A NOVEL MISSENSE MUTATION IN THE *HSD3B2* GENE, UNDERLYING NONSALT-WASTING CONGENITAL ADRENAL HYPERPLASIA. NEW INSIGHT INTO THE STRUCTURE-FUNCTION RELATIONSHIPS OF 3β-HYDROXYSTEROID DEHIDROGENASE TYPE II.

María Sonia Baquedano[1], Marta Ciaccio[1], Roxana Marino[1], Natalia Perez Garrido[1], Pablo Ramirez[1], Mercedes Maceiras[1], Adrian Turjanski[2], Lucas A. Defelipe[2], Marco A. Rivarola[1], Alicia Belgorosky[1]

1 Endocrine Service, Hospital de Pediatria Garrahan, CONICET, Buenos Aires Argentina.; 2 Biological Chemistry Department, Instituto de Química Física de los Materiales Medio Ambiente y Energia (INQUIMAE)-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Context: 3βHSD2 is a bifunctional microsomal NAD+-dependent enzyme crucial for adrenal and gonad steroid biosynthesis, converting Δ5-steroids to Δ4-steroids. 3βHSD2 deficiency is a rare cause of congenital adrenal hyperplasia caused by recessive loss-of-function HSD3B2 mutations.

Objective: The aim was to define the pathogenic consequences of a novel missense mutation in HSD3B2 gene.

Patient: We report a 7-month-old 46,XX girl referred because of precocious pubarche and postnatal clitoromegaly. Hormonal profile showed inadequate glucocorticoid levels, increased 17OHP and renin levels and very high DHEAS levels, suggestive of compensated nonsalt-losing 3βHSD2 deficiency.

Design and results: Direct sequencing revealed a novel, homozygous, pG250V HSD3B2 mutation. In vitro analysis in intact COS-7 cells, showed impaired enzymatic activity for the conversion of pregnenolone to progesterone and dehydroepiandrosterone to androstenedione (20% and 27% of WT at 6h, respectively). G250V-3βHSD2 decreased the Vmax for progesterone synthesis without affecting the Km for pregnenolone. Western blot and immunofluorescence suggested that p.G250V mutation has no effect on the expression and intracellular localization of the mutant protein. Molecular homology modeling predicted that mutant V250 affect a L239-Q251 loop next to a β-sheet structure in the NAD+-binding domain.

Conclusions: We identified a novel p.G250V mutation of HSD3B2 which causes an incomplete loss of enzymatic activity, explaining the compensated non-salt loss phenotype. In vitro and in silico experiments provided insight into the structure-function relationship of the 3βHSD2 protein suggesting the importance of L239-Q251 loop for the catalytic activity of otherwise stable 3βHSD2 enzyme.

Type II 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3βHSD2) is a bifunctional microsomal nicotinamide adenine dinucleotide $(NAD)^+$-dependent membrane-bound enzyme that catalyzes the conversion of the three principal endogenous Δ^5-steroid precursors, pregnenolone, 17α-hydroxypregnenolone (17OHPreg)

Abbreviations:

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received June 17, 2014. Accepted October 9, 2014.

and dehydroepiandrosterone (DHEA) into their respective Δ^4-ketosteroids, namely progesterone, 17α-hydroxyprogesterone (17OHP) and androstenedione (Δ^4-A), all with similar efficiency (Apparent K_m and Apparent V_{max}) (1, 2). The first reaction is the oxidation of the 3β-hydroxyl group to the ketone by dehydrogenase activity; during this process, NAD+ is reduced to NADH. The intermediate Δ^5, 3-ketosteroid remains tightly bound to the enzyme with nascent NADH, and the presence of NADH in the cofactor-binding site activates the Δ^5-Δ^4-isomerase activity, residing on the same enzyme that adapts different conformation (3, 4). Therefore, 3βHSD2 is essential for the biosynthesis of all classes of active steroid hormones including aldosterone, and cortisol in the adrenal cortex and sex steroids in the adrenals and gonads.

Recessive loss-of-function *HSD3B2* mutations cause a rare form of congenital adrenal hyperplasia (CAH) characterized by varying degrees of salt loss and incomplete masculinization in males and mild virilization or normal external genitalia in females (5). Deficiency of 3βHSD2 activity in the adrenals precludes normal aldosterone and cortisol synthesis, causing a rise in ACTH and the flooding of cortisol precursors along the Δ^5-pathway and with marked rise in DHEA and DHEAS production. In humans there are two 3βHSD genes and cognate enzymes. Type I gene (*HSD3B1*) is expressed in placenta and peripheral tissues. Type II gene (*HSD3B2*) encodes the adrenal and gonadal 3βHSD2 enzyme (6). The relatively high levels of 17OHP, Δ^4-A and testosterone are explained by normal activity of the peripheral isoenzyme 3βHSD1, capable of metabolizing elevated levels of Δ5-steroids into Δ4-steroids (7).

We are reporting the clinical phenotype, hormonal pattern and molecular studies of a novel homozygous missense (p.G250V) mutation in the *HSD3B2* gene, found in 46,XX girl with suspected diagnosis of 3βHSD2 deficiency. Functional studies argued for the pathogenicity of the mutation. Molecular homology modeling of 3βHSD2 predicted, for the first time, that the reported HSD3B2 mutations, A245P (7), R249X (8), G250V (present study) and Y253N (7) would affect the Leu239 to Gln251 loop of the protein, emphasizing the importance of this loop for the catalytic activity of otherwise stable 3βHSD2 enzyme.

Subjects and Methods

Case Report

The affected 46,XX patient is an adopted child, reportedly the result of a consanguineous relationship. She was referred to our Hospital at 7 months of age because of precocious pubarche and clitoromegaly with apparent normal external genitalia at birth. At clinical examination, she presented pubic hair Tanner stage II-III, clitoral emlargement (2.8 $\times$ 1 cm, Figure S1 in the Supplemental Data), two orifices and nonpalpable gonads, body length 66.3 cm (-0.31 SDS), weight 7800g (0.53 SDS) and advanced bone age (18 months). Ultrasonography revealed enlarged adrenal glands and presence of uterus and ovaries. Laboratory tests revealed normal serum electrolytes, low serum cortisol levels and high serum ACTH, dehydroepiandrosterone sulfate (DHEAS), 17OHP, and plasma renin levels (TABLE S1 in the Supplemental Data). Dexamethasone suppression test showed good suppression of adrenal androgens and eliminated suspicion of adrenal tumor (Supplemental TABLE S1). The patient was started on hydrocortisone (11 mg/body surface area (BSA)/d) and fludrocortisone (0.1 mg/d) therapy. During follow up adequate growth velocity and skeletal bone maturation was observed (Figure S2 in the Supplemental Data).

The study was approved by the Ethical Review Board of the Garrahan Pediatric Hospital. Written informed consent was obtained from the adoptive parents.

METHODS are described in the Supplementary appendix

Genetic analysis

The entire coding region and splice junctions of *HSD3B2* were amplified and sequenced (for details, see the Supplementary appendix)

In vitro and in silico studies of mutant 3βHSD2

We characterized the functional and structural consequences of the *HSD3B2* mutation (for detail, see the Supplementary appendix)

Results

Genetic analysis

DNA sequencing of the patient´s *HSD3B2* gene revealed a novel homozygous c.749G>T mutation in exon 4, predicting a p.G250V amino acid change (Figure S3 in the Supplemental Data). This sequence variation has not been detected in 120 independent *HSD3B2* alleles. Unfortunately, parent´s DNAs were unavailable for genetic studies.

In vitro functional analysis of mutant *G250V-3βHSD2*

To assess the effect of the mutation on 3βHSD2 expression and / or activity, COS-7 cells were transfected with either wild-type (WT) or mutant G250V-3βHSD2 generated by site directed mutagenesis. Western blot analysis confirmed similar levels of expression of both the mutant G250V and the WT 3βHSD2 protein (Figure 1A), suggesting that the mutation does not interfere with protein translation and that the enzyme stability is unaffected. No endogenous immunoreactive 3βHSD protein was detected in mock-transfected COS-7 cells with the empty pcDNA3 vector (Figure 1A). Both WT-3βHSD2 and mu-

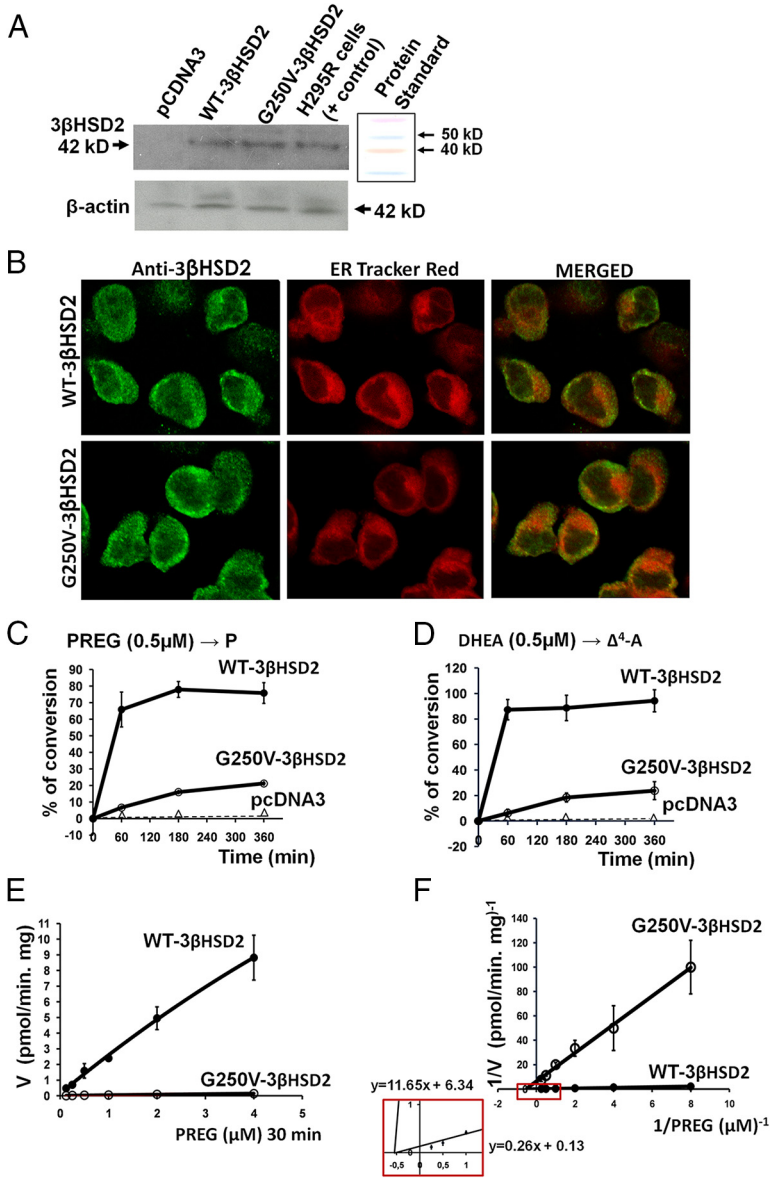

Figure 1. *In* vitro studies of mutant 3βHSD2. COS-7 cells were transiently transfected with empty pcDNA3 vector and pcDNA3 vectors carrying WT 3βHSD2 cDNA and mutant G250V-3βHSD2 cDNA, generated by site-directed mutagenesis. **A) Western blot analysis.** A 42-KDa 3βHSD2 specific-band was detectable in the homogenates from transfected COS-7 cells expressing the WT-3βHSD2 and mutant G250V-3βHSD2 proteins. The mock pcDNA3 vector alone showed no 3βHSD2 specific-band. H295R cell lysate was used as 3βHSD2 positive control. An anti β-actin antibody was used to assure equivalent protein load. **B) Laser scanning confocal microscopy of transfected COS-7 cells.** Cells were grown and incubated with ER Tracker Red (red, *middle column*) and antihuman 3βHSD2 polyclonal antiserum as primary antibody and the antirabbit Alexa Fluor 488 antibody as secondary antibody (green, *left column*). Image overlay (*right* column) shows intracellular colocalization of the endoplasmic reticulum and 3βHSD2 in COS-7 cells expressing WT-3βHSD2 and mutant G250V-3βHSD2. Scale bar = 20.0 μm. **C and D) Comparison between mutant G250V-3βHSD2 and WT-3βHSD2 enzyme activities in intact transfected COS-7 cells, based on the time course of enzymatic conversion of unsaturated concentrations of pregnenolone (0.5 μM) into progesterone (C) and DHEA (0.5 μM) into Δ^4-A (D) in the culture medium.** The control vector carrying no 3βHSD2 cDNA exhibited no enzyme activity. **E and F) Kinetic properties of the expressed mutant G250V-3βHSD2 and WT-3βHSD2 proteins in intact transfected COS-7 cells.** 48 hours after transfection, COS-7 cells were incubated with 125 nM up to 4 μM pregnenolone for 30 minutes. Under these conditions, first-order kinetics was always maintained. Data are displayed as Km plot of V (pmol/min.mg total protein in each well) against pregnenolone **(E)** and Lineweaver-Burk plot **(F),** used to obtain kinetic constants. The lines intersect the abscissa at approximately the same point **(insert)**, yielding apparent Km values of 1.96 (WT-3βHSD2) and 1.84 μM (G250V-3βHSD2) for pregnenolone. Results are given as mean ± SD for three independent experiments, each performed in triplicate. PREG, pregnenolone.

tant G250V-3βHSD2 protein colocalized with ER Tracker Red, a marker of endoplarmic reticulum (Figure 1B). This indicates that the p.G250V mutation has no effect on the intracellular localization of the mutant protein. 3βHSD2 activity was measured as Progesterone or Δ^4-A production. The time course and amount of formation of Progesterone from Pregnenolone and of Δ^4-A from DHEA in intact COS-7 cells are depicted in Figure 1C and D. G250V-3βHSD2 activity using Pregnenolone as substrate for 60, 180 and 360 minutes was 8.7% (± 2.4%), 20.5% (± 2.7%) and 32% (± 0.3%) of WT-3βHSD2, respectively. Similarly, compared to the WT-3βHSD2, the G250V-3βHSD2 mutant produced 7%–27% Δ^4-A from DHEA over the observation period of 60–360 minutes. The G250V-3βHSD2 mutant had an affinity for Pregnenolone comparable to that of WT-3βHSD2 enzyme, with apparent Michaelis constant (K_m) values of 1.84 ± 0.7 μM and 1.96 ± 0.5 μM, respectively. However, the G250V mutation showed a remarkably decreases V_{max} for the synthesis of Progesterone (0.16 ± 0.05 pmol/min.mg total proteins vs 7.58 ± 1.3 pmol/min.mg total proteins for WT-3βHSD2), which results in a catalytic efficiency (V_{max}/K_m) of about 2.3% WT (Figure 1E and F).

In silico structural analysis of the novel p.G250V mutation in 3βHSD2

To obtain a better understanding of the structure–function relationship of p.G250V mutation in 3βHSD2 we performed *in silico* protein modeling studies. The three-dimensional (3D) structure of 3βHSD2 protein were modeled using the crystal structure of galactose mutarotase/UDP-galactose 4-epimerase from Saccharomyces cerevisiae

(PDB ID 1Z45) as a template on the basis that it had the highest identity to 3βHSD (22%) and has NAD+ bound in the structure (Figure S4 in the Supplemental Data). Glycine at position 250 is located in a flexible region next to a beta sheet structure in the region of the NAD+ binding domain forming a L239 to Q251 loop (Figure 2A). An exchange of Glycine with Valine would result in a rearrangement of the loop to accommodate the more voluminous side chain group, but otherwise no major structural changes were apparent (Figure 2B, a and b).

Discussion

The present data describe the characterization of the novel misssense p.G250V mutation found in the *HSD3B2* gene of a female patient with compensated nonsalt-losing 3βHSD2 deficiency referred because of precocious pubarche and postnatal clitoromegaly at 7 months of age. Deficiency of 3βHSD2 activity in the gonads precludes normal testosterone synthesis in male fetuses, explaining undervirilization of males (9). The reason for no or lesser virilization of females compared to girls with 21-hydroxylase deficiency (21-OHD) is less clear. Recently, postnatal activity of the backdoor pathway to DHT in patients with 21-OHD was demonstrated and it has been suggested that the backdoor pathway is the major source of virilizing androgens in 21-OHD (10). In this line, this alternate pathway could not be activated in pre- and postnatal 3βHSD2 deficiency due to very low intraadrenal 17OHP substrate level explaining almost normal sexual differentiation at birth in our patient, as it has been previously suggested (11).

Biochemical data for the patient suggested a subclinical salt loss probably compensated by a limited capacity of aldosterone biosynthesis at the expense of high rennin synthesis (Supplemental TABLE S1). In 3βHSD2 deficiency, the severity of salt wasting usually shows good correlation with the in vitro enzymatic activity (6, 12). Accordingly, our in vitro data confirmed the partial 3βHSD2 inactivation by p.G250V for both the conversion of Pregnenolone and DHEA to Progesterone and Δ^4-A, respectively. Although protein instability was reported to be one factor that may lead to reduced enzyme activity (12, 13), it seemed apparent by Western blot analysis that p.G250V mutation does not impaired protein expression. This finding was substantiated by immunofluorescent analysis in COS-7 cells where colocalization of endoplasmic reticulum and mutant protein was observed, indicating the correct intracellular expression.

Because the crystal structure of 3βHSD has not been determined, a 3D model structure of the 3βHSD2 was generated by the comparative modeling technique to study the potential impact of p.G250V mutation on 3βHSD2 structure. Like other published molecular models for 3βHSD2, galactose mutarotase/UDP-galactose 4-epimerase from Saccharomyces cerevisiae (PDB ID 1Z45) was used as template (14). Comparative modeling could confirm that 3βHSD2 contained a protein folding pattern of a Rossmann-fold cofactor-binding domain common to NAD (P)-binding proteins (15). Thus, the overall fold of the model would be correct and the cofactor-binding domain of the model would be reliable. However, a comparison of the substrate binding domain is difficult because the substrate specificity is highly variable among protein family members. The Glycine residue at position 250 of the 3βHSD2 protein is highly conserved across species (Figure 2C). Our 3D model analysis predicted that G250 forms a flexible loop next to a beta sheet structure in the NAD+ binding Rossmann-fold domain and hence, the mutant residue V250 in the 3βHSD2 protein does not obviously interfere with the ligand binding. According to this, WT and G250V mutant proteins had a similar K_m, indicating similar pregnenolone affinities. However, the V_{max} of the G250V mutant was significantly impaired suggesting a more complex conformational change in the mutant protein which would result in a significantly reduced catalytic efficiency. Nevertheless, determining the actual crystal structure of 3βHSD2 would greatly help to understand the structural biology of the enzyme, including the ligand binding domain. With the amino acid residue of the present study, four amino acid residues (A245, R249, G250 and Y253) involved in the L239 to Q251 loop in 3βHSD2 structure have been reported to have mutations that affect the enzymatic activity (7, 8). Although Y253 does not form the loop structure itself, it could lose the interaction when mutated (Figure 2A). A complete loss-of-function missense mutation, p.Y253N, was found in a male neonate with classic salt-losing disorder as a compound heterozygote with the p.T187HfsX202 mutation (7, 16). The missense mutation p.A245P has been identified in a 4-year-old boy with the non salt-losing form of 3βHSD2 deficiency (7, 17) consistent with partial enzyme activity as demonstrated by in vitro assays (7). Similar to our observation for the p.G250V mutation, Simard et al (7) showed, by immunoblot analysis, that the amount of mutant recombinant 3βHSD2 p.A245P and p.Y253N proteins was similar to that of the WT-3βHSD2 after transient expression in COS-1 cells. These findings suggest that this region would have an important role in the enzyme function without impairing protein stability. We hypothesize that the p.G250V mutation might affect the NADH-induced conformational change needed to activate the isomerase activity (3, 4). The nonsense mutation

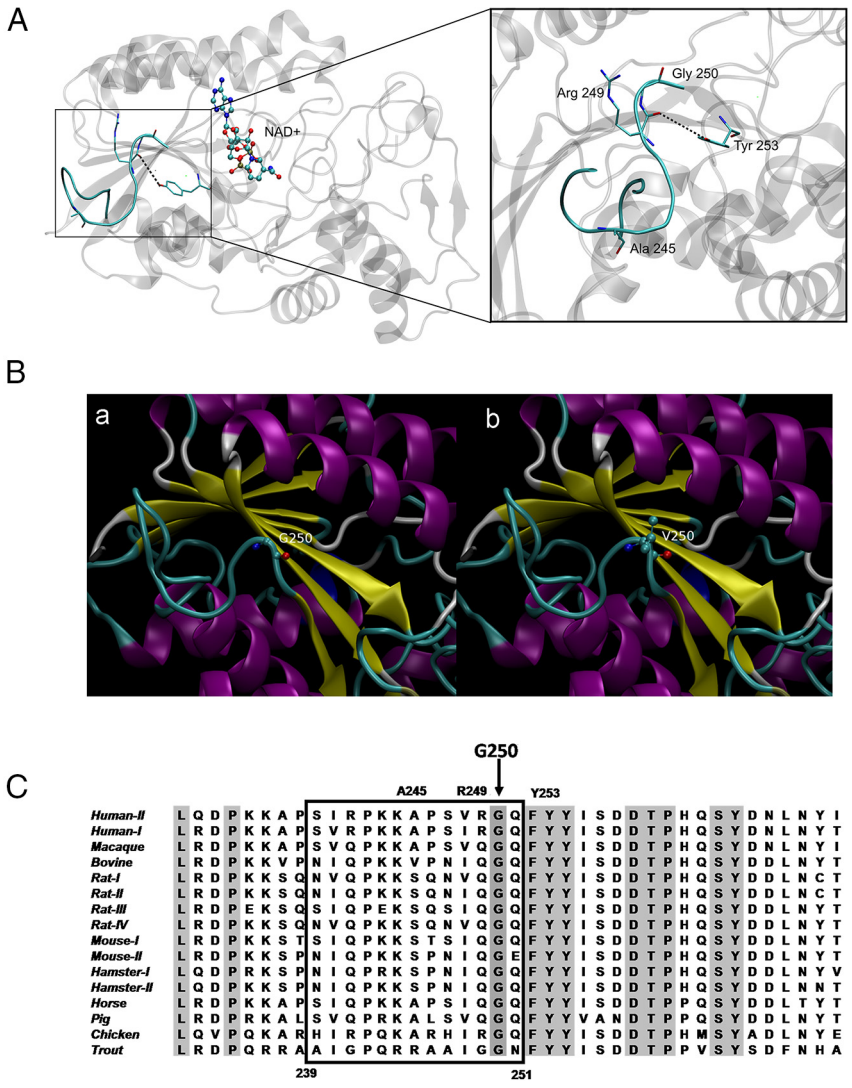

Figure 2. *In silico* structural analysis of the p.G250V mutation in 3βHSD2. A) Model of 3βHSD2 protein showing the L239–Q251 loop next to a beta sheet structure in the NAD+ binding Rossmann-fold domain. In the closeup view of the site the locations of reported amino acid residues at the L239–Q251 loop whose substitution causes mutations that affect 3βHSD2 enzymatic activity are depicted. A245P, R249X and G250V (present study) form the loop structure and Y253N may lose the interaction when mutated. **B) A closeup of both G250 (a) and mutant V250 (b) at the L239–Q251 loop demonstrating the more prominent side-chain group of the mutant valine as compared to the side-chain group Glycine. C) A partial sequencing alignment of human 3βHSD2 amino acid sequence with members of the 3βHSD gene family.** Different orthologous and paralogous members are specified in the left side of the protein sequences. The numbers indicated refer to the human type II sequence. The amino acid residues forming the L239–Q251 loop are boxed. Conserved residues among 3βHSD gene family proteins are shaded. The amino acid residue G250 is very well conserved across species. The amino acid residues at the L239–Q251 loop whose substitution causes mutations associated with 3βHSD2 deficiency are depicted.

R249X described in a homozygous male patient with the salt-losing form is predicted to result in a complete loss of 3βHSD2 activity due to a premature truncation of the protein (8), but protein stability was not analyzed.

In summary, we report a female patient with compensated nonsalt-losing 3βHSD2 and nearly normal external genitalia associated to a previously undescribed missense p.G250V mutation of the *HSD3B2* gene in the NAD+ binding Rossmann-fold domain. In vitro and *in silico* experiments argued for its functional impact and also provided insight into the structure-function relationship of the 3βHSD2 protein. Finally, this clinical case represents an experimental model of nature that reinforces the concept of the significance of the intrauterus activation of adrenal backdoor pathway for the virilization of the external genitalia in the female fetus.

Acknowledgments

Address all correspondence and requests for reprints to: Alicia Belgorosky, MD, PhD, Research Career Award, Principal Investigator National Research Council of Argentina Chairperson of Endocrine Service Hospital de Pediatria Garrahan Ciudad Autonoma de Buenos Aires Pozos 1881 (1245), Argentina, Phone: +5411 4308 0034, FAX: +5411 4308 5325. Email: abelgo@netizen.com.ar.

Disclosure Summary: All authors have nothing to declare.

This work was supported by This study was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Fondo para la Investigación Científica y Tecnológica (FONCYT).

References

1. **Lee TC1, Miller WL, Auchus RJ.** Medroxyprogesterone acetate and dexamethasone are competitive inhibitors of different human steroidogenic enzymes. *J Clin Endocrinol Metab*. 1999;84:2104–2110.
2. **Rhéaume E1, Lachance Y, Zhao HF, Breton N, Dumont M, de Launoit Y, Trudel C, Luu-The V, Simard J, Labrie F.** Structure and expression of a new complementary DNA encoding the almost exclusive 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase in human adrenals and gonads. *Mol Endocrinol*. 1991;5:1147–1157.
3. **Thomas JL, Frieden C, Nash WE, Strickler RC.** An NADH-induced conformational change that mediates the sequential 3β-hydroxysteroid dehydrogenase/isomerase activities is supported by affinity labeling and the time-dependent activation of isomerase. *J Biol Chem*. 1995;270:21003–21008.
4. **Thomas JL, Boswell EL, Scaccia LA, Pletnev V, Umland TC.** Iden-

tification of key amino acids responsible for the substantiality higher affinities type 1 3β-hydroxysteroid dehydrogenase/ isomerase (3βHSD1) for substrates, coenzymes and inhibitors relative to 3βHSD2. *J Biol Chem*. 2005;280:21321–21328.

5. **Miller WL, Auchus RJ.** The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*. 2011;32:81–151.
6. **Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH.** Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. *Endocr Rev*. 2005;26:525–82.
7. **Simard J, Rheaume E, Sanchez R, Laflamme N, de Launoit Y, Luu-The V, Van Seters AP, Gordon RD, Bettendorf M, Heinrich U, Moshang T, New M-I, Labrie F.** Molecular basis of congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency. *Mol Endocrinol*. 1993;7:716–728.
8. **Tajima T, Fujieda K, Nakae J, Shinohara N, Yoshimoto M, Baba T, Kinoshita E, Igarashi Y, Oomura T.** Molecular analysis of type II 3β-hydroxysteroid dehydrogenase gene in Japanese patients with classical 3β-hydroxysteroid dehydrogenase deficiency. *Hum Mol Genet*. 1995;4:969–971.
9. **Pang S.** The molecular and clinical spectrum of 3β-hydroxysteroid dehydrogenase deficiency disorder. *Trends Endocrinol Metab*. 1998;9:82–86.
10. **Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA.** Increased activation of the alternative "backdoor" pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab*. 2012;97:E367–375.
11. **Auchus RJ, Chang AY.** 46,XX DSD: the masculinised female. *Best Pract Res Clin Endocrinol Metab*. 2010;24:219–242.
12. **Moisan AM, Ricketts ML, Tardy V, Desrochers M, Mebarki F, Chaussain JL, Cabrol S, Raux-Demay MC, Forest MG, Sippell WG, Peter M, Morel Y, Simard J.** New insight into the molecular basis of 3β-hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene eleven patients from seven new families and comparison of the functional properties of twenty five mutant enzymes. *J Clin Endocrinol Metab*. 1999;84:4410–4425.
13. **Pang S, Wang W, Rich B, David R, Chang YT, Carbunaru G, Myers SE, Howie AF, Smillie KJ, Mason JI.** A novel nonstop mutation in the stop codon and a novel missense mutation in the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) gene causing, respectively, nonclassic and classic 3β-HSD deficiency congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2002;87:2556–2563.
14. **Rabbani B, Mahdieh N, Haghi Ashtiani MT, Setoodeh A, Rabbani A.** In silico structural, functional and pathogenicity evaluation of a novel mutation: an overview of HSD3B2 gene mutations. *Gene*. 2012;503:215–221.
15. **Thomas JL, Duax WL, Addlagatta A, Brandt S, Fuller RR, Norris W.** Structure/function relationships responsible for coenzyme specificity and the isomerase activity of human type 1 3 beta-hydroxysteroid dehydrogenase/isomerase. *J Biol Chem*. 2003;278:35483–35490.
16. **Wolthers BG, Volmer M, van Seters AP.** Detection of 3β-hydroxysteroid-dehydrogenase deficiency by urinary steroid profiling: solvolysis of urinary samples should be a necessary prerequisite. *Clin Chim Acta*. 1985;145:319–323.
17. **Heinrich UE, Bettendorf M, Vecsei P.** Male pseudohermaphroditism caused by nonsalt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase (3βHSD) deficiency. *JSteroid Biochem Mol Biol*. 1993;45:83–85.