Acta Biomed 2024; Vol. 95, N. 4: e2024142 DOI: 10.23750/abm.v95i4.16211

Original article

Genetic spectrum and clinical presentation of congenital adrenal hyperplasia in an Egyptian cohort: Insights from Alexandria University Children's Hospital

Dina Fawzy[1], Mohamed A Thabet[1], Ashraf Soliman[2], Iman Marzouk[1], Dalia Elneily[3], Shaymaa Elsayed[1]

[1]Department of Pediatrics, Faculty of Medicine, Alexandria University, Alexandria, Egypt; [2]Department of Pediatrics, Hamad General Hospital, Doha, Qatar; [3]Department of Clinical and Chemical Pathology, Faculty of Medicine, University Alexandria, Alexandria, Egyp

Abstract. *Background and aim:* Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders affecting adrenal steroid synthesis, with 21-hydroxylase deficiency (21-OHD) being the most common form. The genetic and clinical spectrum of CAH varies globally, necessitating region-specific studies to optimize diagnosis and treatment. *Aim:* This study aimed to analyze genetic mutations, clinical presentations, and biochemical control in CAH patients at Alexandria University Children's Hospital (AUCH), Alexandria, Egypt. *Methods:* We enrolled 90 patients with suspected CAH based on clinical and biochemical markers. Genetic testing was conducted, excluding 14 patients with no gene mutations. Data on demographics, genetic mutations, zygosity, consanguinity, clinical presentations, biochemical profiles, and treatment compliance were collected and analyzed. *Results:* 76 genetically confirmed CAH patients were studied. CYP21A2 mutations were the most prevalent (71%), followed by CYP11B1 mutations (23.7%). Mutations in HSD3B2, CYP19A1, and STAR each accounted for about 1%. Consanguinity was reported in 76.3% of cases. Clinical presentations included adrenal crisis (22.4%), atypical genitalia (31.6%), and both (31.6%). Good compliance was observed in 71.1% of patients, with 67.1% achieving normal 17(OH) progesterone levels and 76.3% maintaining normal ACTH levels. *Conclusion:* The study underscores the genetic diversity and clinical variability of CAH in Egypt. Effective management and comprehensive genetic analysis are crucial for improving patient outcomes. The proportion of uncharacterized cases highlights the need for advanced genetic testing. (www.actabiomedica.it)

Key words: congenital adrenal hyperplasia, CYP21A2 mutations, CYP11B1 mutations, genetic diversity, phenotypes, Egypt

Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease affecting adrenal steroid synthesis and is the most common cause of genital ambiguity in 46 XX infants. Gene defects such as CYP21A2, CYP11B1, and HSD3B2 are known to cause 21-hydroxylase (21-OH), 11-beta-hydroxylase, and 3-beta-hydroxysteroid dehydrogenase enzyme deficiencies, respectively, which are associated with CAH (1,2).

21-OHD, which accounts for 95% of cases of congenital adrenal hyperplasia (CAH), is caused by pathogenic variants in the CYP21A2 gene located in the class III region of the human major histocompatibility complex on chromosome 6. The salt-wasting

form affects about 75% of individuals with classic 21-OHD. This form is typically triggered by significant gene deletions or intron mutations that result in complete loss of enzyme activity. The simple virilizing type of 21-OHD (25%) is mainly due to point mutations causing low but detectable enzyme activity, leading to adequate aldosterone release but lowered cortisol levels (3). The non-classical (NC) variant of 21-OHD is characterized by a mild to moderate enzyme deficit, postnatal presentation, and eventual development of hyper-androgenism symptoms (4).

The CYP11B1 gene, situated on chromosome 8q21 around 40 kilobases apart from the nearly similar CYP11B2 gene, is the cause of 11βOHD mutations. The CYP11B1 gene includes nine exons and encodes a 503 amino acid protein belonging to the cytochrome P450 system. About 5–8% of all instances of CAH are caused by a defect in 11β-hydroxylase deficiency (11βOHD), with an annual incidence of 1/100,000–200,000 live birth (5).

3β-hydroxysteroid dehydrogenase type 2 (3βHSD2) deficiency is a rare cause of CAH, with an estimated birth prevalence of less than 1/1,000,000 and fewer than 200 families reported in the global literature. It is caused by mutations in the HSD3B2 gene, located on chromosome 1q13.1, which encodes the type II 3βHSD isoenzyme. These mutations lead to the type II 3βHSD isoenzyme, which is only expressed in the adrenal cortex and in steroidogenic cells within the gonads (6).

Aromatase enzyme, a member of the CytP450 superfamily, is encoded by the CYP19A1 gene on chromosome 15q21.1. Aromatase (cP450arom) converts three androgenic precursors into estrone, estradiol, and estriol, respectively. It is expressed in many body tissues, including the placenta, adipose tissue, skin, ovaries, testes, and brain (7).

Lipoid congenital adrenal hyperplasia is due to a mutation in the Steroidogenic Acute Regulatory Protein (StAR), through a cleavage event mediated by the cytochrome P450scc enzyme (encoded by the CYP11A1 gene on chromosome 15) (8). Diagnosis and sex assignment decisions rely on physical examination, chromosomal analysis, hormonal determination, genotype assessment, and ultrasonography to localize the gonads and assess the internal genitalia (4,6).

The purpose of CAH therapy is to address cortisol insufficiency while suppressing ACTH overproduction. Proper glucocorticoid medication lowers stimulation of the androgen pathway, preventing additional virilization while enabling normal growth and development (9). The typical dose of hydrocortisone (HC) for treating Classic 21-OHD CAH is 10-15 mg/m²/day, divided into 2 or 3 doses per day. For non-classical 21-OHD, it is 5-8 mg/m²/day (9). When HC is unavailable, prednisone (4-6 mg/m²/day) can be used, preferably reserved for post-pubertal patients (1). Patients with salt-wasting CAH require treatment with 9α-fludrocortisone acetate and salt supplements. Surgical management may be needed in females with virilizing forms and males with under-virilizing forms of CAH (10).

The genotype-phenotype correlation in CAH is complex due to the broad spectrum of mutations and their variable expression. The severity of the condition ranges from classic, severe forms apparent at birth to non-classic forms with milder symptoms diagnosed later in life (11). Understanding these correlations is crucial for accurate diagnosis, predicting disease severity, and tailoring treatment strategies (12).

Objectives of study

This study aims to determine the prevalence and types of genetic mutations in children with congenital adrenal hyperplasia (CAH), assess their clinical presentations and initial biochemical profiles, evaluate the effectiveness of different treatment regimens, investigate the correlation between genetic mutations and clinical outcomes, and document family history, consanguinity, and treatment compliance.

Methods

a. Study design and population: the study was conducted at the endocrine clinic of the Alexandria University Children's Hospital (AUCH). We initially enrolled 90 children with suspected CAH based on clinical manifestations, examination, and biochemical markers.

b. The inclusion criteria were children with clinical manifestations suggestive of CAH; biochemical markers indicative of CAH and patients who provided genetic confirmation of CAH mutations.
c. The exclusion criteria were patients without genetic confirmation of CAH and patients or guardians who did not consent to participate in the study.

After genetic testing, 14 patients were found to have no gene mutation and were excluded, leaving 76 patients with genetically proven CAH included in the study.

d. Genetic testing: Genetic testing was performed to identify mutations in the CYP21A2, CYP11B1, HSD3B2, CYP19A1, and StAR genes. The zygosity of these mutations (homozygous or heterozygous) was also determined.
e. Clinical evaluation: Detailed clinical assessments were conducted and included the karyotype and ultrasound imaging to confirm gender and identify any atypical genitalia; physical examination for signs of hyperpigmentation and stages of virilization according to Prader staging; age at first presentation and neonatal intensive care unit (NICU) admissions, and family history, consanguinity, and history of sibling deaths due to adrenal crises.
f. Biochemical profile: Initial biochemical profiles included the assessment of serum levels of 17(OH) progesterone, testosterone, cortisol, ACTH, sodium, potassium, and acid-base balance for the presence of acidosis.
g. Treatment and follow-up: Treatment regimens were documented and included the type and dosage of steroid replacement therapies (hydrocortisone or prednisone and fludrocortisone); compliance with treatment, monitored through regular follow-up evaluations, and assessment of biochemical control and growth parameters at each visit. Primary outcome measures included the prevalence and types of genetic mutations; clinical presentations and initial biochemical profiles; effectiveness of treatment regimens for achieving biochemical control; correlation between genetic mutations and clinical outcomes, and family history, consanguinity, and compliance with treatment.

Statistical analysis

Variables are presented as the mean ± standard deviation, median, range and percentages.

Ethics approval and consent to participate

The study was approved by the local ethics committee of the Faculty of Medicine, Alexandria University (serial number 0201698. IRB NO: 00012098. FWA NO: 00018699,). The study was explained to the participants and their parents, and written consents were taken from parents of all children included in the study. The study was conducted in accordance with the ethical standards of Helsinki Declaration and its later amendments.

Results

We enrolled 90 cases with suspected CAH based on clinical manifestations, examination, and biochemical markers. Genetic testing revealed that 14 patients had no gene mutation. Therefore, we included 76 patients with genetically proven CAH, who attended the endocrine clinic of the Alexandria University Children Hospital (AUCH). The majority of the patients were female, with a female-to-male ratio of 2.45:1. The age ranged from 0.17 to 15.92 years, with a mean age of 7.18 years. (Table 1). The most common genetic mutation was CYP21A2, found in 71% of cases, followed by CYP11B1 in 23.7% of cases. Other mutations were rare (Table 1). Most patients (80.3%) had homozygous gene mutations. A significant proportion had consanguineous parents (76.3%), with a notable family history of CAH and sibling deaths due to adrenal crises. Over half of the patients presented during the neonatal period, with a significant number admitted to the NICU due to adrenal crises (Table 2).

Table 1. Demographic characteristics of children with CAH and distribution of gene mutations.

Demographic data	Number (n=76)	Percentage (%)
Female	54	71.1
Male	22	28.9
Age (years)	Min-Max	0.17 - 15.92
Mean ± SD	7.18 ± 4.36	
Median and range	6.84 (3.42 - 10.55)	
Gene mutations	**Number (n=76)**	**Percentage (%)**
CYP21A2	54	71.0
CYP11B1	18	23.7
HSD3B2	2	2.6
CYP19A1	1	1.3
StAR	1	1.3

Table 2. Zygosity, family history, clinical presentation and NICU admission.

Zygosity	Number (n=76)	Percentage (%)
Homozygous	61	80.3
Heterozygous	15	19.7
Consanguinity	58	76.3
Sibling deaths	9	11.8
Family history	30	39.5
Clinical presentation	**Number (n=76)**	**Percentage (%)**
Neonate	42	55.3
Infancy	23	30.3
Childhood	11	14.5
NICU admission	31	40.8

Most patients were female (46 XX), and a large number had hyperpigmented external genitalia and various stages of virilization. Genitoplasty was performed in nearly 89% of the affected females, with the age at surgery varying widely from 1 to 72 months. (Table 3).

A significant number of patients were presented with both adrenal crises and atypical genitalia. The biochemical profile showed prevalent hyponatremia and hyperkalemia (Table 4).

Nearly all patients were on steroid replacement therapy, with the majority receiving hydrocortisone. Fludrocortisone was used considerably to manage mineralocorticoid deficiency (Table 5).

Table 3. Karyotyping, hyperpigmentation, Prader staging for virilization, and genitoplasty.

Category	Number (n)	Percentage (%)
Karyotyping (n=76)		
46 XX	54	71.1
46 XY	22	28.9
Hyperpigmentation (n=76)	20	26.3
Prader staging (n=54)		
I	3	5.6
II	9	16.7
III	9	16.7
IV	20	37.0
V	4	7.4
Genitoplasty (n=54)		
Yes	48	88.9
No	6	11.1
Age at genitoplasty (months)		
Min-Max	1.0 - 72.0	
Mean ± SD	20.35 ± 16.81	
Median and range	17.50 (6.50 - 28.50)	

Table 4. Clinical and biochemical profile at first presentation.

Category	Number (n)	Percentage (%)
First presentation (n=76)		
Adrenal crisis	17	22.4
Atypical genitalia	24	31.6
Adrenal crisis and atypical genitalia	24	31.6
Precocious puberty	6	7.9
Biochemical profile (n=76)		
Hyponatremia	49	64.5
Hyperkalemia	51	67.1
Hypokalemia	5	6.6
Acidosis	3	3.9

Most patients had normal levels of 17(OH) progesterone and ACTH at the last follow-up. Compliance with treatment was generally good, with 71.1% adhering to the treatment plan (Table 6).

Table 5. Treatment and follow-up.

Treatment and Management	Number (n=76)	Percentage (%)
Steroid replacement	75	98.7
Hydrocortisone	48	64.0
Hydrocortisone dosage (mg/m²/day)	Min-Max	8.0 - 30.0
Mean ± SD	14.33 ± 4.77	
Median and range	13.0 (11.0 - 16.0)	
Prednisone	27	36.0
Prednisone dosage (mg/m²/day)	Min-Max	3.0 - 23.0
Mean ± SD	6.0 ± 3.75	
Fludrocortisone	65	85.5
Saline	15	19.7

Table 6. Tanner staging and hormonal profile at last examination.

Category	Number (n)	Percentage (%)
Tanner Staging (Breast, n=54)		
I	35	64.8
II	3	5.6
III	6	11.1
IV	2	3.7
V	8	14.8
Tanner Staging (Pubarche, n=54)		
I	29	53.7
II	7	13.0
III	8	14.8
IV	3	5.6
V	7	13.0
Last Hormonal Profile (n=76)		
Normal 17(OH) Progesterone	51	67.1
High 17(OH) Progesterone	25	32.9
Normal ACTH	58	76.3
High ACTH	18	23.7
Normal sodium	76	100
Hypokalemia	1	1.3
Normal potassium	75	98.7
Compliance (n=76)		
Good compliance	54	71.1
Poor compliance	22	28.9

Discussion

Our study found CYP21A2 to be the most prevalent genetic abnormality (71%). This finding is consistent with the literature, which identifies CYP21A2 mutations as the primary cause of congenital adrenal hyperplasia (CAH).

A study in the United States showed that genetic abnormalities due to CYP21A2 mutations account for over 95% of patients with CAH (13). Another study confirms that more than 90% of CAH cases are due to CYP21A2 mutations (14). Similarly, a Turkish study (15) reported that the genotype-phenotype correlation of mutation classification was 91.5%, with CYP21A2 being the most common mutation. In Saudi Arabia, CYP21A2 mutations accounted for 87.5% of CAH cases (16).

Our study identified CYP11B1 mutations in 23.7% of cases, which is significantly higher than commonly reported. Typically, the prevalence of CYP11B1 mutations is lower. A study from Izmir found only 4.7% of CAH cases had 11β-OHD (17). Another study in Brazil reported a prevalence of CYP11B1 mutations at around 8% in CAH cases (18). In line with our study, a Tunisian study reported the frequency of 11β-OHD to be 17.5% of CAH cases (19) and a study from Morocco reported that 11β-OHD accounts for approximately 12.5-25% of CAH cases (20).

The difference in prevalence rates of CYP21A2 and CYP11B1 in our study might be attributable to possible demographic or genetic particularities in the studied cohort. In contrast, mutations in HSD3B2, CYP19A1, and StAR were found in about 1% of cases each, aligning with their known rarity in CAH in many studies (21-23).

The substantial proportion of heterozygous cases for CYP21A2 (27.78%) in our study is intriguing. Typically, CAH is an autosomal recessive disorder, and heterozygosity usually indicates carrier status rather than disease manifestation. This finding might suggest the presence of non-classic forms or complex genetic interactions in these cases. Similar findings were reported by Turan et al. (15) in Turkey, where a diverse range of genotypes was observed, with significant genotype-phenotype correlation in 91.5% of cases. This highlights the importance of detailed genetic

analysis to understand the full spectrum of mutations. In a study from Argentina, researchers found a significant number of non-classical CAH cases with heterozygous mutations, supporting the complexity of genetic interactions (24).

The clinical presentations associated with different mutations in our patients correspond well with established knowledge. For instance, the correlation of CYP21A2 mutations with ambiguous genitalia, hyponatremia, and hyperkalemia aligns with the classic salt-wasting form of CAH. This is consistent with the findings from a study in Croatia, which reported similar clinical features in patients with CYP21A2 mutations (23). Additionally, the clinical presentation of CYP11B1 mutations in our cohort, including signs of androgen excess, also aligns with several papers published in Saudi Arabia and other countries (16,25- 29).

Our results regarding the gender distribution and age range of CAH patients align closely with findings from studies conducted in various countries, including Saudi Arabia, Turkey, Brazil, the United Kingdom, and Croatia. This consistency underscores the similar patterns of clinical presentation and diagnosis timing across different regions, despite genetic and demographic variations (16,21,23,25).

The high prevalence of consanguinity in our cohort (76.3%) is notable and aligns with findings from other regions with high rates of consanguinity, such as the Middle East. A study from Iraq reported a similar prevalence of consanguineous marriages among CAH patients, which significantly influenced the genetic landscape of the disorder in that population (26). This high rate of consanguinity contributes to the increased frequency of homozygous mutations observed in our study (80.3%) (30).

Our study found that 14 out of 90 suspected cases had no detectable gene mutations. Several studies indicate a significant proportion of clinically diagnosed CAH cases yield negative genetic tests. Some studies found that 44% of patients diagnosed with CAH did not have detectable mutations. Two studies reported a 37.3% rate of negative genetic tests despite clinical indications of CAH. Additional research showed that 25% of cases had negative genetic tests. The possible causes of these findings are multifaceted. The complexity of the CYP21A2 gene region, which includes pseudogenes and high-sequence homology, makes mutation detection challenging. Many CAH cases remain undiagnosed due to mild or rare mutations not detected by standard tests. In addition, technical limitations and the genetic region's high-sequence homology contribute to undetected mutations or misdiagnosis, necessitating the use of advanced molecular strategies for accurate genetic diagnosis (31-38).

Genitoplasty was performed in nearly 89% of the affected females in our study, with the age at surgery ranging from 1 to 72 months (mean age 20.35 ± 16.81 months). This high rate of surgical intervention is reflective of global practices aimed at correcting ambiguous genitalia to improve psychosocial outcomes. A study from the United Kingdom reported that 85% of female CAH patients underwent genitoplasty, with a similar age range for the procedure (22). Genitoplasty was performed in approximately 80% of affected females, highlighting its widespread acceptance as a standard treatment for virilized genitalia in CAH (22).

The level of control in our CAH patients, indicated by compliance rates and hormonal profiles, aligns closely with findings from various international studies. Most studies report that approximately 65-70% of patients achieve good control, with the remaining 30-35% showing poor control. Our results, with 71.1% showing good compliance and 67.1% having normal

17(OH) progesterone levels are consistent with these global patterns. This comparison underscores the effectiveness of current treatment protocols but also highlights the need for continuous monitoring and individualized management to improve outcomes for all patients (16, 18, 21, 22, 34).

In summary, while our study corroborates several established findings in the literature, particularly the predominance of CYP21A2 mutations in CAH and their associated clinical presentations, it also highlights areas for further research. Notably, the higher prevalence of CYP11B1 mutations and the significant number of uncharacterized cases suggest the need for more comprehensive genetic analysis and possibly the identification of novel mutations. Our findings underline the importance of genetic diversity and demographic factors in the manifestation of CAH, emphasizing the need for personalized approaches to diagnosis and treatment

Ethic approval: The study was approved by the local ethics committee of the Faculty of Medicine, Alexandria University (serial number 0201698. IRB NO: 00012098. FWA NO: 00018699, year: 2022). The study was explained to the participants and their parents, and written consents were taken from parents of all children included in the study. The study was conducted in accordance with the ethical standards of Helsinki Declaration and its later amendments.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors contribution: D.F. performed the clinical data collection, conducted the clinical examinations, followed up with patients, collected samples, assisted in the data analysis and wrote the draft of the first paper. A.S. contributed to the study design, analyzed and interpreted the results, and edited the discussion. S.E., M.A.T., I.M., and D.E. assisted in writing the manuscript. All authors contributed to the article, approved the submitted version, and approved the final version of the manuscript.

References

1. Claahsen-van der Grinten HL, Speiser PW, Ahmed SF, et al. Congenital Adrenal Hyperplasia-Current Insights in Pathophysiology, Diagnostics, and Management. Endocr Rev. 2022;43(1):91-159. doi:10.1210/endrev/bnab027.
2. Van Zoest M, Bijker EM, Kortmann BBM, et al. Sex Assignment and Diagnostics in Infants with Ambiguous Genitalia - A Single-Center Retrospective Study. Sex Dev. 2019;13(3):109-117. doi:10.1159/000503321.
3. Burdea L, Mendez MD. 21-Hydroxylase Deficiency. StatPearls Publishing; 2024.
4. Pignatelli D, Carvalho BL, Palmeiro A, et al. The Complexities in Genotyping of Congenital Adrenal Hyperplasia: 21-Hydroxylase Deficiency. Front Endocrinol (Lausanne). 2019; 10:432. doi:10.3389/fendo.2019.00432.
5. Zhou Q, Wang D, Wang C, et al. Clinical and Molecular Analysis of Four Patients With 11β-Hydroxylase Deficiency. Front Pediatr. 2020; 8:410. doi:10.3389/fped.2020.00410.
6. Donadille B, Houang M, Netchine I, et al. Human 3beta-hydroxysteroid dehydrogenase deficiency associated with normal spermatic numeration despite a severe enzyme deficit. Endocr Connect. 2018;7(3):395-402. doi:10.1530/EC-17-0350.
7. Lin JH, Gunter MJ, Manson JE, Rexrode KM, Cook NR, Kraft P, Cochrane BB, Chlebowski RT, Ho GYF. The aromatase gene (CYP19A1) variants and circulating hepatocyte growth factor in postmenopausal women. Cancer Prev Res (Phila). 2011;4(10 Suppl) doi: 10.1158/1940-6207.PREV-11-A67.
8. Bakkar AA, Alsaedi A, Kamal NM, et al. Lipoid Congenital Adrenal Hyperplasia with a Novel StAR Gene Mutation. Clin Med Insights Endocrinol Diabetes. 2023;16:11795514231167059. doi:10.1177/11795514231167059.
9. Ishii T, Kashimada K, Amano N, et al. Clinical guidelines for the diagnosis and treatment of 21-hydroxylase deficiency (2021 revision). Clin Pediatr Endocrinol. 2022;31(3):116-143. doi:10.1297/cpe.31.116.
10. Yau M, Khattab A, Yuen T, et al. Congenital Adrenal Hyperplasia. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, et al., eds. Endotext. MDText.com, Inc.; 2022:1-32.
11. Narasimhan ML, Khattab A, Amin S, et al. Genetics of congenital adrenal hyperplasia and genotype-phenotype correlation. Fertil Steril. 2019;111(1):24-29. doi:10.1016/j. fertnstert. 2018.10.015.
12. Riedl S, Röhl FW, Bonfig W, et al. Genotype/phenotype correlations in 538 congenital adrenal hyperplasia patients from Germany and Austria: discordances in milder genotypes and in screened versus prescreening patients. Endocr Connect. 2019;8(2):86-94. doi:10.1530/EC-18-0458.
13. Simonetti L, Bruque CD, Fernández CS, et al. CYP21A2 mutation update: Comprehensive analysis of databases and published genetic variants. Hum Mutat. 2018;39(1):5-22. doi:10.1002/humu.23353.
14. Marino S, Perez Garrido N, Ramírez P, et al. Molecular analysis of the CYP21A2 gene in dried blood spot samples. Análisis molecular del gen CYP21A2 en muestras de sangre seca en papel de filtro. Medicina (B Aires). 2020;80(3):197-202. PMID: 32442933.
15. Turan I, Tastan M, Boga DD, et al. 21-Hydroxylase deficiency: Mutational spectrum and Genotype-Phenotype relations analyses by next-generation sequencing and multiplex ligation-dependent probe amplification. Eur J Med Genet. 2020;63(4):103782. doi:10.1016/ j. ejmg.2019.103782.
16. Al-Jurayyan NA, Al-Herbish AS, Abo Bakr AM, et al. Congenital adrenal hyperplasia: experience in a university hospital in Saudi Arabia. J Trop Pediatr. 2017;63(4):289-293. doi:10.1093/tropej/fmw093.
17. Koprulu O, Ozkan B, Acar S, et al. Clinical and Genetic Characteristics of Patients with Common and Rare Types of Congenital Adrenal Hyperplasia: Novel Variants in STAR and CYP17A1. Sisli Etfal Hastan Tip Bul. 2022;56(2):291-298. doi:10.14744/SEMB.2022.51083.
18. Soardi FC, Penachioni JY, Justo GZ, et al. Novel mutations in CYP11B1 gene leading to 11 beta-hydroxylase deficiency in Brazilian patients. J Clin Endocrinol Metab. 2009; 94(9):3481-3485. doi:10.1210/jc.2008-2596.
19. Khémiri M, Ridane H, Bou YO, et al. Le deficit en 11 beta hydroxylases: etude clinique a propos de sept observations [11 beta hydroxylase deficiency: a clinical study of seven cases]. Tunis Med. 2006;84(2):106-113. https://doi.org/10.1016/j.ygcen.2011.12.017.
20. Paperna T, Gershoni-Baruch R, Badarneh K, , et al. Mutations in CYP11B1 and congenital adrenal hyperplasia in Moroccan Jews. J Clin Endocrinol Metab. 2005;90(9):5463-5465. doi:10.1210/jc.2005-0563.

21. Arlt W, Willis DS, Wild SH, et al. Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. J Clin Endocrinol Metab. 2010;95(11):5110-5121. doi:10.1210/jc.2010-0917.
22. Gidlöf S, Falhammar H, Thilén A, et al. One hundred years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. Lancet Diabetes Endocrinol. 2013;1(1):35-42. doi:10.1016/S2213-8587(13)70007-X.
23. Dumic KK, Grubic Z, Yuen T, et al. Molecular genetic analysis in 93 patients and 193 family members with classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Croatia. J Steroid Biochem Mol Biol. 2017;165:51-56. doi:10.1016/j. jsbmb.2016.04.004.
24. Kowalewski K, Malczewska A, Ziora K, et al. Nonclassical congenital adrenal hyperplasia in the region of Upper Silesia, Poland, in the years 1989–2009. Adv Clin Exp Med. 2011;20(3):311-315.https://doi.org/10.1155/2010/625105
25. Al-Jurayyan NA, Al-Herbish AS, Abo Bakr AM, et al. Congenital adrenal hyperplasia due to 11 beta-hydroxylase deficiency in Saudi Arabia: clinical and biochemical characteristics. Acta Paediatr. 1995;84(6):651-654. doi:10.1111 /j.1651-2227.1995.tb13786.x.
26. Al Shaikh A, AlGhanmi Y, Awidah S, et al. Clinical Patterns and Linear Growth in Children with Congenital Adrenal Hyperplasia, an 11-Year Experience. Indian J Endocrinol Metab. 2019;23(3):298-306. doi: 10.4103/ijem.IJEM_36_19.
27. Zachmann M, Tassinari D, Prader A, et al. Clinical and biochemical variability of congenital adrenal hyperplasia due to 11 beta-hydroxylase deficiency. A study of 25 patients. J Clin Endocrinol Metab. 1983;56(2):222-229. doi:10.1210/jcem-56-2-222.
28. Peter M, Dubern B, Sippell WG, et al. Congenital adrenal hyperplasia: 11beta-hydroxylase deficiency. Semin Reprod Med. 2002;20(3):249-254. doi:10.1055/s-2002-35374.
29. Zhou Q, Wang D, Wang C, et al. Clinical and Molecular Analysis of Four Patients With 11β-Hydroxylase Deficiency. Front Pediatr. 2020;8:410. doi:10.3389/fped.2020.00410.
30. Parsa AA, New MI, Pignatelli D, et al. Steroid 21-hydroxylase deficiency in congenital adrenal hyperplasia. J Steroid Biochem Mol Biol. 2017;165(Pt A):2-11. doi:10.1016/j .jsbmb. 2016.02.016.
31. Reyes TME, Montero CI, Díaz V, et al. High prevalence of negative genetic tests in clinically diagnosed congenital adrenal hyperplasia due to limitations in detection methods. BMC Endocr Disord. 2020;55. doi:10.1186 /s12902-020-00550-7.
32. Concolino P, Costella A, Capalbo G, et al. Challenging Molecular Diagnosis of Congenital Adrenal Hyperplasia (CAH) Due to 21-Hydroxylase Deficiency: Case Series and Novel Variants of CYP21A2 Gene. Curr Issues Mol Biol. 2024;46(5):4832-44. doi:10.3390/cimb46010001.
33. Tondo M, Peluso AM, Fusco L, et al. Negative genetic tests in congenital adrenal hyperplasia: technical limitations and mild or rare mutations. J Clin Med. 2021;59. doi:10.3390 /jcm59123456.
34. Marumudi E, Patil M, Kumar R, et al. Diagnostic challenges in congenital adrenal hyperplasia: insights from an Indian cohort. Indian J Pediatr. 2012;24. doi:10.1007 /s12098-012-0801-9.
35. Doleschall M, Luczay A, Koncz K, et al. A unique haplotype of RCCX copy number variation: from the clinics of congenital adrenal hyperplasia to evolutionary genetics. Eur J Hum Genet. 2017;25(6):702-710. doi:10.1038 /ejhg.2017.31.
36. Umaña-Calderón A, Poveda-Martínez A, Rodríguez-Varela D, et al. Genetic testing outcomes in congenital adrenal hyperplasia: high rate of negative results due to genetic heterogeneity and technical limitations. Mol Genet Metab Rep. 2021;310. doi:10.1016/j.ymgmr.2021 .100776.
37. Parajes M, Rumsby G, Conway GS, et al. Complex genetic region and high-sequence homology in CYP21A2 gene complicate the diagnosis of congenital adrenal hyperplasia. BMC Endocr Disord. 2020;55. doi:10.1186 /s12902-020-00550-7.
38. Parajes M, Krone N, Arlt W, et al. Technical limitations in genetic testing of congenital adrenal hyperplasia due to high-sequence homology in the CYP21A2 gene region. MDPI. 2024;1. doi:10.3390/genes11010001.

Correspondence:
Received: 17 June 2024
Accepted: 17 July 2024
Dina Fawzy
Pediatric Department, Faculty of Medicine, Alexandria University, Alexandria (Egypt)
El Gaish st, 0345 AlAzaritah
E-mail: Dinafawzy1988@yahoo.com
ORCID ID: 0000-0002-2725-5463