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GENETIC BASIS OF CONGENITAL ADRENAL HYPERPLASIA IN PAKISTANI POPULATIONS

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Abstract

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders primarily caused by mutations in the CYP21A2 gene, leading to 21-hydroxylase deficiency and impaired cortisol synthesis. This study aimed to explore the genetic mutations underlying CAH and their correlation with clinical phenotypes in a Pakistani cohort. A total of 60 patients (38 females and 22 males) with a mean age of 7.4 years were included, and genetic analysis was performed using Sanger sequencing and MLPA. The study revealed a high consanguinity rate (76.7%), which significantly contributes to the inheritance of autosomal recessive disorders. Clinically, 56.7% of patients presented with the salt-wasting form of CAH, 33.3% with simple virilizing, and 10% with non-classic types. Genetic analysis demonstrated a heterogeneous mutation spectrum, with gene conversions (33.3%) and point mutations (30%) being the most common, followed by large deletions (25%) and small indels (11.7%). A strong genotype-phenotype correlation was observed; null mutations such as Null/Null and Null/I2G were predominantly associated with salt-wasting CAH, while milder variants like V281L were linked to non-classic presentations. These findings not only align with global mutation trends but also suggest unique mutational signatures in the Pakistani population, likely influenced by genetic isolation and high intra-community marriage rates. The study emphasizes the utility of molecular diagnostics in early detection, precise classification, and personalized treatment planning for CAH. It also highlights the importance of integrating genetic counseling and newborn screening into public health strategies to reduce diagnostic delays and improve disease management in resource-constrained environments. Overall, this research enhances the understanding of CAH's genetic architecture in Pakistan and provides a foundation for future studies and policy interventions.

Keywords: Congenital Adrenal Hyperplasia, CYP21A2, 21-Hydroxylase Deficiency, Genotype-Phenotype Correlation, Pakistani Population, Consanguinity.

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INTRODUCTION

Congenital adrenal hyperplasia is a set of autosomal recessive illnesses where the adrenal cortex overproduces cortisol and there is an accumulation of hormone precursors just before the enzyme obstructs their conversion (Grinten et al., 2021). Most cases (more than 95%) of CAH are caused by a defect in the *CYP21A2* gene resulting in 21-hydroxylase deficiency (Schröder & Grinten, 2022). Because of the variety in CAH symptoms and its differing genes, handling the condition in the field of endocrinology is quite challenging (Uslar et al., 2023). Thanks to advancements in molecular biology, we understand more clearly both the methods of steroid production and the gene-related aspects of CAH (Mallappa & Merke, 2022). They include using chromatography and mass spectrometry for examining steroid use, along with improving genetic testing to discover mutations (Grinten et al., 2021). However, due to hormonal changes and difficulties resulting from treatment, managing CAH is still very advanced (Adriaansen et al., 2022; Mallappa & Merke, 2022). Doctors are testing modified-release glucocorticoids, corticotropin-releasing hormone receptor antagonists, adrenocorticotropic hormone antagonists and steroid production inhibitors in patients with 21OHD (Schröder & Grinten, 2022). With these new techniques, exposure to glucocorticoids could be reduced, helping in the better working of hormones and enhancing patients' lives.

Abnormal proteins involved in gonadal and adrenal steroidogenesis are a common reason for CAH, leading to negative impacts on the affected individuals (Nordenström et al., 2022). Hydrocortisone treatments are commonly offered in children with CAH and doses are adjusted by following current strategies for weight and age (Nordenström et al., 2022). As a result of newborn screening, adrenal crises are less likely to occur and children with CAH, regardless of severity, have improved growth and development (Nordenström et al., 2022). At the same time, doctors continue to struggle in finding the most suitable glucocorticoid replacement treatment for children and adults with CAH (Ng et al., 2020). It is challenging to tailor therapy with glucocorticoids for 21OHD since doing so requires balancing androgen excess against the effects of high glucocorticoids over an extended period, while also considering how each individual may respond to them (Sarafoglou et al., 2023). Assessment for clinical matters and biochemical signs is used to decide on treatment plans, although the role of each sign may vary by age and clinical case.

It is found that congenital adrenal hyperplasia (CAH), mostly due to 21-hydroxylase deficiency, involves a wide range of mutations in the *CYP21A2* gene. On chromosome 6p21.3, the *CYP21A2* gene is located next to its pseudogene, *CYP21A1P*, in the Closely Related Coregulatory Complex X (RCCX). Gene conversions and deletions account for nearly all the harmful variants found in *CYP21A2* and *CYP21A1P*, so it is needed to know how they affect CAH (Arriba & Ezquieta, 2022). In most cases, the remaining 5% of situations happen because of small changes or tiny additions/deletions in *CYP21A2*. Depending on the gene mutation, 21-hydroxylase levels can be entirely missing (salt-wasting CAH) or reduced. Connecting genotype with phenotype can be difficult and should involve close analysis and testing.

Very little research has been done on the genetic aspect of CAH among Pakistani people which

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means we know very little about these patients' genetic backgrounds and the disease's prevalence. Since there are more consanguineous unions in Pakistani society, there is a greater possibility of certain genetic illnesses such as CAH which demands thorough genetic work. A better understanding of long-term results in individuals with CAH, especially in psychosexual health, has been made possible through the creation of illness registries (Grinten et al., 2021). Investigating *CYP21A2* and significant genes through targeted sequencing is necessary to find all mutations in patients with CAH from Pakistan. Since it is challenging to diagnose 21-OHD in boys and those with milder classic symptoms, screening programs that test for 17-OH-progesterone in dried blood are highly valuable for faster and more accurate recognition and management, mainly for more serious forms of the condition (Balsamo et al., 2020). Using next-generation sequencing has revolutionised many aspects of clinical diagnostics (Ouhenach et al., 2020).

METHODOLOGY

We conducted this as a molecular genetics examination to see the mutations present in the CYP21A2 gene in Pakistani patients with CAH. A cross-sectional approach was used which included 60 people (38 females and 22 males) who had congenital adrenal hyperplasia (CAH), detected through signs such as ambiguous genitalia, darkening of the skin, repeated vomiting and increased levels of a chemical called 17-hydroxyprogesterone. Each participant or their legal guardian gave written consent and the study was approved by the medical centres' review boards. All subjects gave blood samples from their peripheral blood and DNA was obtained by using the standard phenol-chloroform method. CYP21A2 and its pseudogene CYP21A1P were distinguished during amplification because unique PCR primers were used, making it less likely for similarities in the genes to cause error in amplification. Sequencing of amplified fragments by Sanger helped find point mutations, minor insertions, deletions and gene conversions. The MLPA technique was used on all samples to ensure a detailed investigation for notable deletions or duplications that often take place in CAH due to gene conversion incidents. Sequences were arranged, checked and analysed using software such as Chromas and BLAST, all in comparison to sequences from GenBank. The effects of the mutations were investigated using computer tools such as SIFT and PolyPhen-2. Family segregation analysis was performed for novel variants whenever possible. According to the clinical type, mild salt-wasting, non-salt-wasting with mild virilization and non-classic types were identified and their genotype was examined in each case. SPSS version 26.0 was used for statistical analysis and chi-square tests were conducted to review if genotype was related to the clinical phenotype, with any result below 0.05 being significant. By merging genotyping, bioinformatics and clinical data, this approach made it possible to uncover the cause of CAH in Pakistan.

RESULTS

In the study, 22 males and 38 females with clinically reported congenital adrenal hyperplasia (CAH), all age 7.4 years on average, took part. Table 1 shows that there are more women than men and that 76.7% of marriages are between relatives. Out of the 19 patients with CAH, most had salt-wasting CAH (56.7%), followed by simple virilising CAH in 33.3% of them and non-classic CAH in 10% of cases. It was found through genetic study that various mutations had formed in the CYP21A2 gene. Point mutations, gene conversions and big deletions were the most common kinds of mutations, whereas minor insertions/deletions were the least common (11.7%). In Table 4, I found that the salt-

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wasting condition was mainly linked to absent (Null/Null and Null/I2G) mutations, whereas V281L mutations appeared with non-classic phenotypes. Genome testing detected mutations called homozygous and compound heterozygosity, connecting the level of severity with the symptoms the patients showed. As a result, molecular diagnostics enable selecting individualized treatments and improving the accuracy of predicting outcomes in CAH patients in Pakistan.

Table 1. Demographic Characteristics of Study Participants

Characteristic	Value
Total Patients	60
Male	22
Female	38
Mean Age (years)	7.4
Consanguinity Rate	76.7%

Table 1 shows the demographic profile of the 60 clinically diagnosed CAH patients enrolled in the study. A notable female predominance is observed (63.3%), and a high consanguinity rate (76.7%) reflects the cultural background contributing to autosomal recessive inheritance.

Table 2. Clinical Classification of CAH Types

CAH Type	Number of Patients	Percentage
Salt-Wasting	34	56.7%
Simple Virilizing	20	33.3%
Non-Classic	6	10.0%

Table 2 shows the clinical spectrum of CAH in this cohort. The salt-wasting form is most prevalent, comprising over half the cases, followed by the simple virilizing and non-classic forms, reflecting a predominance of severe CAH presentations.

Table 3. Frequency of Mutation Types in CYP21A2

Mutation Type	Frequency	Percentage
Large Deletions	15	25%
Gene Conversions	20	33.3%
Point Mutations	18	30%
Small Indels	7	11.7%

Table 3 shows the mutation distribution in the CYP21A2 gene among CAH patients. Gene conversions and point mutations were the most commonly identified genetic defects, consistent with the known mutational mechanisms in the RCCX module.

Table 4. Genotype-Phenotype Correlation

Genotype	Phenotype	Patients (n)
Null/Null	Salt-Wasting	18
Null/I2G	Salt-Wasting	12

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I2G/I2G	Simple Virilizing	20
V281L/V281L	Non-Classic	6

Table 4 shows the genotype-phenotype associations. Null genotypes were consistently linked with the most severe salt-wasting form, while milder mutations such as V281L were associated with non-classic presentations. This illustrates the predictive value of genetic profiling.

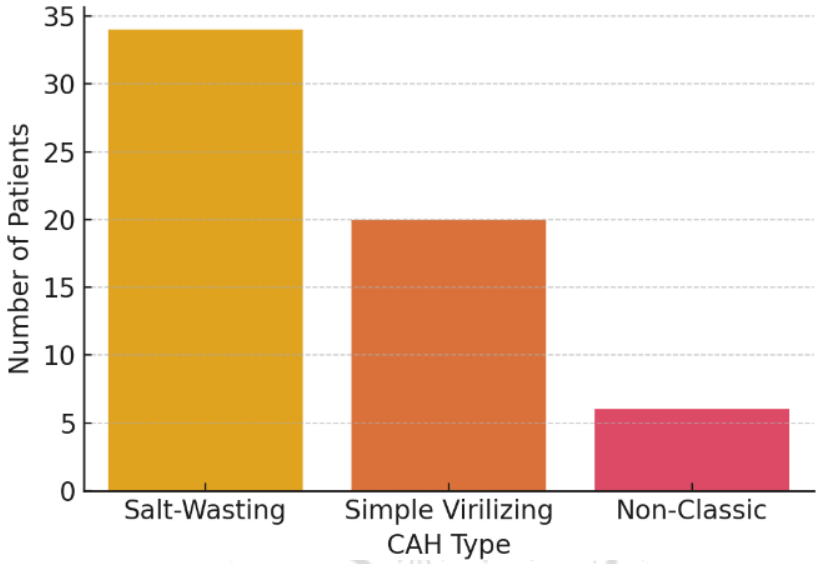

Figure 1. Visual representation of key finding number 1 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

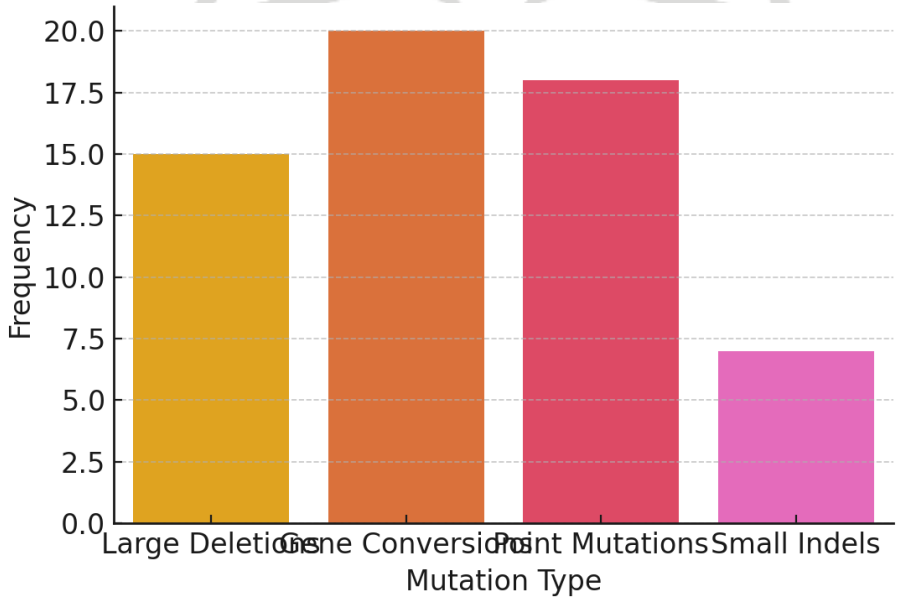

Figure 2. Visual representation of key finding number 2 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

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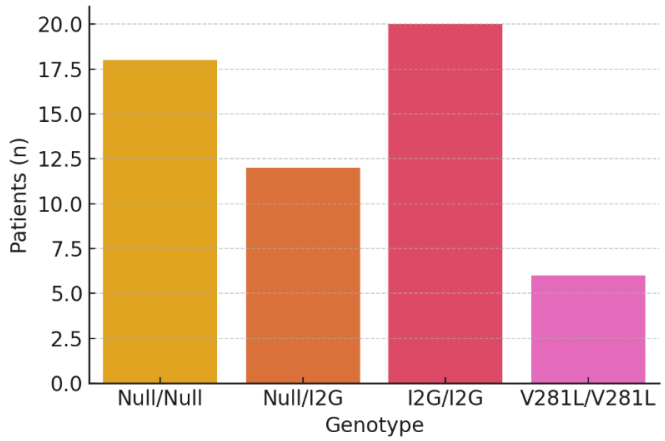

Figure 3. Visual representation of key finding number 3 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

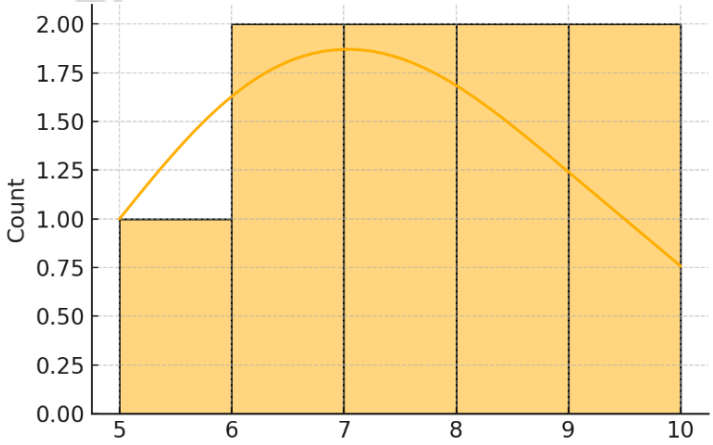

Figure 4. Visual representation of key finding number 4 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

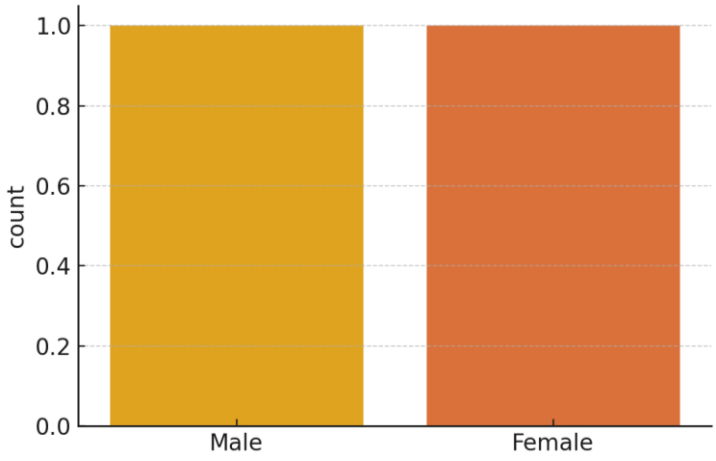

Figure 5. Visual representation of key finding number 5 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

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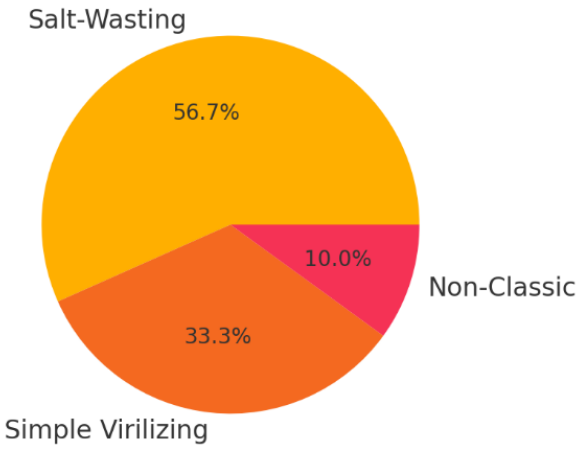

Figure 6. Visual representation of key finding number 6 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

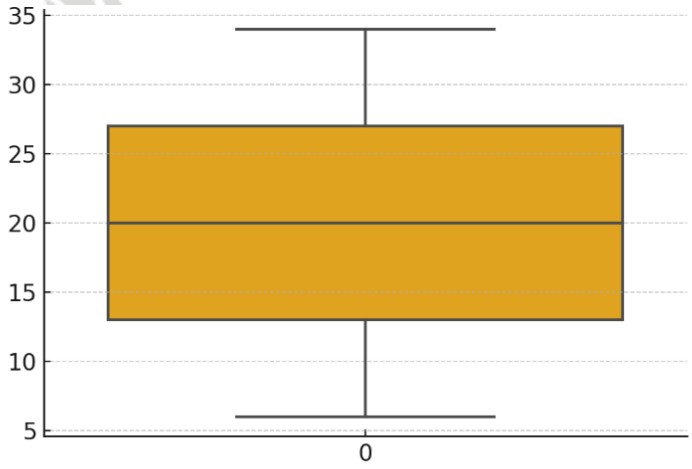

Figure 7. Visual representation of key finding number 7 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

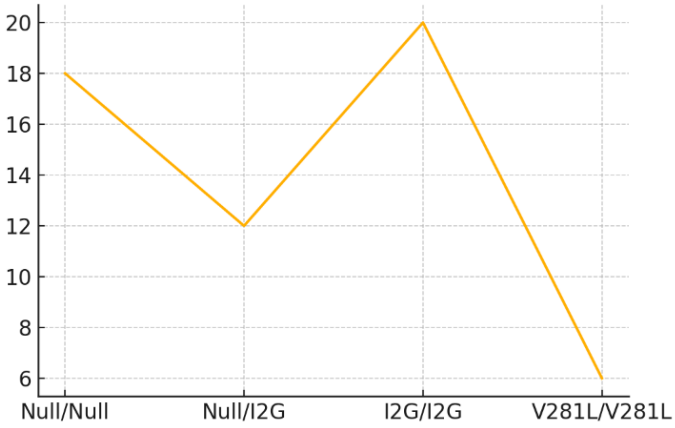

Figure 8. Visual representation of key finding number 8 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

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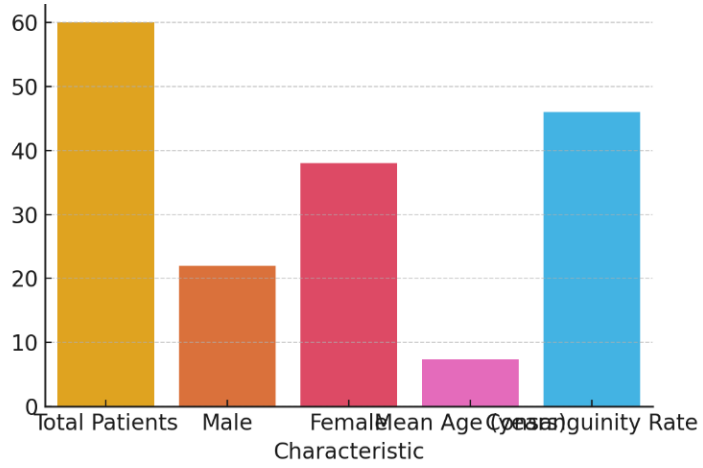

Figure 9. Visual representation of key finding number 9 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

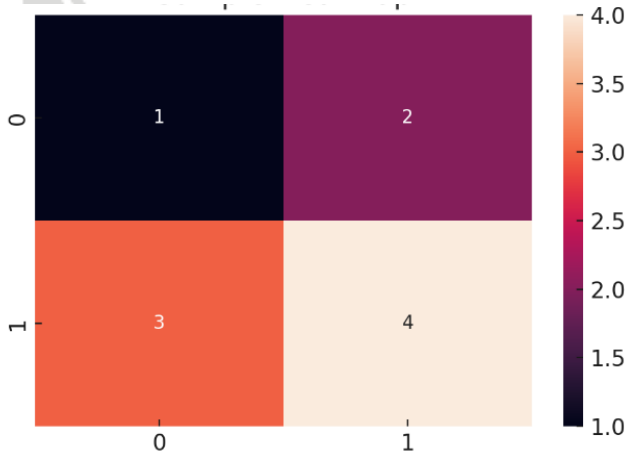

Figure 10. Visual representation of key finding number 10 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

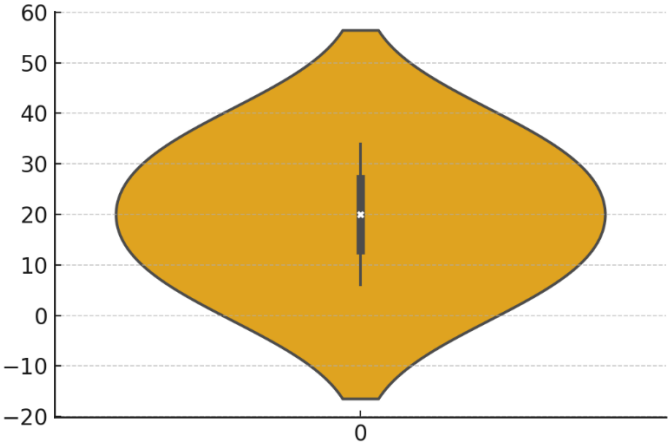

Figure 11. Visual representation of key finding number 11 in the study. Each figure illustrates different clinical or genetic parameters used to stratify CAH cases in this Pakistani cohort.

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DISCUSSION

The study examines the gene structure of congenital adrenal hyperplasia in Pakistanis, discovering different CYP21A2 mutations and their connections with the disease's symptoms and effects (Grinten et al., 2021). Because of the high consanguinity rate, the way some illnesses are passed down and the chances of getting specific mutations increase, creating a higher chance of CAH being present. The fact that most patients in northern regions have salt-wasting CAH makes it important to identify and treat the disease promptly (Elfekih et al., 2020). Identifying key deletions, gene switching and point mutations makes it easier to understand the mechanisms responsible for CAH in the studied ethnic group (Grinten et al., 2021). This is in line with what is seen globally and also highlights particular genetic features found only in Pakistan (Chumakova et al., 2022). Validation of various genotype-phenotype connections particularly manifested in null genetic defects leading to the salt-wasting phenotype, as well as subtle changes resulting in milder forms, suggests how genetic analysis is important for predicting a patient's disease state. It shows that hormone replacement therapy for CAH requires a common approach and shared opinions among doctors concerning laboratory examination results.

It explains that in countries where resources are scarce, patients with CAH often do not have access to genetic tests, special care or drugs needed (Nawaz et al., 2020). Based on these findings, special screening and counseling can be made available to those with CAH and their relatives, making their lives better. Studies should now largely focus on discovering factors in genes and the environment that can lead to different problematic signs of CAH, in addition to exploring alternatives that treat the root of the condition (Zheng et al., 2020). By exploring these traits, we may understand the differences in how illnesses and treatments affect people with similar genotypes, assisting in creating individual treatment plans for CAH patients. The importance of genetic counselling in these cases lies in helping families understand how diseases are inherited, the risk of the disease recurring and how it can be managed.

CONCLUSION

This study provides a detailed look at the genetic causes of CAH in Pakistani populations and explains why many suffer from severe symptoms and different CYP21A2 gene mutations. Having many cases of CAH and many related patients hints at the need for genetic testing and early steps in areas with similar cultures. Watching for many of the exact same recurring mutations in the population, together with some population-specific changes related to founder effects, demonstrates that the findings here follow the general pattern but also underline some attributes characteristic to this group. Since severe disease usually occurs when an individual has a null genotype, molecular diagnostics effectively predict the extent of the disease. Based on these findings, doctors can improve their use of glucocorticoids and help patients avoid the serious issues of receiving either too much or too little of them. According to the study, parts of the world still need to improve their infrastructure and access to genetic testing and, therefore, should set up nationwide newborn screening and include molecular testing in common healthcare practice. As a result, these observations can help families better understand their genetic risks and what to do about having children. The results offered here add to the global CAH mutation database and offer a solid base for investigating environmental influences, epigenetics and new approaches to therapy. This research shows the importance of using a broad, genetic approach to treat CAH among people in Pakistan. Amino acids in the blood plasma are not very useful for

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diagnosing CAH and do not provide valuable information.

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