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Core Gene Identification Of The Congenital Adrenal Hyperplasia (CAH) By Computational Approach

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Abstract

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders that are mostly caused by a deficiency in the enzymes that are necessary for the manufacture of cortisol. Typically, classical studies focus on specific gene changes. The utilisation of a systems-level approach that incorporates a computational approach provides a unique framework for understanding the complicated mechanism of CAH-associated core gene disease. As a result of this procedure, the POMC, CYP21A2, CYP17A1, CYP11B1, CYP11B2, and HSD3B2 core genes have been identified via a computational approach. These screened core genes, POMC, CYP21A2, CYP17A1, CYP11B1, CYP11B2, and HSD3B2, make it possible to illustrate how CAH progression occurs. It can have a negative impact on the entire steroidogenic network. These changes can then result in hormonal imbalances, adrenal hyperplasia, and other symptoms that can be observed in clinical settings. The mapping of protein-protein interactions, regulatory pathways, and downstream metabolic effects explains how this occurs. Furthermore, this approach enables the discovery of new genes that are linked to therapies that are being administered at currently available hormonal therapies. This article provides a summary of how the computational approach is used to focus on the way in which the core genes CYP11B1, CYP11B2, CYP21A2, CYP17A1, POMC, and HSD3B2 work together in the progression of CAH. The study also could contribute to the development of targeted therapies that are more effective in the treatment of this complicated CAH disease.

Keywords: CAH, POMC, CYP21A2, CYP17A1, CYP11B1, CYP11B2, HSD3B2.

Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive illnesses caused by abnormalities in the genes encoding enzymes which are required for the generation of adrenal hormones [1-3].The adrenal glands, which are situated on the top of both kidneys, are impacted by CAH. Typically, adrenal glands are responsible for the secretion of three different hormones like corticosteroids, mineralocorticoids as well as androgens. If the body fails to produce any of the above hormones due to a specific enzyme shortage, the system will overproduce a different kind of hormone precursor for making up the imbalance. There are two types classical CAH (salt-wasting, simple virilization) and non-classical CAH [4]. Out of these CAH, high incidence of CAH is reported in non-classical CAH than classical CAH[5].CAH symptoms are directly linked to the disorder's two hormone effects, which include low cortisol along with aldosterone levels, as well as high androgen levels[6]. Classical CAH symptoms include adrenal shortage, ambiguous genitalia in females, apparent precocious puberty in childhood [7], but non-classical CAH effects appear in late childhood, teenagers,

and eventually later life [8]. Female abnormalities include hirsutism (having excessive facial/body hair) like symptoms [9].

Ninety five percent of CAH cases is attributable to a shortage in 21-hydroxylase, which is the consequence of mutations in the CYP21A2 gene [10]. People who get a diagnosis at pregnancy or in their infancy stages are at a greater chance of having major enzymatic abnormalities.

CAH medication is permanent and requires accurate hormone replacement therapies such as glucocorticoid, mineralocorticoid replacement, surgical removal [11], and new therapies. Corticotropin-releasing factor type 1 (CRF1) receptor [12].A computational approach is new technique that predicts the specific gene target that is responsible for the progression of the disease [13]. The aim of this study to identify the core gene of congenital adrenal hyperplasia (CAH) via computational approach.

Materials and method

Mining of the target gene of CAH

Mining of the target gene of CAH carried out from two disease database like DisGeNet (https://www.disgenet.org/) [14] and Gene Cards (https://www.genecards.org/) database [15]. The genes were taken on the basis of the relevance score.

Intersection and protein protein interaction (PPI) network of the common gene

The common genes of both databases were screened from Venny Tool 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) web tool [16]. After that the common gene visualized through Cytoscape (software version3.10.3) [17] software for the visualization of PPI network at 0.400 confidence.

Selection of the core gene

The core genes of CAH were selected through CytoNCA application that was plugged in Cytoscape software. Three variables like degree, betweenness and closeness were taken for selection of the core gene.

Assessment of Gene ontology (GO) and Kyoto Encyclopaedia of Gene and Genome (KEGG) pathway

The assessment of the GO and KEGG pathways performed by the ShinyGO 0.85 (http://bioinformatics.sdstate.edu/go74/) [18]. Four parameters like biological processes, cellular components, molecular function, and KEGG pathways taken at the False discovery rate (FDR) cutoff 0.05.

Results

Target gene of CAH analysis

A total 2550 target genes were mined CAH mined from Gene Cards and nine genes obtained from the DisGeNet database. Using Venny Tool, nine genesCYP11B1, CYP11B2, CYP21B1, CYP17A1, POMC, HSD3B2, POR, STAR, and TNXB were found to be common in both databases (Fig.1).

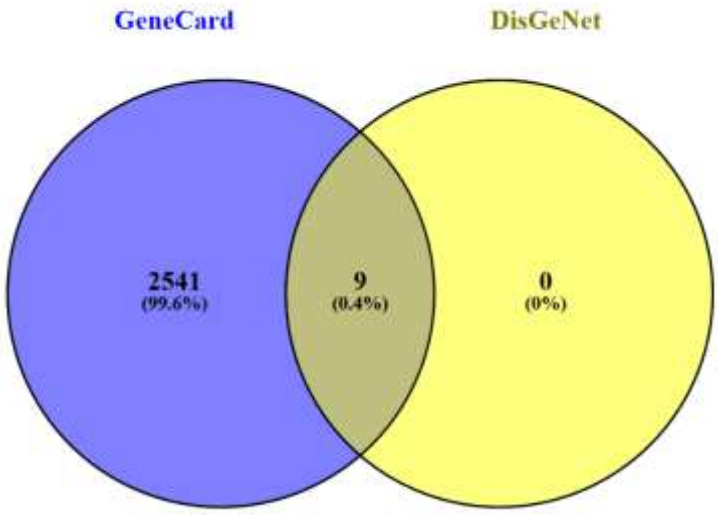

Fig 1. Mining of common gene of CAH from Genecard and DisGeNet database

Protein protein network and core gene analysis

The protein protein network analysis of 9 genes (CYP11B1, CYP11B2, CYP21B1, CYP17A1, POMC, HSD3B2, POR, STAR, and TNXB) was carried out by STRING and Cytoscope software at confidence scores 0.400 shows in Figure 2. The CytoNCA reveals that CYP11B1, CYP11B2, CYP21B1, CYP17A1, POMC and HSD3B2 gene highest degree, closeness and betweenness. These six genes(CYP11B1, CYP11B2, CYP21B1, CYP17A1, POMC and HSD3B2) were considered as coregene (Fig. 3).

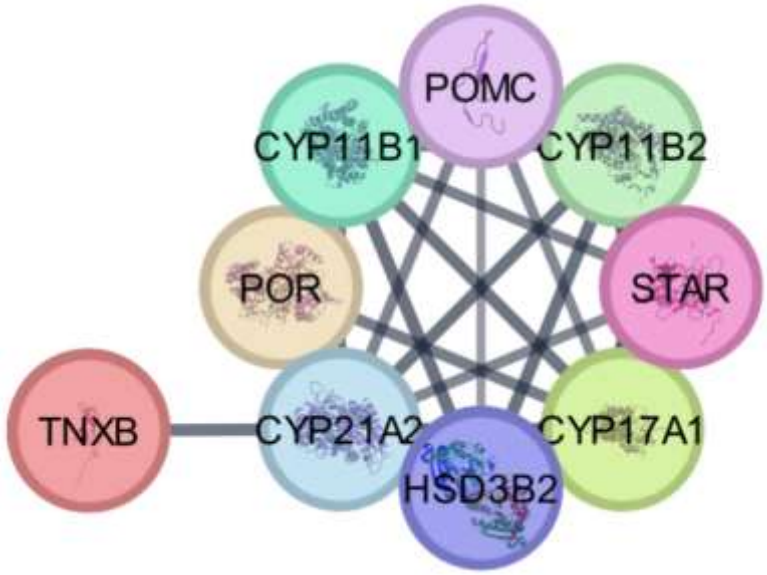

Fig 2. Protein protein interaction network of common gene of CAH

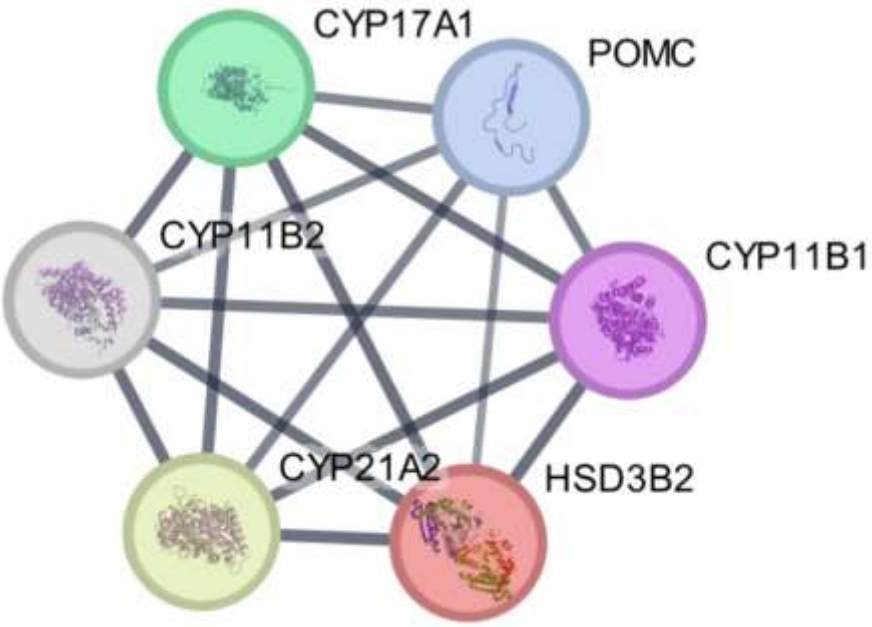

Fig 3. Core gene of CAH

Gene ontology (GO) analysis

In order to investigate the biological functions, cellular component, biological process and pathways, we carried out the GO and KEGG pathway enrichment analysis.

Several biological process Androgen biosynthetic process, Cortisol biosynthetic process, Tertiary alcohol biosynthetic process, Glucocorticoid biosynthetic process, Aldosterone biosynthetic process, Mineralocorticoid biosynthetic process, Mineralocorticoid metabolic process, Aldosterone metabolic process, Cortisol metabolic process, Primary alcohol biosynthetic process, Cellular response to potassium ion, Glucocorticoid metabolic process, C21-steroid hormone metabolic process, Steroid hormone biosynthetic process, Hormone biosynthetic process, Steroid biosynthetic process, Hormone metabolic process, Steroid metabolic process, Regulation of hormone levels and Lipid biosynthetic process were carried out by screen core gene (Fig. 4 and Table 1)

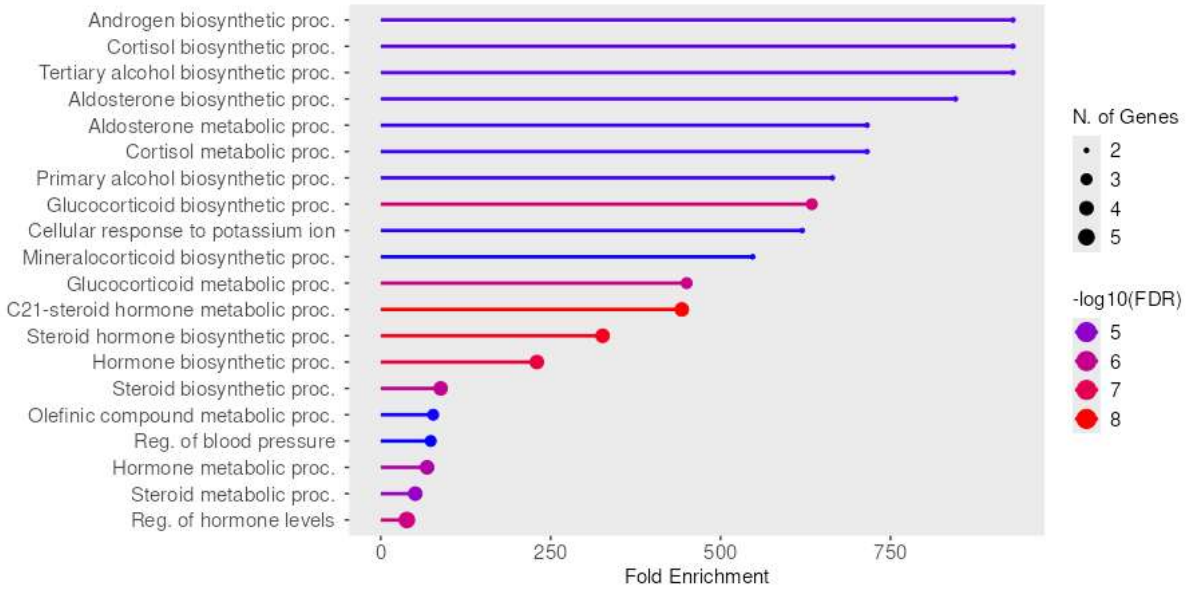

Fig. 4 Biological process of CAH core gene

Table 1. List of core gene in several biological process

Enrichment FDR	Pathway Genes	Fold Enrichment	Pathway	Genes
3.56E-05	10	775.2666667	GO:0006702 Androgen biosynthetic proc.	HSD3B2 CYP17A1
3.56E-05	10	775.2666667	GO:0034651 Cortisol biosynthetic proc.	CYP11B1 CYP11B2
3.56E-05	10	775.2666667	GO:1902645 Tertiary alcohol biosynthetic proc.	CYP11B1 CYP11B2
6.41E-10	22	704.7878788	GO:0006704 Glucocorticoid biosynthetic proc.	CYP11B1 CYP11B2 CYP21A2 CYP17A1
4.08E-05	11	704.7878788	GO:0032342 Aldosterone biosynthetic proc.	CYP11B1 CYP11B2
1.39E-07	17	684.0588235	GO:0006705 Mineralocorticoid biosynthetic proc.	CYP11B1 CYP11B2 CYP21A2
1.80E-07	19	612.0526316	GO:0008212 Mineralocorticoid metabolic proc.	CYP11B1 CYP11B2 CYP21A2
5.14E-05	13	596.3589744	GO:0032341 Aldosterone metabolic proc.	CYP11B1 CYP11B2
5.14E-05	13	596.3589744	GO:0034650 Cortisol metabolic proc.	CYP11B1 CYP11B2
5.68E-05	14	553.7619048	GO:0034309 Primary alcohol biosynthetic proc.	CYP11B1 CYP11B2
6.22E-05	15	516.8444444	GO:0035865 Cellular response to potassium ion	CYP11B1 CYP11B2
2.07E-09	31	500.172043	GO:0008211 Glucocorticoid metabolic proc.	CYP11B1 CYP11B2 CYP21A2 CYP17A1
5.88E-09	42	369.1746032	GO:0008207 C21-steroid hormone metabolic proc.	CYP17A1 CYP11B1 CYP11B2 HSD3B2
9.47E-11	57	340.0292398	GO:0120178 Steroid hormone biosynthetic proc.	CYP11B1 CYP11B2 CYP21A2 CYP17A1 HSD3B2
2.89E-10	81	239.2798354	GO:0042446 Hormone biosynthetic proc.	CYP17A1 CYP11B1 CYP11B2 HSD3B2 CYP21A2
1.07E-08	211	91.85624013	GO:0006694 Steroid biosynthetic proc.	CYP11B1 CYP11B2 HSD3B2 CYP21A2 CYP17A1
3.42E-08	273	70.995116	GO:0042445 Hormone metabolic proc.	CYP17A1 CYP11B1 CYP11B2 HSD3B2 CYP21A2

1.34E-07	367	52.81108084	GO:0008202 Steroid metabolic proc.	CYP17A1 CYP11B1 CYP11B2 HSD3B2 CYP21A2
1.07E-08	605	38.44297521	GO:0010817 Reg. of hormone levels	POMC CYP17A1 CYP11B1 CYP11B2 HSD3B2 CYP21A2
5.87E-06	828	23.40780998	GO:0008610 Lipid biosynthetic proc.	CYP11B1 CYP11B2 HSD3B2 CYP21A2 CYP17A1

The screened core genes were distributed in several cellular component like Smooth endoplasmic reticulum membrane , Mitochondrial inner membrane, Organelle inner membrane, Mitochondrial membrane, Mitochondrial envelope, Endoplasmic reticulum membrane, Endoplasmic reticulum subcompartment, Nuclear outer membrane-endoplasmic reticulum membrane network, Organelle subcompartment, Organelle envelope and its membrane (Fig. 5 and Table2).

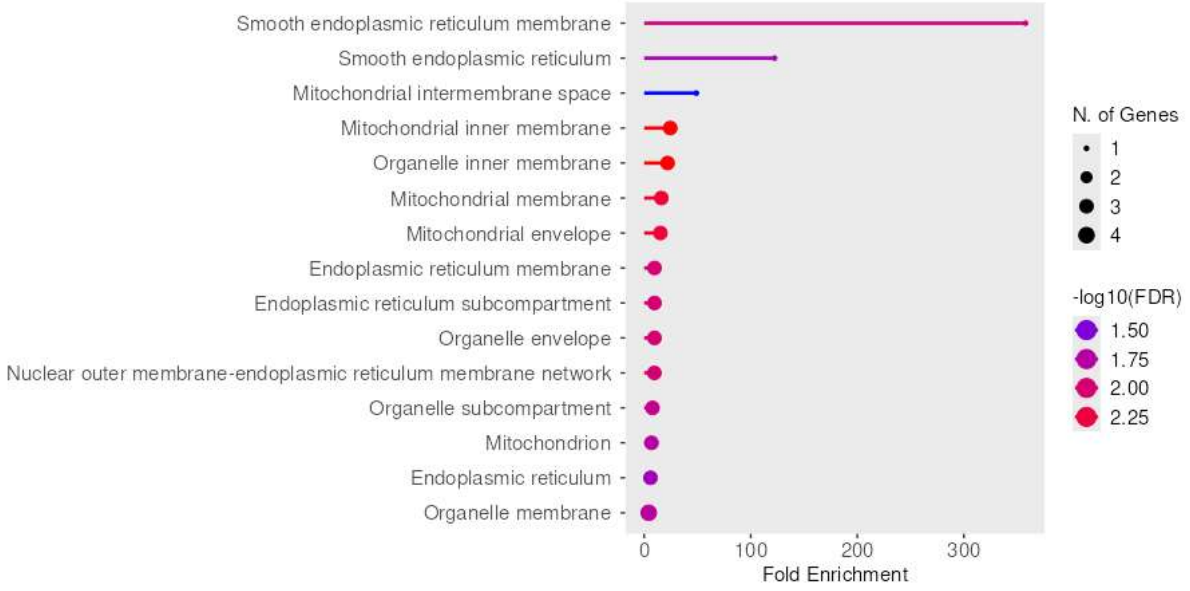

Fig. 5 Cellular component of CAH core gene

Table 2. List of core gene in several components

Enrichment FDR	Pathway Genes	Fold Enrichment	Pathway	Genes
0.010961547	13	298.1794872	GO:0030868 Smooth endoplasmic reticulum membrane	HSD3B2
0.027039238	38	102.0087719	GO:0005790 Smooth endoplasmic reticulum	HSD3B2
0.002494197	570	20.40175439	GO:0005743 Mitochondrial inner membrane	CYP11B1 CYP11B2 HSD3B2
0.002725739	634	18.34227129	GO:0019866 Organelle inner membrane	CYP11B1 CYP11B2 HSD3B2
0.004577805	866	13.42840647	GO:0031966 Mitochondrial membrane	CYP11B1 CYP11B2 HSD3B2

0.004577805	917	12.68157034	GO:0005740 Mitochondrial envelope	CYP11B1 CYP11B2 HSD3B2
0.002494197	1433	10.82019074	GO:0005789 Endoplasmic reticulum membrane	CYP17A1 CYP11B1 HSD3B2 CYP21A2
0.002494197	1439	10.77507528	GO:0098827 Endoplasmic reticulum subcompartment	CYP17A1 CYP11B1 HSD3B2 CYP21A2
0.002494197	1458	10.63465935	GO:0042175 Nuclear outer membrane-endoplasmic reticulum membrane network	CYP17A1 CYP11B1 HSD3B2 CYP21A2
0.002725739	1782	8.701084923	GO:0031984 Organelle subcompartment	CYP17A1 CYP11B1 HSD3B2 CYP21A2
0.012356954	1441	8.070090215	GO:0031967 Organelle envelope	CYP11B1 CYP11B2 HSD3B2
0.00476252	2352	6.592403628	GO:0005783 Endoplasmic reticulum	HSD3B2 CYP17A1 CYP11B1 CYP21A2
0.027869582	2029	5.731394776	GO:0005739 Mitochondrion	CYP11B1 CYP11B2 HSD3B2
0.004577805	4340	4.465821813	GO:0031090 Organelle membrane	CYP11B1 CYP11B2 CYP17A1 HSD3B2 CYP21A2

Various molecular function such as Steroid 17-alpha-monooxygenase activity , Steroid delta-isomerase activity, Type 1, 3 and 4 melanocortin receptor binding, 3-beta-hydroxy-delta5-steroid dehydrogenase (nad+) activity, Melanocortin receptor binding, Oxidoreductase activity acting on paired donors with incorporation or reduction of molecular, Steroid 21-monooxygenase activity, 17-hydroxyprogesterone 21-hydroxylase activity, Progesterone 21-hydroxylase activity, Steroid hydroxylase activity, Intramolecular oxidoreductase activity transposing c=c bonds, Monooxygenase activity, Heme binding, Iron ion binding, Tetrapyrrole binding, Oxidoreductase activity acting on paired donors with incorporation or reduction of molecular, Oxidoreductase activity and Transition metal ion binding govern by core gene (Fig. 6 and Table 3).

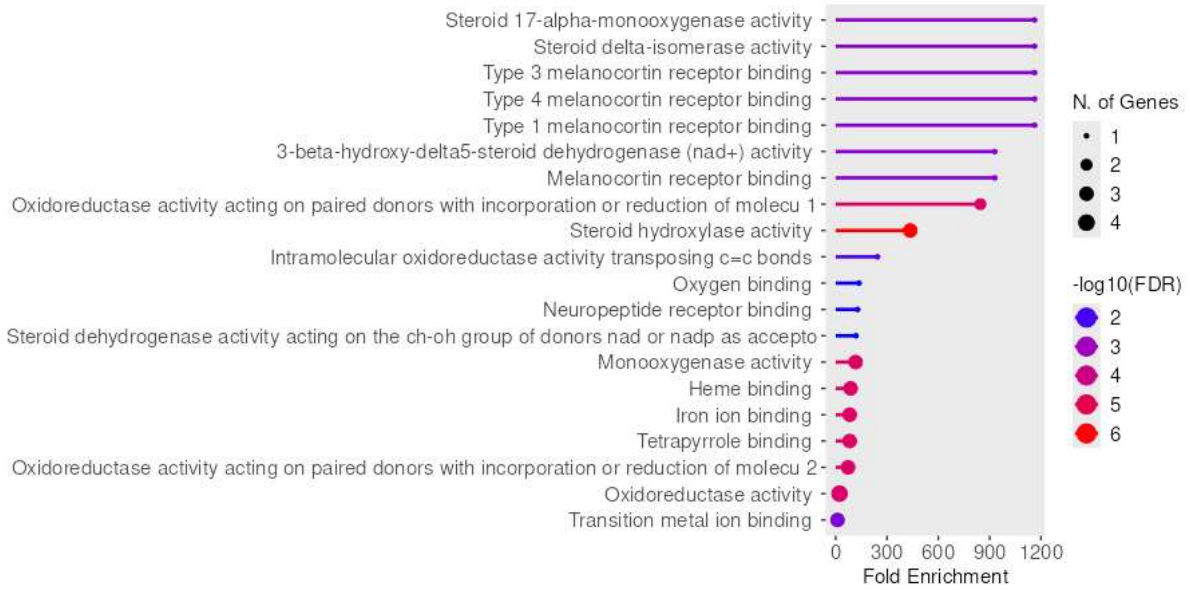

Fig. 6 Molecular function of CAH core gene

Table 3. List of core gene in several molecular function

Enrichment FDR	Pathway Genes	Fold Enrichment	Pathway	Genes
0.003021027	4	969.0833333	GO:0004508 Steroid 17-alpha-monooxygenase activity	CYP17A1
0.003021027	4	969.0833333	GO:0004769 Steroid delta-isomerase activity	HSD3B2
0.003021027	4	969.0833333	GO:0031781 Type 3 melanocortin receptor binding	POMC
0.003021027	4	969.0833333	GO:0031782 Type 4 melanocortin receptor binding	POMC
0.003021027	4	969.0833333	GO:0070996 Type 1 melanocortin receptor binding	POMC
0.003303893	5	775.2666667	GO:0003854 3-beta-hydroxy-delta5-steroid dehydrogenase (nad+) activity	HSD3B2
0.003303893	5	775.266	GO:0031779 Melanocortin receptor binding	POMC
1.56E-05	11	704.787	GO:0016713 Oxidoreductase activity acting on paired donors with incorporation or reduction of molecu	CYP11B1 CYP11B2
0.003338312	6	646.055	GO:0004509 Steroid 21-monooxygenase activity	CYP21A2
0.003338312	6	646.055	GO:0103069 17-hydroxyprogesterone 21-hydroxylase activity	CYP21A2
0.003338312	6	646.055	GO:0106309 Progesterone 21-hydroxylase activity	CYP21A2
1.81E-09	32	484.541	GO:0008395 Steroid hydroxylase activity	CYP17A1 CYP11B1 CYP11B2 CYP21A2
0.010028732	19	204.017	GO:0016863 Intramolecular oxidoreductase activity transposing c=c bonds	HSD3B2
2.06E-07	120	129.211	GO:0004497 Monooxygenase activity	CYP17A1 CYP11B1 CYP11B2 CYP21A2
3.35E-07	160	96.90833333	GO:0020037 Heme binding	CYP21A2 CYP11B1 CYP11B2 CYP17A1

3.35E-07	170	91.20784314	GO:0005506 Iron ion binding	CYP17A1 CYP11B1 CYP11B2 CYP21A2
3.35E-07	170	91.20784314	GO:0046906 Tetrapyrrole binding	CYP21A2 CYP11B1 CYP11B2 CYP17A1
4.55E-07	192	80.75694444	GO:0016705 Oxidoreductase activity acting on paired donors with incorporation or reduction of molecu	CYP17A1 CYP11B1 CYP11B2 CYP21A2
1.87E-06	823	23.55002025	GO:0016491 Oxidoreductase activity	CYP17A1 CYP11B1 CYP11B2 HSD3B2 CYP21A2
0.000565348	1277	12.14199948	GO:0046914 Transition metal ion binding	CYP17A1 CYP11B1 CYP11B2 CYP21A2

KEGG pathways analysis

Several biological pathways like steroid hormone biosynthesis, cortisol synthesis and secretion, aldosterone synthesis and secretion, ovarian steroidogenesis, cushing syndrome, adipocytokine signaling pathway, prolactin signaling pathway, melanogenesis, estrogen signaling pathway and metabolic pathways were controlled by core gene of CAH (Fig. 7 and Table 4).

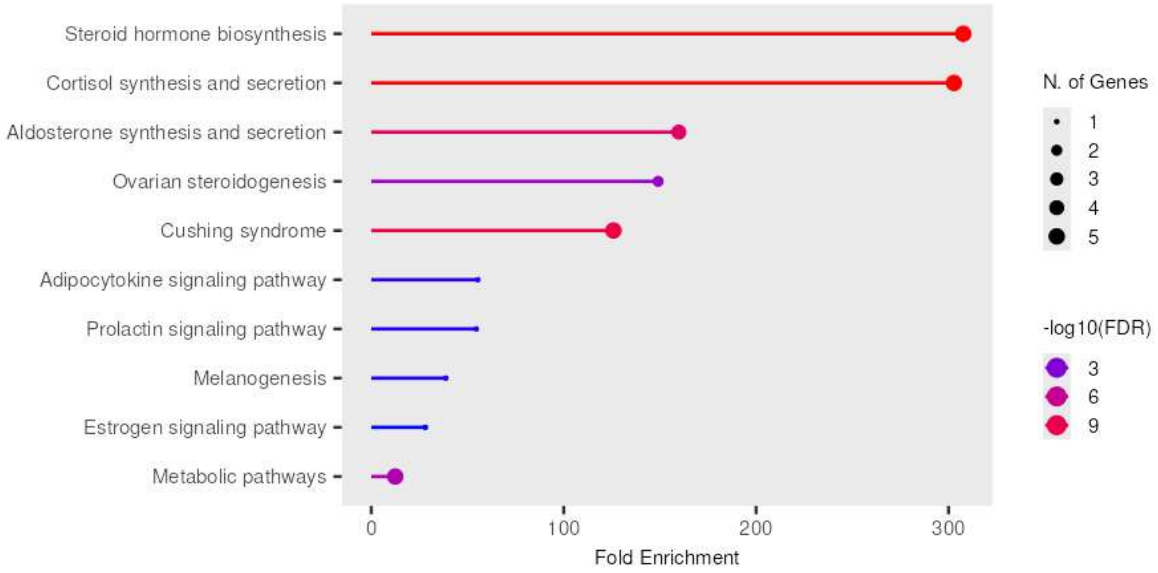

Fig. 7 Involvement of CAH core gene in several biological pathways

Table 5 List of CAH core gene in several biological pathways

Enrichment FDR	Pathway Genes	Fold Enrichment	Pathway	Genes
5.23E-12	63	307.6455026	Path:hsa00140 Steroid hormone biosynthesis	CYP11B1 CYP11B2 CYP17A1 CYP21A2 HSD3B2
5.23E-12	64	302.8385417	Path:hsa04927 Cortisol synthesis and secretion	CYP11B1 CYP17A1 CYP21A2 HSD3B2 POMC
1.38E-08	97	159.8487973	Path:hsa04925 Aldosterone synthesis and secretion	CYP11B2 CYP21A2 HSD3B2 POMC
0.000158431	52	149.0897436	Path:hsa04913 Ovarian steroidogenesis	CYP17A1 HSD3B2
3.08E-10	154	125.8549784	Path:hsa04934 Cushing syndrome	CYP11B1 CYP17A1 CYP21A2 HSD3B2 POMC
0.029540874	70	55.37619048	Path:hsa04920 Adipocytokine signaling pathway	POMC
0.029540874	71	54.59624413	Path:hsa04917 Prolactin signaling pathway	CYP17A1
0.036868832	100	38.76333333	Path:hsa04916 Melanogenesis	POMC
0.045604574	138	28.08937198	Path:hsa04915 Estrogen signaling pathway	POMC
1.96E-05	1556	12.45608398	Path:hsa01100 Metabolic pathways	CYP11B1 CYP11B2 CYP17A1 CYP21A2 HSD3B2

Discussion

Congenital adrenal hyperplasia (CAH) is a series of autosomal recessive illnesses [1] caused by enzyme abnormalities in the adrenal cortex's steroidogenic pathway. Cortisol shortage results in an ensuing rise in adrenocorticotropic hormone (ACTH), which causes adrenal hyperplasia [19] and the buildup of precursors components. With an approximate worldwide frequency of 1 in in 15,000-20000 live births in Western countries [20]. CAH is a crucial for understanding the interactions of genetics, hormone biochemistry, and clinical medicine [21]. The most common variant, accounting for more than 95% of cases, is 21-hydroxylase deficiency (21-OHD), caused by mutations in the CYP21A2 gene [22].

Our study reveals that six core genes like CYP11B1, CYP11B2, CYP21B1, CYP17A1, POMC and HSD3B2 that's directly or indirectly participated in progression of the CAH.

The cytochrome P450 enzyme CYP17A1 is located in the endoplasmic reticulum of steroidogenic cells, which are present in the adrenal glands as well as gonads [23]. It exhibits two different types of enzymatic activities like 17α -hydroxylase and 17,20-lyase activity [24]. Each plays a vital rolein process of steroidogenesis [25]. Deletion and mutations cause a shortage of the both enzymatic activities [26].

CYP11B2 is located in Zona Glomerulosa of Adrenal Cortex [27]. It plays an important role in aldosterone synthesis via regulation of the Renin-Angiotensin-Aldosterone System (RAAS), Potassium signalling pathways. Lack of CYP11B2 causes CAH occurrence [28].

The rare form of 3β-hydroxysteroid dehydrogenase deficient form is caused by the HSD3B2 gene, which is known to have a crucial and non-redundant function in congenital adrenal hyperplasia (CAH)

[29]. In the adrenal glands and gonads, that HSD3B2 gene encodes an enzyme which is required for transforming Δ5-steroids (for example, pregnenolone and 17α-hydroxypregnenolone) into Δ4-steroids (which include progesterone and 17α-hydroxyprogesterone) [30]. This process is a crucial phase in the process of biosynthesis of all categories of active steroid hormones, which include glucocorticoids, mineralocorticoids, and sex steroids. When there are changes in the gene HSD3B2, the outcome is a deficit of an enzyme that it encodes [31]. This absence of the enzyme causes a reduction in the body's capability to make cortisol and aldosterone, whereas at the same time generating an increase in the concentration of precursor androgens, which have a minimal functional impact. Because of this, classic manifestations of the deficiencies during early development are marked by adrenal difficulties that result in the loss of salt from the body because of to a lack in aldosterone, as well as various forms of under virilization in genetic males (due to impaired testosterone synthesis) and moderate virilization in genetic females (due to the peripheral conversion of weak androgens) [32]. Consequently, HSD3B2 plays an essential role in the maintenance of steroidogenic flux[33], and their deficiency leads to an abrupt disruption of the whole hormonal pathway, which results in a multifaceted along with potentially fatal kind of CAH that is characterised through a lack of adrenal hormones and abnormal sexuality.

The pathophysiology of congenital adrenal hyperplasia (CAH) is significantly influenced by the pro-opiomelanocortin (POMC) gene [34], which serves an important but indirect function in the development of the disease. The peptide hormone known as adrenocorticotropic hormone (ACTH) is produced by the cleavage of the POMC gene byproduct that is the precursor of a variety of other hormones [35]. Consequently, the extremely low levels of cortisol present in CAH cause a persistent as well as excess triggering of both the expression and processing of the POMC gene in the pituitary corticotrophs [36]. This ultimately leads to significantly high amounts of ACTH. The abnormally high levels of adrenocorticotropic hormone (ACTH) cause the hyperplastic adrenal glands to secrete an excessive amount of adrenal androgens, which in turn results in the classic signs of congenital adrenal hyperplasia (CAH), including virilisation and other symptoms [36].

The CYP11B1 gene is responsible for the producing 11-beta-hydroxylase which plays a vital role in cortisol production [37]. Cortisol is responsible for the regulation of stressful circumstances [38]. It also plays a critical role in the regulation of blood pressure, metabolism, and immune function [38]. Mutation of this gene causes deficiency of the enzyme responsible for cortisol synthesis as a result CAH progression occurs [39].

Conclusion

Congenital adrenal hyperplasia is not just an endocrine deficit. It is a complex genetic ailment which demands clinicians to carefully balance hormone replacement, a bioethical approach that pushes the limits of surgical procedure and gestational medication, and a chronic health issue that necessitates thorough, permanent, personalised treatment. Discoveries in genes, neonatal examination, and the advancement of personalised therapies continue to improve our strategy, alongside the goal of improving not only longevity but also overall quality of life as well as medical benefits for people with CAH.

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