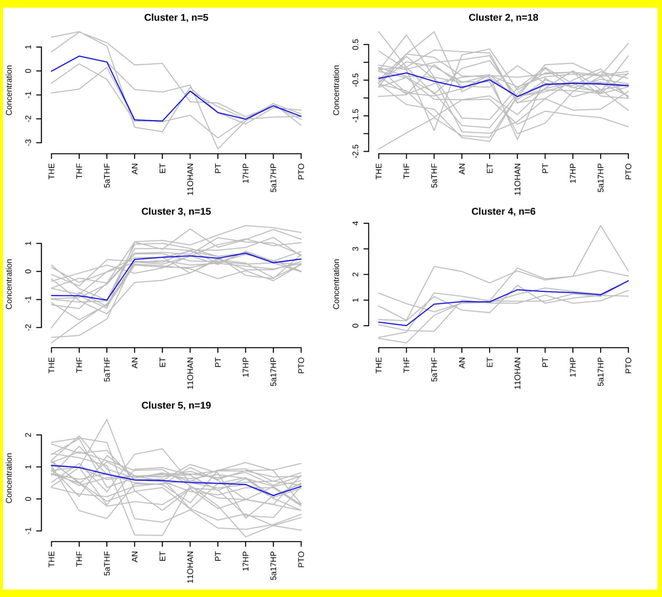
We evaluated 24-hour urinary steroid metabolome analyses of 63 prepubertal children aged 6.9 ± 1.5 years with classic CAH due to 21-hydroxylase deficiency treated with hydrocortisone (HC) and fludrocortisone. Steroid metabolites quantities were z-transformed based on sex and age-adjusted references of treated children with CAH [1]. Clusters were generated by k-means clustering algorithm using the MetaboAnalyst 4.0 software. **Fig. 1** gives an overview of analyzed urinary steroid metabolites.

Results

Five unique clusters were generated (**Fig. 2+3**). A subsequent principal component analysis showed that 58.8% of metabolite levels variations were explained by the 1st, and 25% by the 2nd principal component (**Fig 4**).

The HC dose differed significantly between the 5 clusters (ANOVA; P < .0001) (**Table 1**). Cluster #1 (N=5 (8%)) showed over-treatment through a combination of high urinary cortisol metabolites and low metabolites of androgens and 17-hydroxyprogesterone (17OHP). The daily HC dose was relatively high (14.5 ± 3.0 mg/m² BSA). Cluster #2 (N=18 (29%)) revealed good disease control due to moderate cortisol metabolites and suppressed androgen and 17OHP metabolites. The daily HC dose was 12.7 ± 2.2 mg/m² BSA. Cluster #3 (N=15; 24%) demonstrated under-treatment through a combination of low cortisol metabolites and very high metabolites of androgens and 17OHP. The daily HC dose was relatively low (10.5 ± 1.9 mg/m² BSA) and the HV was increased (1.96 ± 1.83 z). Cluster #4 (N=6 (10%)) and cluster #5 (N=19 (30%) both revealed treatment failure. Cluster #4 revealed despite appropriate urinary cortisol metabolites and high HC dose (14.8 ± 3.9 mg/m² BSA) unsuppressed very high androgen- and 17OHP metabolites. In cluster #5, metabolites of androgens and 17OHP were moderate to slightly elevated, although cortisol metabolites were markedly increased, and HC dose was high (14.4 ± 3.5 mg/m² BSA).

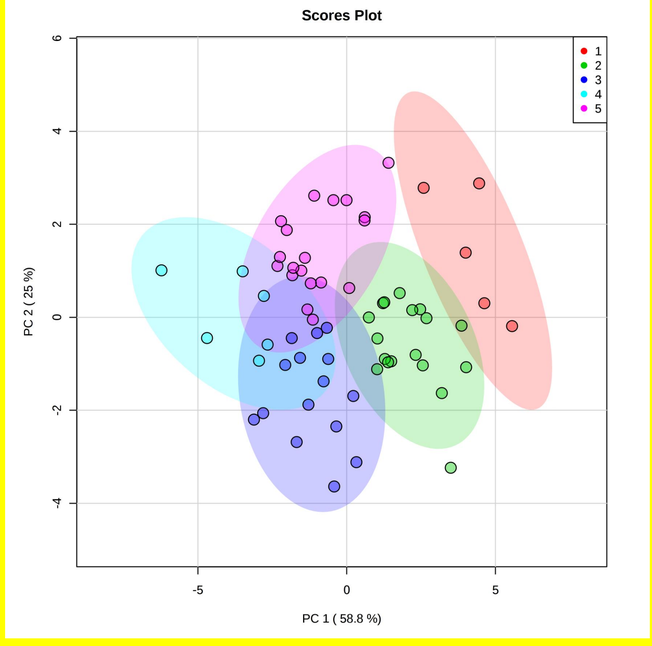
Figure 3. Steroid signature of each cluster.



The x-axis includes the analyzed ten major steroid metabolites. The y-axis indicates the z-transformed 24-h urinary quantities of steroid metabolites of children with CAH treated with hydrocortisone and fludrocortisone (N = 63). Median values of subjects in each cluster are connected by blue lines.

Disclosure: The authors have nothing to disclose.

Figure 4. Principal component analysis of the 24-h urinary steroid metabolomes



Each dot represents one of the 63 samples projected on the principal plane formed by the first and second principal axes. The dots are colored semitransparent according to the subject's classification group (cluster #1-5). The color-filled area around the individual points reflects the 95% confidence interval of each cluster.

Conclusions

Software-based analysis of urinary steroid metabolomes helps to monitor treatment of children with CAH. This method allows classification in under-, over-, and adequate-treated children as well as in patients with treatment failure.

Figure 1. Schematic overview of steroidogenesis and steroid metabolism in CAH.

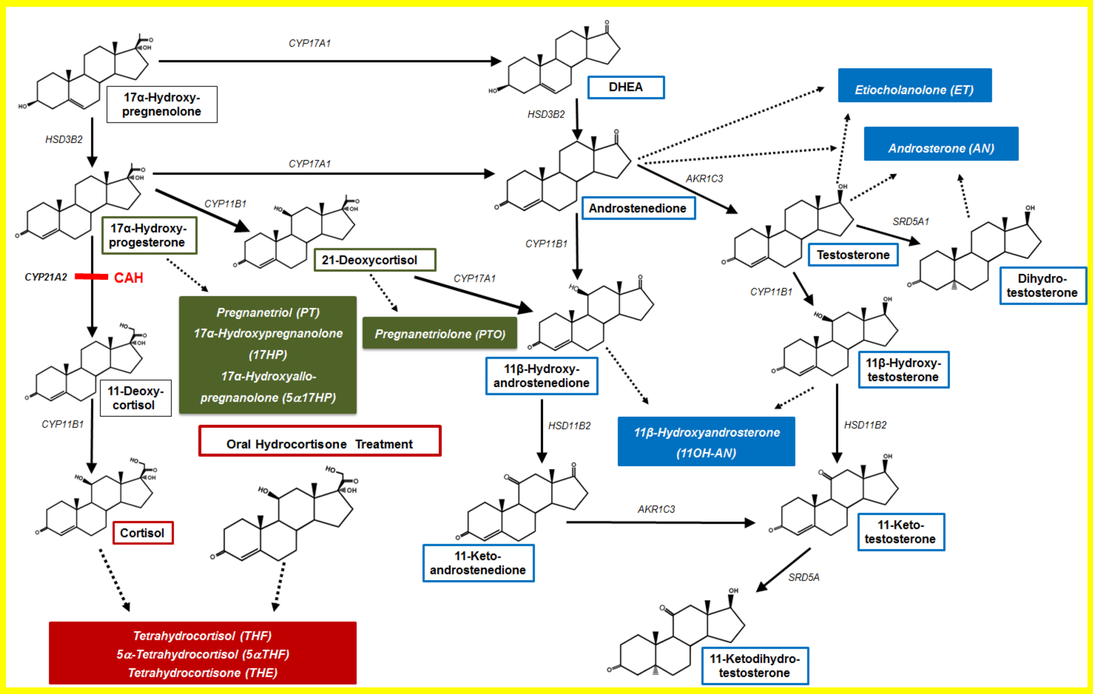
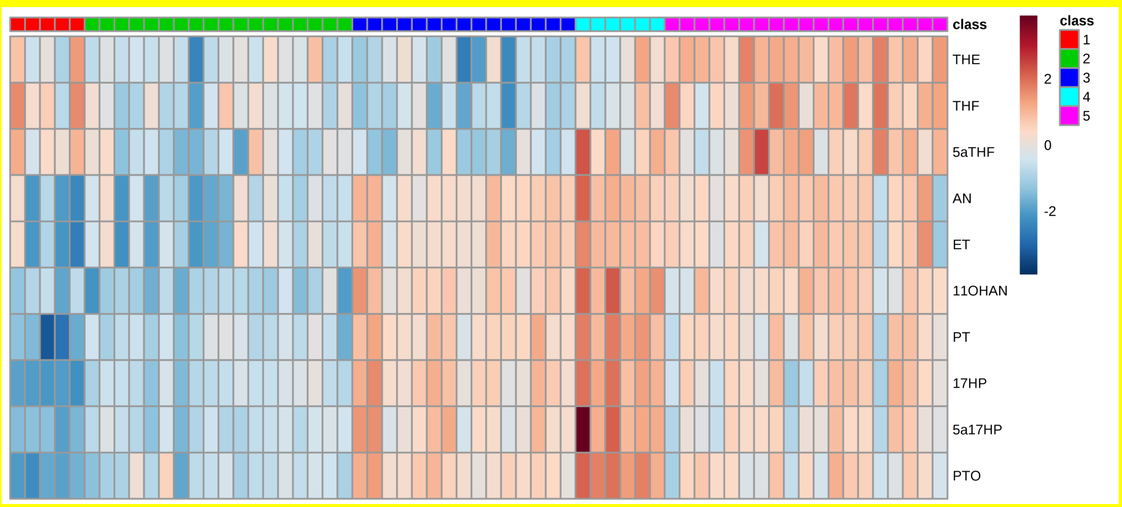


Figure 2. Heatmap-transformation and clustering of 24-h urinary steroid metabolomics data of 63 treated children with CAH.



Each column represents one individual patient with CAH treated with hydrocortisone and fludrocortisone (N = 63). Each row represents the z-transformed metabolite concentration of one of the ten analyzed steroids. The colors in the heatmap are red to blue indicating high to low concentration of the metabolites. Colors at the top (first row) indicate the five clusters.

Table 1. Descriptive data of 63 treated children with CAH according to generated clusters.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	p-value
N (%)	5 (8%))	18 (29%)	15 (24%)	6 (10%)	19 (30%)	
Sex (F/M)	3/2	4/14	8/7	2/4	8/11	
Age (years)	8.3 ± 1.2	6.6 ± 1.6	6.7 ± 1.5	6.3 ± 1.5	7.3 ± 1.3	0.09
BMI (z-score)	0.25 ± 0.14	0.64 ± 1.2	0.59 ± 1.33	0.37 ± 0.40	1.21 ± 0.95	0.21
Height (z-score)	0.23 ± 0.79	0.49 ± 1.52	0.11 ± 1.23	0.15 ± 1.26	0.46 ± 0.84	0.87
Height velocity (z-score)	0.87 ± 2.65	-0.13 ± 2.05	1.96 ± 1.83	0.47 ± 2.15	0.92 ± 2.21	0.10
Hydrocortisone (mg/m²/d)	14.5 ± 3.0	12.7 ± 2.2	10.5 ± 1.9	14.8 ± 3.9	14.4 ± 3.5	0.001

Reference:

1. Kamrath C, Wettstaedt L, Boettcher C, Hartmann MF, Wudy SA. The urinary steroidome of treated children with CAH. *J Steroid Biochem Mol Biol* 2017; 165: 396–406.