Biochemical and Molecular Modeling Analyses Explain the Functional Loss of 17β-HSD3 Mutant G133R in Three Tunisian Patients

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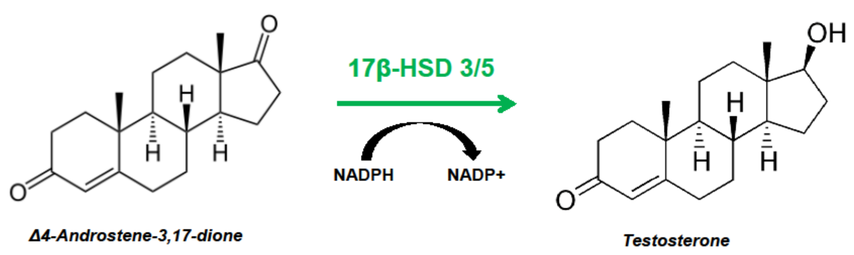
Introduction

17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3) catalyzes the conversion of Δ4-androstene-3,17-dione to testosterone in testicular Leydig cells and has a key role in male sexual development[1]. Mutations in the *HSD17B3* gene can result in reduced enzyme activity and decreased testosterone synthesis, leading to a rare autosomal recessive disease named 46, XY disorder of sex development (46, XY DSD)[2].



Patients with 46, XY DSD show undervirilization of external genitalia, which often appear female[3]. They are usually raised as females until a virilization occurs at puberty due to extra testicular testosterone synthesis[4] by the enzyme 17β-HSD5, which is not expressed in early development.

We characterized three Tunisian patients from non-consanguineous families with 46, XY DSD and investigated 17β-HSD3 deficiency.

Patients Clinical History

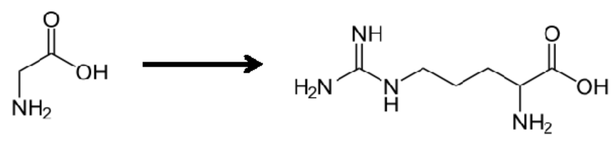
Genomic DNA from patient 1 was directly analyzed for mutations in the *HSD17B3* gene by DNA sequencing because of a familial history, recording a paternal cousin with *HSD17B3* deficiency. For the patients P2 and P3 a human chorionic gonadotropin stimulation test was performed, due to signs of virilization observed at puberty and the absence of a complete hormonal profile. The results revealed 17β-HSD3 deficiency.

	Patient 1 (P1)	Patient 2 (P2)	Patient 3 (P3)
Age (years)	7	14	
Height cm	155	-	-
Weight Kg	45	-	-
Tanner stage	P1B1	P4B1	P5B1
Testosterone ng/ml	0.8	3	4
LH, IU/L	12.5	-	-
FSH, IU/L	4.7	40	42
Caryotype analysis	46, XY	46, XY	46, XY

References values: LH: 1.24-8.62 mUI/ml; FSH: 1.27-19.26 mUI/ml; T: 1.75 – 7.61 ng/mL

Genetic Analysis

Genetic analysis of the *HSD17B3* gene revealed two compound heterozygous mutations, *i.e.* a novel missense mutation (**G133R**) and a premature stop codon (**C206X**).

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17bHSD1      68 RDSKSVAAARERVT-EGR----VDVLVCNAG-LGLLGPL-EALGE
17bHSD2     140 TKPVQIKDAYSKVA-AMLQDRGLWAVINNAGVLGFPTDG-ELLLM
17bHSD3     107 FTKDDIYEHIKEKL-AGLEIG---ILVNNVGMLPNLLPS-HFLNA
17bHSD4      71 ANYDSVEEGEKVVKTALDAFGRIDVVVNNAG---ILRDR-SFARI
17bHSD6      85 TKMESIAAATQWVK-EHVGDRGLWGLVNNAGILTPITLC-EWLNT
17bHSD7      65 SNLQSVFRASKELK---QRFQRLDCIYLNAGIMPNPQLNIKALFF
17bHSD8      77 SEARAARCLLEQVQ-ACFSRP-PSVVVSCAG----ITQD-EFLLH
17bHSD9      84 TDPQSVQQAAKWVE-MHVKEAGLFGLVNNAGVAGIIGPT-PWLTR
17bHSD10     66 TSEKDVQTALALAK---GKFGRVDVAVNCAGIAVASKTYNLKKGQ
17bHSD11     95 SNREDIYSSAKKVK-A--EIGDVSILVNNAG---VVYTS-DLFAT
17bHSD12    109 FASEDIYDKIKTGL-AGLEIG---ILVNNVGMS-YEYPE-YFLDV
17bHSD13     70 -----------KVK-K--EVGDVTIVVNNAG---TVYPA-DLLST
17bHSD14     64 TQEDDVKT---LVSETIRRFGRLDCVVNNAGHHPPPQRP-----E
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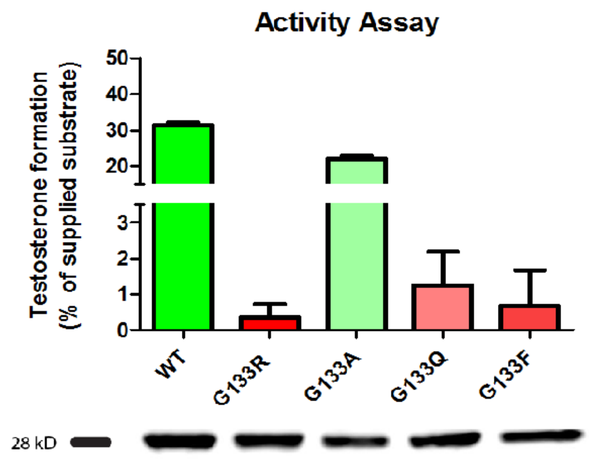
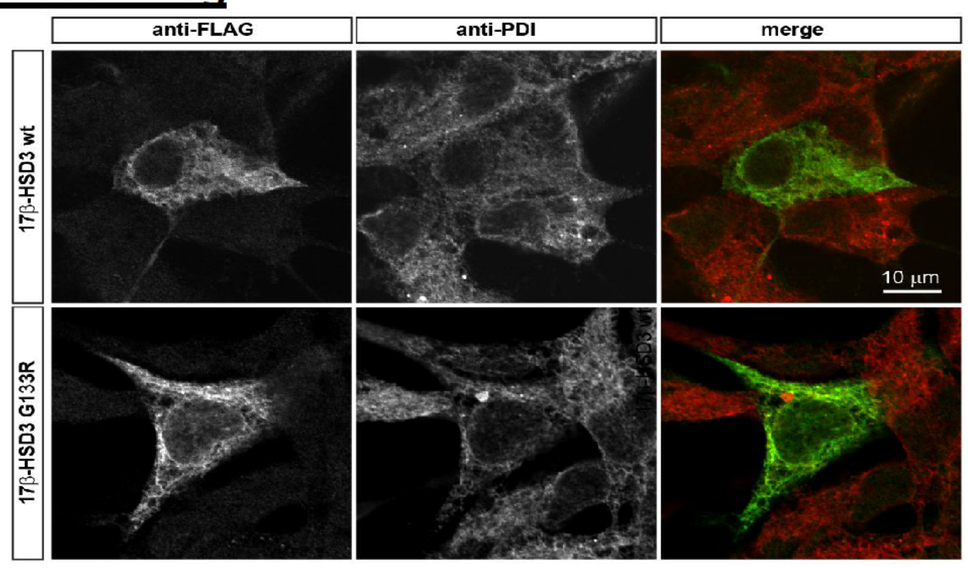
Alignment of related 17β-HSD's showed that the residue G133 and prior amino acids are highly conserved among the subfamily of short chain dehydrogenase/reductase.

Activity Assay

Using site-directed mutagenesis, an expression plasmid for 17β-HSD3 G133R was constructed. Additionally, expression plasmids for substitutions of glycine 133 to alanine (G133A), to phenylalanine (G133F), and to glutamine (G133Q) were created. Wild-type and mutant enzymes were expressed in HEK-293 cells, followed by assessment of the conversion of radiolabeled Δ4-androstene-3,17-dione to testosterone. Mutants G133R, G133Q and G133F were almost completely inactive, whereas G133A retained more than 80% of wild-type activity. Western blot analysis showed comparable expression of all mutants.

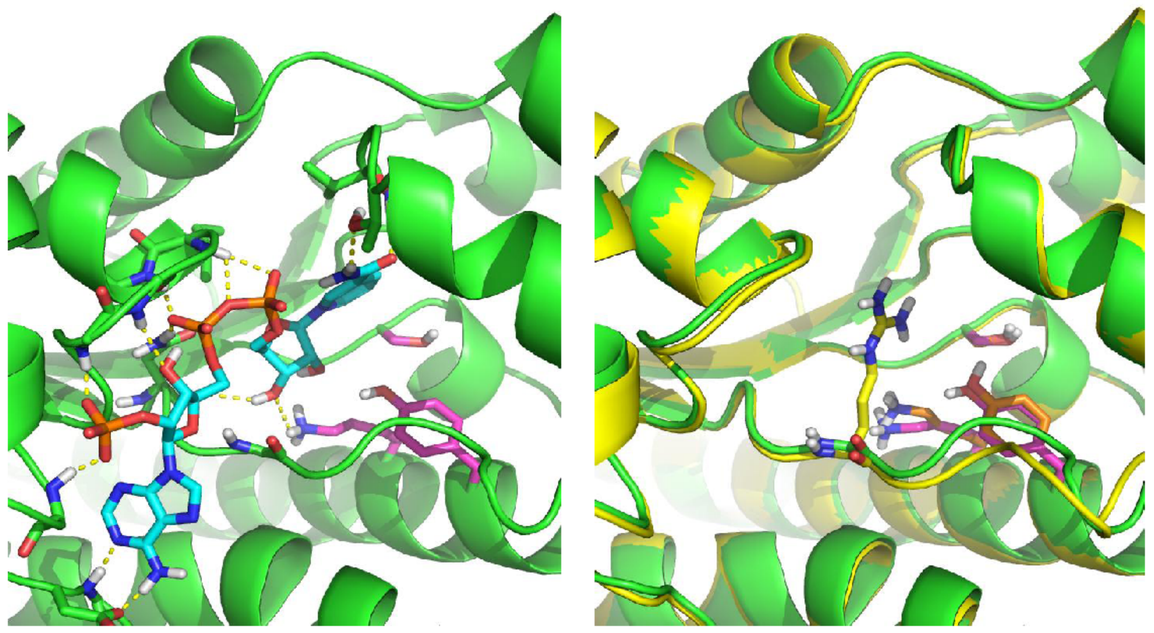


Immunostaining



Immunostaining results of 17β-HSD3-FLAG and 17β-HSD3 G133R-FLAG confirmed the expression of both enzymes at the endoplasmic reticulum membrane. No indication of dislocation nor degradation was evident.

Molecular Modeling Prediction



A homology model of 17β-HSD3 predicted that the loss of activity is due to a disruption of the cofactor binding site. While an alanine at position 133 was still tolerated, more bulky side-chains led to steric hindrance thus preventing cofactor binding.

Conclusion

We characterized three Tunisian patients from non-consanguineous families with 46, XY DSD due to 17β-HSD3 deficiency. Genetic analysis of the *HSD17B3* gene revealed two compound heterozygous mutations, *i.e.* a novel missense mutation (**G133R**) and a premature stop codon (**C206X**). In activity measurements, mutants **G133R**, G133Q, and G133F were almost completely inactive, whereas G133A retained >80% of wild-type activity. A homology model of 17β-HSD3 predicted that the loss of activity is due to a disruption of the cofactor binding site. While an alanine at position 133 was still tolerated, more bulky side-chains led to steric hindrance thus preventing cofactor binding. The functional analysis and homology modeling revealed an important role of this residue in the structural arrangement of the cofactor binding pocket. The results provide an improved mechanistic understanding of the 17β-HSD3 structure-function relationship and explained the 17β-HSD3 deficiency observed in the patients.

Acknowledgement

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Steroids, developmental and paediatric endocrinology

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