Establishment of clinical and lab algorithms for the identification carriers of **mutations in CYP21A2**

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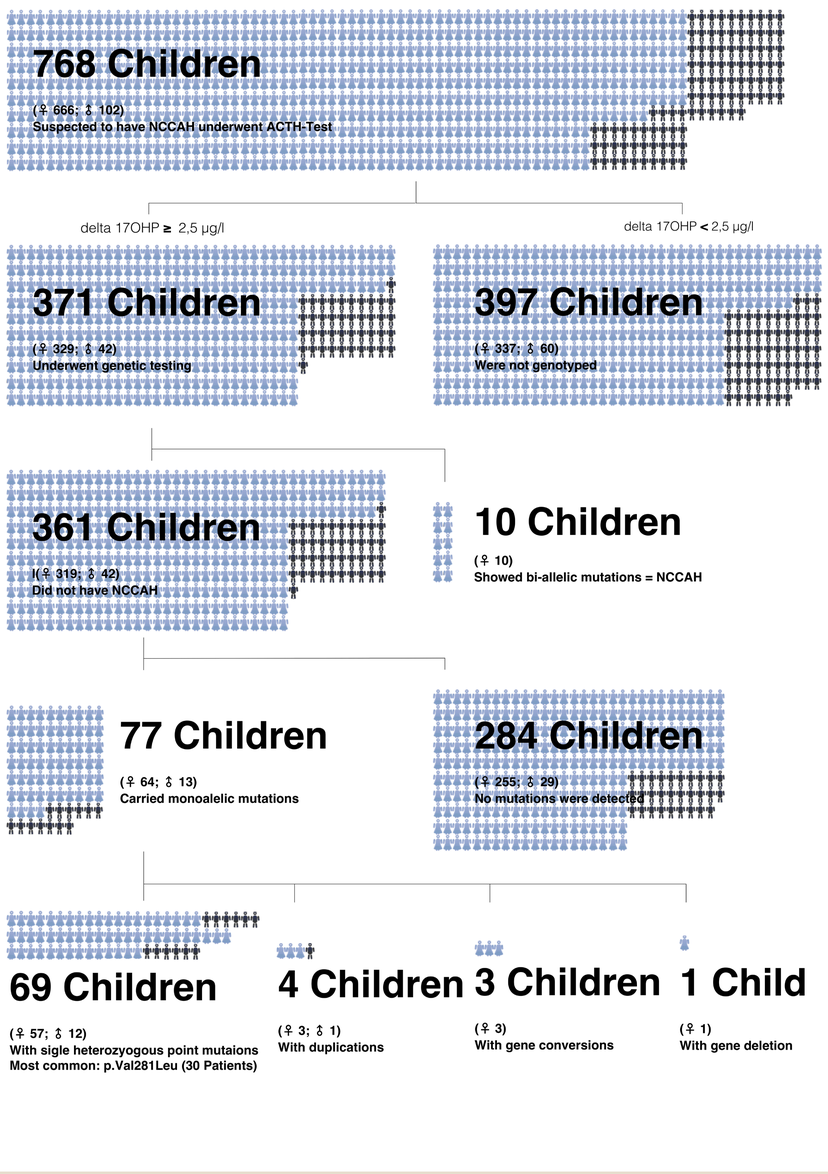


background

Bi-allelic mutations of CYP21A2 encoding 21-hydroxylase are the most frequent cause of congenital adrenal hyperplasia (CAH). Non-classical CAH (NCCAH) or even just hyperandrogenism may be caused by mild or single heterozygous mutations of CYP21A2. These mutations are associated either with elevated basal or ACTH-stimulated levels of 17-hydroxyprogesterone (17OHP) in blood.

objective

The objective of this study was to identify the most suitable of six different test algorithms and the determination of appropriate cut-off levels for that test to recognize patients with NCCAH and carriers of clinically relevant mutations in CYP21A2. Test algorithms were composed around 17OHP measurements.

patients

Between July 2006 and July 2015 ACTH-tests were conducted in 768 children and adolescents (Age ≤ 20 y) suspected to have NCCAH. Inclusion criteria were premature pubarche with accelerated bone age, hyperandrogenemia, hirsutism, or menstrual irregularities.

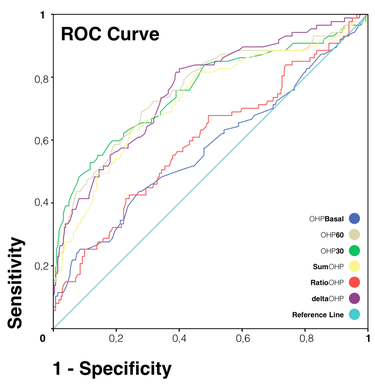
methods

The compared test algorithms were:

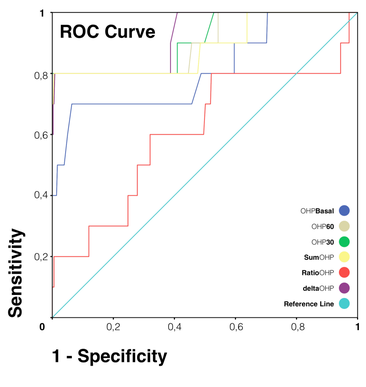
- 17OHPBasal: **Basal** 17OHP levels
- 17OHP30: Stimulated 17OHP levels **30 min** after ACTH was given
- 17OHP60: Stimulated 17OHP levels **60 min** after ACTH was given
- delta17OHP: **Difference** between basal and stimulated 17OHP levels
- sum 17OHP: **Sum** of basal and stimulated 17OHP levels
- ratio 17OHP: **Quotient** of stimulated 17OHP divided by basal 17OHP

Receiver operating characteristics (ROC) were plotted and the most suitable test was identified by the greatest area under the curve (AUC). For 371 patients with delta17OHP≥ 2.5 µg/l genomic blood DNA of the subjects CYP21A2 gene was analyzed by DNA-sequencing and MLPA. Cut-off levels were determined from ROC by pythagorean theorem.

mutation on at least one parental allele



mutation on both parental allele



Diagonal segments are produced by ties.

results

Among the six tested algorithms, delta17OHP revealed the highest AUC with respect to identifying both patients carrying single (0.762) or bi-allelic (0.920) mutations. Basal 17OHP values by themselves proved weak in identifying single heterozygous and bi-allelic carriers.

	At least one parental allele		Both parental alleles	
Test Variable	**Area under the curve (AUC)**	**Asymptotic Significance**	**Area under the curve (AUC)**	**Asymptotic Significance**
17OHPBasal	0,573	0,039	0,811	0,001
17OHP30	0,756	0,000	0,908	0,000
17OHP60	0,758	0,000	0,900	0,000
Delta 17OHP	**0,762**	0,000	**0,920**	0,000
Sum 17OHP	0,728	0,000	0,888	0,000
Ratio 17OHP	0,599	0,005	0,609	0,238

conclusion

Not only bi-allelic but also single heterozygous mutations of CYP21A2 may be associated with clinical signs such as premature pubarche and hyperandrogenism. Our results suggest calculating the difference between stimulated 17OHP values 60 minutes after ACTH was given and basal 17OHP before ACTH was given. Genotyping of CYP21A2 is proposed in patients with values exceeding 3.4 µg/l diff.17OHP. Bi-allelic mutations, i.e. CAH or NCCAH are associated with diff.17OHP >10 µg/l.

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P 1 / A d r e a n a l
nothing to disclose

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