Case Report

Genetic Testing for a Patient with Suspected 3 Beta-Hydroxysteroid Dehydrogenase Deficiency: A Case of Unreported Genetic Variants

Elisa Menegatti 1,2,*, Daniele Tessaris 3, Alice Barinotti 2,*, Patrizia Matarazzo 3 and Silvia Einaudi 4

1 Division of Medical Genetics, Azienda Ospedaliera Città della Salute e della Scienza, University of Turin, 10126 Turin, Italy
2 Clinical Pathology Unit, Department of Clinical and Biological Sciences, University of Turin, 10125 Turin, Italy
3 Department of Pediatric Endocrinology, Regina Margherita Children’s Hospital, University of Turin, 10126 Turin, Italy
4 Federazione Malattie Rare Infantili (FMRI), Regina Margherita Children’s Hospital, 10126 Torino, Italy
* Correspondence: elisa.menegatti@unito.it

Abstract: 3beta-hydroxysteroid dehydrogenase type II deficiency (HSD3B2 deficiency), a rare form of congenital adrenal hyperplasia (CAH), is characterized by varying degrees of salt loss and incomplete masculinization in males and mild virilization or normal external genitalia in females. We report the case of a patient (46XY) showing salt loss and incomplete masculinization, markedly elevated levels of 17OHP (17 hydroxyprogesterone), ACTH (Adreno Cortico Tropic Hormone), testosterone and delta4androstenedione (delta4A), low levels of cortisol and absence of bone skeletal alterations that frequently characterize POR (Cytochrome P450 oxidoreductase) deficiency. Mutation analysis by Sanger sequencing of the HSD3B2 gene showed that the patient presented with a compound heterozygote for two novel variants c.370A>G p.Ser124Gly and c.308-6 G>A. The two HSD3B2 gene variants were also present in the patient’s older brother showing only incomplete masculinization. The in silico analysis revealed a probable damaging effect of c.370A>G p.Ser124Gly: residue p.Ser124 is highly conserved among species and seems to be located in the catalytic site of the enzyme, playing a pivotal role in NAD(H) binding to its substrate. Intronic c.308-6G>A variant is predicted to be likely pathogenic; the substitution seems to cause a change in the splice acceptor site located 6bp downstream of the variant. The two siblings seem to be affected by 3β-HSD2 deficiency; nevertheless, the two novel variants are likely to cause variable expressivity of the disease.

Keywords: congenital adrenal hyperplasia; 3β-hydroxysteroid dehydrogenase; case report

1. Introduction

3β-hydroxysteroid dehydrogenase (3β-HSD) is one of the key enzymes of steroid biosynthesis in adrenal glands and gonads. 3β-HSD is a 42-kDa microsomal enzyme catalysing the conversion of the hydroxyl group to a keto group on carbon 3 and the isomerisation of Δ5steroids precursors into Δ4 ketosteroids [1–3].

3β-HSD is responsible for the conversion of pregnenolone to progesterone, 17β-hydroxyprogrenenolone to 17β-hydroxyprogesterone, dehydroepiandrosterone (DHEA) to androstenedione and androstenediol to testosterone. 3β-HSD is encoded by two closely related genes located on chromosome 1 (1p13.1), HSD3B2 and HSD3B1, coding for type II and I isozymes, respectively. Type I isozyme (HSD3B1) is mainly expressed in the placenta and peripheral tissues, and type II isozyme (HSD3B2) is mainly expressed in the adrenal gland and gonads [4,5].

Deleterious mutations in the HSD3B2 gene cause classical 3β-HSD2 deficiency (OMIM # +201810), which is an extremely rare autosomal recessive form of congenital adrenal hyperplasia. 3β-HSD2 deficiency has a prevalence of 1:1,000,000 and is associated with...
an impairment in the steroidogenesis in both the adrenals and gonads. The effects of classic 3β-HSD2 deficiency include dysfunction in the synthesis of glucocorticoids and mineralocorticoids in adrenal glands, with an excess of Δ5-steroids, resulting in adrenal insufficiency with or without salt wasting [5–9].

In addition to adrenal insufficiency, gonadal function is also impaired in patients with 3β-HSD2 deficiency. Affected male individuals encounter incomplete virilization, with clinical signs ranging from mild hypospadias to genital ambiguity due to impaired testosterone biosynthesis. 46XX individuals have normal female genitalia or show moderate signs of virilization of the external genitalia with clitoromegaly. These features are linked to an overproduction of DHEA by the foetal adrenals, which in turn can be converted to testosterone by extra-adrenal 3β-HSD1 [10–16].

Consequently, the presence of peripheral 3β-HSD1 activity often complicates the hormonal diagnosis of this disorder [17].

Herewith, we report the presence of 3β-HSD2 deficiency in two siblings who attended the Pediatric Endocrinology Centre of the Regina Margherita Children’s Hospital of Turin and who presented with compound heterozygote for one novel missense variant and a splicing variant in the HSD3B2 gene. We performed in silico analysis to assess the effects of the missense variant on enzyme activity and the effect of the intronic variant on splicing.

2. Case Report
2.1. Patients, Clinical Presentation and Hormonal Analysis
2.1.1. Patient n.1
A full-term newborn (gestational age = 41 weeks) was born after an uneventful pregnancy by spontaneous vaginal delivery. The birth weight and length were 3330 g and 51.5 cm, respectively. Apgar scores were 9 and 9 at 1 and 5 min, respectively. This patient showed hyperpigmentation and perineal hypospadias: both testes of 2 mL were palpable in a bifid severely hyperpigmented scrotum. At the age of 3 days, they were screened for hormonal abnormalities: their tests showed markedly elevated 17OHP levels (86 ng/mL n.v. 10 ng/mL), ACTH 250 pg/mL (n.v. 8–53), cortisol 42 µg/L (n.v. 60–230), and DHEAS 1970 mcg/L (560–2360). Testosterone and delta4-androstenedione (delta4A) were 1.3 ng/mL (n.v. <0.4) and 18.5 ng/mL (n.v. 0.60–5.60) respectively. Renin, aldosterone and electrolytes were within normal range (as shown in Table 1).

The karyotype was 46 XY. Given the presence of adrenal insufficiency in congenital adrenal hyperplasia, a substitutive therapy with hydrocortisone at the initial dosage of 35 mg/mg/day was started. At the age of 18 days, the patient showed some signs of salt wasting with mild hyponatremia (Na = 130 mEq/L); hence fludrocortisone 0.05 mg/day and oral NaCl supplementation of 1 g/day were added to the therapy.

We decided to test the patient for HSD3B2 gene mutations in order to investigate a possible genetic cause for the presence of salt loss and incomplete masculinization in the absence of bone skeletal alterations that are characterized more frequently in POR deficiency. A high level of testosterone (1.3 ng/mL), measured on the third day of life, should reflect maternal hormonal interference.

2.1.2. Patient n.2
Patient n.2 was the older brother of patient n.1. At the time of clinical and biochemical screening, they were 2.5 years old. They were a full-term newborn (gestational age = 40 weeks), born after an uneventful pregnancy by vaginal delivery. Their weight at birth was 3110 g and their length was 51.5 cm. Their Apgar scores were 10 and 10 at 1 and 5 min, respectively. Neurological exam was normal. Neither asthenia nor other health problems were reported. Weight was 12.5 kg (−0.56 SDS), height was 89 cm (−0.39 SDS). They showed the same severe perineal hypospadias as the brother, supporting the need for surgical correction, with normal scrotal gonads of 2.5 mL bilaterally (pubertal stage G1PH1). Neonatal hyperpigmentation was referred by the parents. Hormonal data did not show adrenal insufficiency (details are reported in Table 1).
Table 1. Details regarding the hormonal tests of patient n.1 at the age of three days and patient n.2 at the age of two and a half years.

| Hormone                  | Patient n.1 Values | Patient n.2 Values | Normal Levels                                      |
|--------------------------|--------------------|--------------------|---------------------------------------------------|
| 17-OHP ng/mL             | 86                 | -                  | <10 (full term newborn)987654<2 (after 6 months)  |
| ACTH pg/mL               | 250                | 53                 | 8–53                                              |
| Cortisol µg/L            | 42                 | 157                | 60–230                                            |
| Aldosterone ng/mL        | 514                | 197                | 70–550                                            |
| PRA ng/mL/h              | 2.07               | 12.8               | 1.31–3.95                                         |
| Dihydrotestosterone pg/mL| 26                 | -                  | 300–1200                                          |
| Testosterone ng/mL       | 1.3                | <0.2               | <0.4                                              |
| Delta4androstenedione ng/mL| 18.5              | -                  | 0.60–5.60                                         |
| DHEAS mcg/L              | 1970               | 779                | 560–2360                                          |
| LH U/L                   | 1.2                | -                  | <1.3                                              |
| FSH U/L                  | 0.4                | -                  | <2                                                |
| Na mEq/L                 | 136                | 139                | 136–146                                           |
| K mEq/L                  | 5.3                | 4.6                | 3.5–5.3                                           |
| Cl mEq/L                 | 105                | -                  | 97–110                                            |
| Glucose                  | 64                 | 78                 | 70–110                                            |

17-OHP = 17 hydroxyprogesterone (defined in the abstract). ACTH = Adreno Cortico Tropic Hormone (defined in the abstract). PRA = Plasma renin activity. DHEAS = Dehydroepiandrosterone. LH = Luteinizing hormone. FSH = Follicle-stimulating hormone. Na = Sodium. K = Potassium. Cl = Chloride.

3. Materials and Methods

3.1. Hormonal Testing

Hormonal tests were realized as previously reported [18]. In particular, blood samples were taken in the morning between 08:00–09:00 h, following an overnight fast. Plasma ACTH levels (pg/mL) were measured in duplicate by immunoradiometric assay (Diasorin, Saluggia, Italy). The sensitivity of the assay was 1.2 pg/mL. The mean inter- and intra-assay coefficients of variation were 4.2 and 8.4%. Serum cortisol levels (µg/dL) were measured in duplicate by radioimmunoassay (Pantec, Torino, Italy). The sensitivity of the assay was 3.6 µg/dL. The mean inter- and intra-assay coefficients of variation were 9.2 and 5.8%, respectively. Serum 17α-OHP levels (ng/mL) were measured in duplicate by radioimmunoassay (RADIM, Pomezia, Italy). The sensitivity of the assay was 0.01 ng/mL. The mean inter- and intra-assay coefficients of variation were 9.0 and 8.9%, respectively.

3.2. DNA Sequencing

After we obtained informed consent, total genomic DNA was purified from EDTA-collected peripheral blood leukocytes using the QIAamp DNA Blood Midi Kit (Qiagen, Milan, Italy), according to the manufacturer’s instructions.

Direct sequencing by the Sanger method of HSD3B2 exons 1–4 and exon-intron splicing junction boundaries was performed (Ref. Seq.: NM_000198; NP_000189) (Table 2).

PCR reactions were treated with exonuclease I and shrimp alkaline phosphatase (ExoSAP-IT, USB Corporation, Cleveland, OH, USA) and sequence reactions were performed by ABI Big Dye Terminator (Applied Biosystems, Foster City, CA, USA) chemistry and analysed by ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).
Table 2. Oligonucleotides designed for the HSD3B2 mutation analysis.

| Primer            | Sequence                     |
|-------------------|------------------------------|
| HSD3B2_1-2_FW     | GCTCCAGTCCTCCTCCAGG          |
| HSD3B2_1-2_REV    | AGGTCACACTCCCCACACCC         |
| HSD3B2_3_FW       | GGAATGTGTAACAATTCACTGC       |
| HSD3B2_3_REV      | TCTTCTGATCCTCATTTAACCAA      |
| HSD3B2_4_FW       | CATGCTGGTGACGCTCTTT          |
| HSD3B2_4_REV      | GAAGAAGACATAAGTTGGG          |
| HSD3B2_4INT_FW *  | ACCTTTGACACTTTGTC            |
| HSD3B2_4INT_REV * | TGTTGCGCTTGAAGGG             |

* Internal primers for sequencing. FW = forward. REV = reverse.

Sequence data were analysed using Mutation Surveyor DNA variant analysis software (Softgenetic, State College, PA, USA).

3.3. In Silico Analysis

The following databases and integrated tools were used to predict the effect of the two mutations:
- Alamut v1.4 (http://www.interactive-biosoftware.com (accessed on 8 June 2022));
- Varsome 11.3 (https://varsome.com/ (accessed on 8 June 2022));
- Franklin by Geeox (https://help.genoox.com/en/collections/2077313-franklin-variant-interpretation (accessed on 8 June 2022)).

With respect to the missense mutation, the evaluation of the risk of pathogenicity was thus the result of the following individual tools integration:
- MetaLR;
- REVEL;
- DEOGEN2;
- FATHMM;
- M-CAP;
- MVP;
- MutPred;
- Mutation assessor;
- PROVEAN;
- SIFT4G;
- PolyPhen2.

With respect to the intronic mutation, the integrated tools included:
- SplitSiteFinder;
- MaxEntScan;
- NNSPLICE;
- GeneSplicer.

4. Results

Sequencing of the HSD3B2 gene showed that Patient n.1 and their brother (Patient n.2) carried two mutations that have never been previously described: c.370A>G p.Ser124Gly and c.308-6 G>A (Figure 1a–d). Conversely, both parents were clinically healthy and heterozygous carriers of the mutations; the father carried the missense mutation, while the mother carried the intronic mutation (Figure 1e,f).
4. Results

Sequencing of the HSD3B2 gene showed that Patient n.1 and their brother (Patient n.2) carried two mutations that have never been previously described: c.370A>G p.Ser124Gly and c.308-6 G>A (Figure 1a–d). Conversely, both parents were clinically healthy and heterozygous carriers of the mutations; the father carried the missense mutation, while the mother carried the intronic mutation (Figure 1e,f).

Figure 1. Sequences from Mutation Surveyor v3.30 reporting the mutations found in Patient n.1, Patient n.2 and their parents. (a) Patient n.1 missense mutation HSD3B2 c.370 A>G. (b) Patient n.1 intronic mutation HSD3B2 c.308-6 G>A. (c) Patient n.2 missense mutation HSD3B2 c.370 A>G. (d) Patient n.2 intronic mutation HSD3B2 c.308-6 G>A. (e) Father’s missense mutation HSD3B2 c.370 A>G. (f) Mother’s intronic mutation HSD3B2 c.308-6 G>A.

4.1. HSD3B2 c.370 A>G p.Ser124Gly

The missense mutation on exon 4 c.370 A>G, leading to serine substitution in position 124 with a glycine (p.Ser124Gly), was inherited from the father and never described in the literature.

According to all three main tools used, the variant is of uncertain significance (ACMG classification) [19]:

- Alamut: variant of uncertain significance (PM1, PM2, BP4);
- Varsome: variant of uncertain significance (PM2, PP3);
- Franklin: variant of uncertain significance, likely pathogenic (PM2, PM1 e PP2).

When focusing on each predictor and meta predictor, 11 out of 15 reported the variant as being damaging/pathogenic. Table 3 summarizes the obtained results.
Table 3. A detailed summary of the predictors and meta predictors outcomes.

| Predictor                  | Outcome                      |
|----------------------------|-------------------------------|
| REVEL                      | Pathogenic (score: 0.6129)   |
| DEOGEN2                    | Damaging (score: 0.6196, 0.7482) |
| FATHMM                     | Damaging (score: −0.34)       |
| M-CAP                      | Damaging (score: 0.1732)      |
| MVP                        | Pathogenic (score: 0.9976)    |
| MutPred                    | Pathogenic (score: 0.935)     |
| Mutation assessor          |                               |
| PROVEAN                    | Damaging (score: −3.02, −3.49) |
| SIFT4G                     | Damaging (score: 0.002, 0.003) |
| PolyPhen2                  | HDivPred = probably damaging (score: 0.986)/HVarPred = possibly damaging (score: 0.898) |
| EIGEN                      | Neutral (score = −0.1586)     |
| LRT                        | Neutral (score = 0.06347)     |
| Mutation taster            | Polymorphism (score: 0.9774)  |
| PrimateAI                  | Tolerated (score: 0.3522)     |

REVEL = rare exome variant ensemble learner. DEOGEN2 = this is the name. FATHMM = Functional Analysis through Hidden Markov Models. PROVEAN = Protein Variation Effect Analyzer. SIFT4G = Sort Intolerant From Tolerant version 4G. EIGEN = this is the name. LRT = this is the name. AI = the name is “Primate AI”.

4.2. HSD3B2 c.308-6 G>A

The variant on intron 3 (c.308-6 G>A) consists of the substitution of guanine to adenine located six nucleotides upstream from exon 4 with a frequency of 0.000004 according to gnomAD and a likely pathogenic effect (PP3, PM2) according to Varsome. When analysed using Alamut™ Visual Software (Interactive Biosoftware), three models out of four mimicking the splicing predicted a change at the acceptor site 6bp downstream to the variant:

- MaxEnt: −67.1%
- NNSPLICE: −0.9%
- SSF: −100.0%

5. Discussion

We reported the medical history of two siblings attending the Pediatric Endocrinology Centre of Regina Margherita Children’s Hospital of Turin who presented with two heterozygous mutations on the HSD3B2 gene that have never been previously described in the literature. Patient n.1 presented with a more severe condition, showing both an altered adrenal hormonal profile and hyperpigmentation and perineal hypospadias. On the other hand, while hormonal tests in Patient n.2 did not show adrenal insufficiency, they still presented with severe perineal hypospadias.

The first variant found in the two siblings was inherited from the healthy father and was on exon 4 (c.370 A>G), resulting in a missense mutation leading to the change of serine in position 124 to a glycine. The in silico analysis revealed a probable damaging effect of this variant: residue p.Ser124 indeed is highly conserved among species and seems to be located in the catalytic site of the enzyme, playing a pivotal role in NAD(H) binding to its substrate [20]. The first reaction during the conversion of steroid precursors is, in fact, the oxidation of the 3β-hydroxyl group to the ketone by the dehydrogenase activity, a process during which NAD+ is reduced to NADH. The intermediate Δ5,3-ketosteroid remains bound to the enzyme with nascent NADH, and the actual presence of NADH in the cofactor-binding site leads to the activation of the Δ5-Δ4-isomerase activity [5,7].

The second variant was found on intron 3 (c.308-6 G>A) and was inherited from a healthy mother. According to the ACMG recommendation, the intronic variant is classified as a VUS with the following grade: PM2 (moderate), due to the extremely low frequency in gnomAD population databases. Nonetheless, the in silico analysis revealed a likely pathogenic effect since the substitution of a guanine to an adenine seems to form a new
dinucleotide resulting in a change in the splice acceptor site located 6bp downstream of
the variant and consequently in a frameshift or in the activation of a new cryptic site,
potentially resulting in a non-functional enzyme.

Due to the rarity of the disease, the phenotype–genotype correlation has not been fully
investigated in a large case series. Albeit, in general, the functional and biochemical data are
in agreement with the expressed phenotype, the clinical phenotype in the affected patients
is significantly heterogeneous; moreover, identical DNA variants have been found in the
HSD3B2 gene in patients showing a different clinical outcome [3,21,22]. Among others,
in a recent work by Ladjouze et al., the authors report the finding of the same mutation
c.665C>A both in a patient showing a salt-wasting form and in a patient showing a
non-salt-wasting form [21].

Also, in our case, the possibility of some residual canonical splicing that may occur at
some extent cannot be excluded.

Taking the above into account and also considering the role of other factors on gene
expression (such as epigenetic modifications and other modifying genes), the observation
of diverse clinical presentations in these two siblings sharing the same variants is not
surprising [23].

6. Conclusions

We report the presence of two novel variants on the HSD3B2 gene in the compound
heterozygous form that likely caused the 3βHSD2 deficiency with variable expressivity
in two siblings, a missense mutation on exon 4 (c.370 A>G) and an intronic mutation on
intron 3 (c.308-6 G>A), whose potential pathogenetic effect were evaluated by in-silico
analysis and need functional validation. This study contributes to a better understanding
of the molecular defects of 3β-HSD and of the phenotypic heterogeneity associated with
this enzymatic deficit.

Author Contributions: Conceptualization, E.M. and S.E.; methodology, E.M., D.T. and P.M.; software,
E.M. and A.B.; writing—original draft preparation, E.M., S.E. and D.T.; writing—review and editing,
A.B. and P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study. All
the experiments were performed to define the clinical diagnosis.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The contributing author can share data upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Lorence, M.C.; Murry, B.A.; Trant, J.M.; Mason, J.I. Human 3 beta-hydroxysteroid dehydrogenase/delta 5—4isomerase from
placenta: Expression in nonsteroidogenic cells of a protein that catalyses the dehydrogenation/isomerisation of C21 and C19
steroids. Endocrinology 1990, 126, 2493–2498. Available online: https://pubmed.ncbi.nlm.nih.gov/2139411/ (accessed on 31 May
2022). [CrossRef]

2. Lachance, Y.; Van Luu-The Labrie, C.; Simard, J.; Dumont, M.; De Launoit, Y.; Guerin, S.; Leblanc, G.; Labrie, F. Characterization
of human 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase gene and its expression in mammalian cells. J. Biol.
Chem. 1990, 265, 20469–20475. Available online: http://www.jbc.org/article/S0021925817305288/fulltext (accessed on 31 May
2022). [CrossRef]

3. Simard, J.; Ricketts, M.L.; Gingras, S.; Soucy, P.; Feltus, F.A.; Melner, M.H. Molecular biology of the 3beta-hydroxysteroid
dehydrogenase/delta5-delta4 isomerase gene family. Endocr. Rev. 2005, 26, 525–582. Available online: https://pubmed.ncbi.nlm.
nih.gov/15632317/ (accessed on 1 June 2022). [CrossRef]

4. Wang, L.; Salavaggione, E.; Pellemounter, L.; Eckloff, B.; Wieben, E.; Weinsilboum, R. Human 3beta-hydroxysteroid dehydroge-
nase types 1 and 2: Gene sequence variation and functional genomics. J. Steroid Biochem. Mol. Biol. 2007, 107, 88–99. Available
online: https://pubmed.ncbi.nlm.nih.gov/17689071/ (accessed on 1 June 2022). [CrossRef]
5. Lachance, Y.; Luu-the, V.; Verreault, H.; Dumont, M.; Rhéaume, E.; Leblanc, G.; Labrie, F. Structure of the human type II 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerases (3 beta-HSD) gene: Adrenal and gonadal specificity. DNA Cell Biol. 1991, 10, 701–711. Available online: https://pubmed.ncbi.nlm.nih.gov/1741954/ (accessed on 1 June 2022). [CrossRef]

6. Baquedano, M.S.; Guercio, G.; Costanzo, M.; Marino, R.; Rivarola, M.A.; Belgorosky, A. Mutation of HSD3B2 Gene and Fate of Dehydroepiandrosterone. Vitam. Horm. 2018, 108, 75–123. Available online: https://pubmed.ncbi.nlm.nih.gov/30029738/ (accessed on 7 June 2022).

7. Baquedano, M.S.; Ciaccio, M.; Marino, R.; Garrido, N.P.; Ramirez, P.; Maceiras, M.; Turjanski, A.; Defelipe, L.A.; Rivarola, M.A.; Belgorosky, A. A novel missense mutation in the HSD3B2 gene, underlying nonsalt-wasting congenital adrenal hyperplasia. New insight into the structure-function relationships of 3β-hydroxysteroid dehydrogenase type II. J. Clin. Endocrinol. Metab. 2015, 100, E191–E196. Available online: https://pubmed.ncbi.nlm.nih.gov/25322271/ (accessed on 7 June 2022). [CrossRef]

8. Guran, T.; Kara, C.; Yildiz, M.; Bitkin, E.C.; Haklar, G.; Lin, J.C.; Keskin, M.; Barnard, L.; Anik, A.; Catli, G.; et al. Revisiting Classical 3β-hydroxysteroid Dehydrogenase 2 Deficiency: Lessons from 31 Pediatric Cases. J. Clin. Endocrinol. Metab. 2020, 105, E1718–E1728. Available online: https://pubmed.ncbi.nlm.nih.gov/31950145/ (accessed on 7 June 2022). [CrossRef]

9. Wang, W.; Han, R.; Yang, Z.; Zheng, S.; Li, H.; Wan, Z.; Qi, Y.; Sun, S.; Ye, L.; Ning, G. Targeted gene panel sequencing for molecular diagnosis of congenital adrenal hyperplasia. J. Steroid Biochem. Mol. Biol. 2021, 211, 105899. [CrossRef]

10. Simard, J.; Moisan, A.M.; Morel, Y. Congenital adrenal hyperplasia due to 3beta-hydroxysteroid dehydrogenase/Delta(5)-Delta(4) isomerase deficiency. Semin. Reprod. Med. 2002, 20, 255–276. Available online: https://pubmed.ncbi.nlm.nih.gov/12428206/ (accessed on 8 June 2022). [CrossRef]

11. Turcu, A.; Smith, J.M.; Auchus, R.; Rainey, W.E. Adrenal androgens and androgen precursors-definition, synthesis, regulation and physiologic actions. Compr. Physiol. 2014, 4, 1369–1381. Available online: https://pubmed.ncbi.nlm.nih.gov/25428847/ (accessed on 8 June 2022).

12. Rainey, W.E.; Nakamura, Y. Regulation of the adrenal androgen biosynthesis. J. Steroid Biochem. Mol. Biol. 2008, 108, 281–286. Available online: https://pubmed.ncbi.nlm.nih.gov/17945481/ (accessed on 8 June 2022). [CrossRef]

13. Al Alawi, A.M.; Nordenstroom, A.; Falhammar, H. Clinical perspectives in congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase type 2 deficiency. Endocrone 2019, 63, 407–421. Available online: https://pubmed.ncbi.nlm.nih.gov/30719691/ (accessed on 15 June 2022). [CrossRef]

14. Podgórski, R.; Aeberisher, D.; Stompor, M.; Podgórska, D.; Mazur, A. Congenital adrenal hyperplasia: Clinical symptoms and diagnostic methods. Acta Biochim. Pol. 2018, 65, 25–33. Available online: https://pubmed.ncbi.nlm.nih.gov/29543924/ (accessed on 15 June 2022). [CrossRef]

15. Miller, W.L.; Auchus, R.J. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr. Rev. 2011, 32, 81–151. Available online: https://pubmed.ncbi.nlm.nih.gov/21051590/ (accessed on 5 July 2022). [CrossRef]

16. Baronio, F.; Ortolano, R; Menabò, S.; Cassio, A.; Baldazzi, L.; Di Natale, V.; Tonti, G.; Vestrucci, B.; Balsamo, A. 46,XX DSD due to Androgen Excess in Monogenic Disorders of Steroidogenesis: Genetic, Biochemical, and Clinical Features. Int. J. Mol. Sci. 2019, 20, 4605. Available online: https://pubmed.ncbi.nlm.nih.gov/31533537/ (accessed on 5 July 2022). [CrossRef]

17. Johannsen, T.H.; Mallet, D.; Dige-Petersen, H.; Müller, J.; Main, K.M.; Morel, Y.; Forest, M.G. Delayed diagnosis of congenital adrenal hyperplasia with salt wasting due to type II 3beta-hydroxysteroid dehydrogenase deficiency. J. Clin. Endocrinol. Metab. 2005, 90, 2076–2080. Available online: https://pubmed.ncbi.nlm.nih.gov/15671104/ (accessed on 5 July 2022). [CrossRef]

18. Einaudi, S.; Napolitano, E.; Restivo, F.; Motta, G.; Baldi, M.; Tuli, G.; Grosso, E.; Migone, N.; Menegatti, E.; Manieri, C. Genotype, phenotype and hormonal levels correlation in non-classical congenital adrenal hyperplasia. J. Endocrinol. Investig. 2011, 34, 660–664. Available online: https://pubmed.ncbi.nlm.nih.gov/21169730/ (accessed on 29 July 2022).

19. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gautier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for MoleculaPathology. Genet. Med. 2015, 17, 405–424. Available online: https://pubmed.ncbi.nlm.nih.gov/25741868/ (accessed on 22 July 2022). [CrossRef]

20. Yang, J.; Yan, R.; Roy, A.; Xu, D.; Poisson, J.; Zhang, Y. The I-TASER Suite: Protein structure and function prediction. Nat. Methods 2015, 12, 7–8. Available online: https://pubmed.ncbi.nlm.nih.gov/25549265/ (accessed on 25 July 2022). [CrossRef]

21. Rhéaume, E.; Simard, J.; Morel, Y.; Mebarki, F.; Zachmann, M.; Forest, M.G.; New, M.I.; Labrie, F. Congenital adrenal hyperplasia due to point mutations in the type II 3 beta-hydroxysteroid dehydrogenase gene. Nat. Genet. 1992, 1, 239–245. [CrossRef] [PubMed]

22. Ladjouze, A.; Donaldson, M.; Plotton, I.; Djenane, N.; Mohammeci, K.; Tardy-Guidelot, V.; Mallet, D.; Boulesnane, K.; Bouzerar, Z.; Morel, Y.; et al. Genotype, Mortality, Morbidity, and Outcomes of 3β-Hydroxysteroid Dehydrogenase Deficiency in Algeria. Front. Endocrinol. 2022, 13, 867073. [CrossRef] [PubMed]

23. Pecori Giraldi, F.; Einaudi, S.; Sesta, A.; Verna, F.; Messina, M.; Manieri, C.; Menegatti, E.; Ghizzoni, L. POR polymorphisms are associated with 21 hydroxylase deficiency. J. Endocrinol. Investig. 2021, 44, 2219–2226. [CrossRef]