All-in-one solution for single gene disorders with complex rearrangement

Congenital Adrenal Hyperplasia

Background

Congenital adrenal hyperplasia (CAH) refers to a group of autosomal recessive disorders characterized by enzyme deficiencies in the adrenal steroidogenesis pathway, resulting in impaired cortisol biosynthesis. [1] Over 90% of CAH cases are due to 21-hydroxylase deficiency (21-OHD), which can be clinically classified into three subtypes: classic salt-wasting (SW), classic simple-virilizing (SV), and non-classic CAH (NCCAH). [2]

Most classic CAH is often diagnosed early under biomedical screening in neonatal stage, but has a false-positive rate of 0.4% to 9.3% [3] and somehow missing some SV and NCCAH patient.

~20%

simple-virilizing CAH are unidentified under newborn screening [4]

~90%

of non-classic CAH women never receive a diagnosis [5]

Particularly individuals with NCCAH are frequently asymptomatic or present with relatively mild, late-onset signs such as hyperandrogenism and infertility, which often missed diagnosed or misdiagnoses as other conditions.

CAH type	Frequency	Genes
21-hydroxylase deficiency (21-OHD) Classic CAH: 75% SW and 25% SV Non-classic CAH (NCCAH)	90-95%	*CYP21A2*
11β-Hydroxylase deficiency (11β-OHD)	5-8%	*CYP11B1*
17α-Hydroxylase deficiency (17α-OHD)	1%	*CYP17A1*
3β-Hydroxysteroid dehydrogenase type 2 deficiency (3β-HSD)	1%	*HSD3B2*
Lipoid CAH	Rare	*STAR*
POR deficiency	Rare	*POR*
SCC deficiency	Rare	*CYP11A1*

Information derived from reference [2]

Since over 90% of 21-hydroxylase deficiency (21-OHD) genotypes correlate with their respective phenotypes [6], accurate genotyping can play a key role in early intervention, management, and carrier screening. However, routine assays like MLPA and next-generation sequencing (NGS) struggle with precise genotyping, particularly for chimeric genes and micro-conservation variants. Moreover, some assays focus solely on 21-OHD and are not available for other minority form of CAH.

Limitation of current molecular assays includes:

- False results due to pseudogene interference and mutations in the binding region
- Inaccurate typing of chimeric genes
- Fails to detect duplication-masked deletion
- Cannot identify *cis/trans* configurations

Comprehensive Analysis of Congenital Adrenal Hyperplasia (CACAH)

identifies the most variants using single molecule real-time (SMRT) sequencing

CACAH utilizes advanced long-read SMRT sequencing for precise phenotyping, excelling in variant identification within homologous regions and *CYP21A2-TNXB* chimeric patterns with precise breakpoint detection. It serves as an all-in-one solution for the most cases, including NCCAH screening, carrier screening, and parental testing.

Patent (China): ZL 202111118646.6

Technology: Single molecule real-time (SMRT) sequencing
Platform: PacBio Vega, Sequel II, and Sequel IIe system
Sample type: Dried blood spot (DBS), gDNA, blood, buccal swab, and amniotic fluid
Operation hour: 69 hours
(starting from sample preparation to report generation)

Gene	Basic panel	Comprehensive panel	Prenatal comprehensive panel
CYP21A2-TNXB	12 deletions	Detects P/LP/some VUS SNVs/InDels, selected large intragenic deletions in 7 genes, and specific CNVs in the *CYP21A2*	Detects P/LP SNVs/InDels, selected large intragenic deletions in 7 genes, and specific CNVs in the *CYP21A2*
	273 SNVs/InDels		
	12 CNVs		
CYP11B1	1 deletion		
	79 SNVs/InDels		
CYP17A1	55 SNVs/InDels		
HSD3B2	36 SNVs/InDels		
STAR	46 SNVs/InDels		
POR	--		
CYP11A1	--		

Comprehensive Analysis of Congenital Adrenal Hyperplasia

Precise breakpoints identification - Clearly distinguish different chimeras

Routine methods struggle to distinguish *CYP21A1P/CYP21A2* and *TNXA/TNXB* chimeric patterns. Since different types of chimeras and structural variants influence phenotypic severity, CACAH provides a key advantage with precise chimeric typing, including CAH-X, through breakpoint detection via SMRT sequencing. This enhances genetic insights, improving patient management and clinical outcomes.

IGV display (with SMRT sequencing)

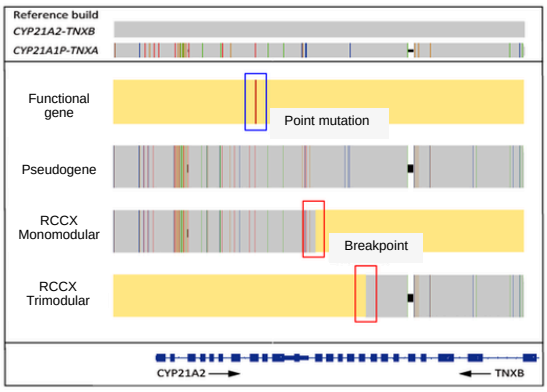
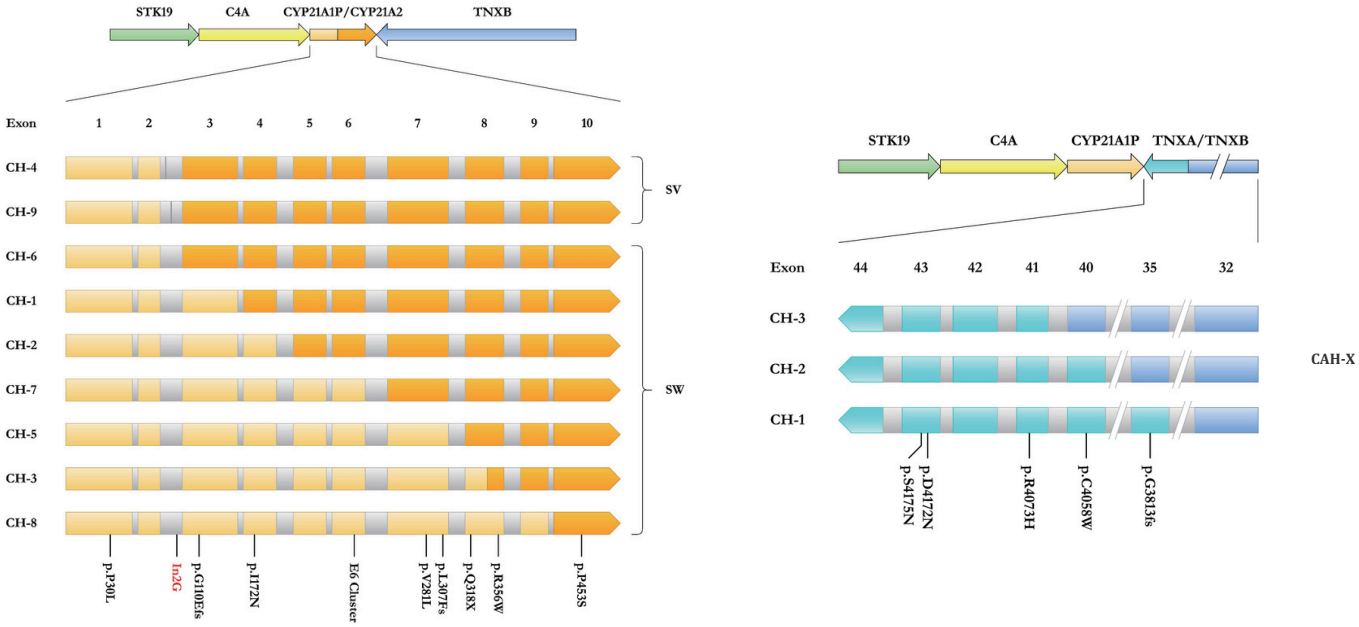


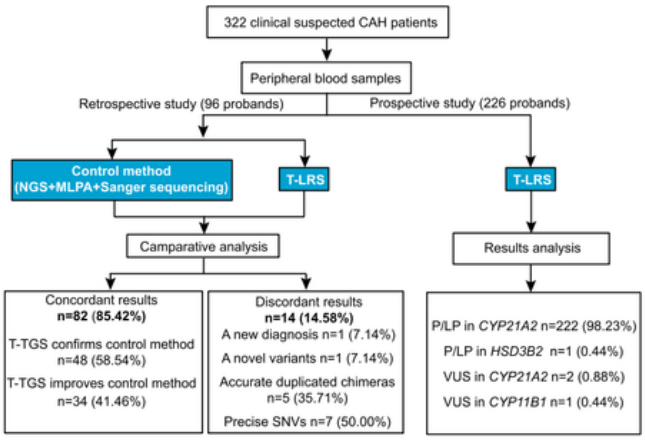
Diagram of *CYP21A1P/CYP21A2* and *TNXA/TNXB* chimeric patterns resulting from rearrangement



CACAH proband testing outperforms trio-based assays (NGS + MLPA + Sanger), demonstrating superior accuracy, cost-effectiveness, and clinical potential

In a cohort of 562 participants [7] —including a retrospective group (96 probands and 191 family members from 95 families) and a prospective group (226 probands):

- In the retrospective analysis, CACAH corrected discordant results in 14.58% of probands, including detecting missed variants and improve chimera classifications
- In the prospective analysis, CACAH successfully identified all the relevant CAH variants in all probands and enabled the establishment of an allele frequency distribution chart



Study Design. T-LRS refers to CACAH proband test.

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