

## Poster D-12

### MOLECULAR HOMOLOGOUS MODELING OF 3B-HSD2 MUTANT ENZYME: STRUCTURE-FUNCTION ASPECTS OF PRO222GLN MUTATION CORRELATES WITH THE EXPERIMENTAL DATA FROM A PATIENT WITH CONGENITAL ADRENAL HYPERPLASIA.



#### Authors:

LUCIO FABIO CALDAS FERRAZ (*CBMEG, UNICAMP*)  
FERNANDA SOARDI (*CBMEG, UNICAMP*)  
PAULA FALCÃO (*EMBRAPA*)  
GORAN NESHICH (*EMBRAPA*)  
MARICILDA PALANDI DE MELLO (*CBMEG, UNICAMP*)

**Short Abstract:** We describe the mutation Pro222Gln in HSD3B2 gene on a patient with congenital adrenal hyperplasia. Molecular modeling of 3B-HSD2 mutant allowed us to identify critical role of residue Pro222 on the folding pattern and catalytic activity of the enzyme. The proposed models correlate with the experimental data previously reported.

#### Long Abstract:

Type II 3B-hydroxysteroid dehydrogenase/D5-D4-isomerase (3B-HSD2), encoded by the HSD3B2 gene, is a key enzyme involved in the biosynthesis of steroid hormones. Inherited deleterious mutations on this gene cause the classical deficiency of 3B-HSD2, an autosomal recessive inherited form of congenital adrenal hyperplasia (CAH) that impairs steroidogenesis in the adrenals and gonads. In the present study we described the clinical and molecular characterization of a male patient diagnosed to be suffering from classical form of CAH due to 3B-HSD2 deficiency. Patient's genomic DNA was isolated from blood leukocytes and used to PCR amplified the coding region of HSD3B2 gene. PCR products were sequenced in both orientations using ABI PRISM 377 Automated DNA Sequencer. Sequencing revealed the homozygous missense mutation Pro222Gln in exon 4. Molecular homologous modeling of the 3B-HSD2 mutant enzyme was performed to obtain a better understanding of the effect of this mutation on the enzyme activity. The three-dimensional structure of both mutant and native 3B-HSD2 were modeled using the crystal structure of UDP-galactose-4-epimerase from *Escherichia coli* (Protein Data Bank code 1A9Z) as a template. The models were created and validated by using the default settings and parameters of the Modeller 8v0 web-served program. The images were produced and analyzed by using the web-based program Diamond STING. The Brazilian male patient described here harbors the Pro222Gln mutation and presents typical clinical features of CAH due to 3B-HSD2 deficiency. The missense mutation Pro222Gln has already been described and leads to a complete loss of 3B-HSD2 activity, which is in agreement with the severe form of CAH observed on the present patient. Two different missense mutations have been identified on codon 222. The C>A transversion in the first nucleotide of codon 222 leads to the amino acid replacement from proline (CCA) to threonine (ACA). Alternatively, the C>A transversion in the second nucleotide of codon 222 converts codon CCA, encoding

proline, to CAA, encoding a glutamine. Both mutations on Pro222 residue abolish severely the 3B-HSD2 activity. In fact, the importance of this residue is evidenced by the fact that Pro222 residue is predicted to be in the membrane-spanning domains, thus suggesting a putative role on the ultimate folding pattern of the enzyme. In addition, besides being highly conserved on that particular position, Pro222 residue is located adjacent to the substrate binding domains. Although P222Q and P222T mutations render no detectable enzyme activity, they probably do so by different manners. The P222Q enzyme shows no evidence of protein instability. Thus, the absence of enzymatic activity may be due to alterations on the catalytic activity of the enzyme, such as disruption of the substrate binding domain introduced by the presence of glutamine on codon 222. On the other hand, P222T enzyme shows no detectable level on Western blottings despite mRNA detection, thus suggesting being a totally unstable protein. This severe instability of the enzyme caused by the presence of threonine on codon 222 seems to be the detrimental mechanism that leads to the profoundly decreased 3B-HSD2 activity. The consequence of amino acid replacements from Proline222 to glutamine or threonine can be inferred according to the distinct biochemical properties of those amino acids and their putative interactions with surrounding residues. Proline has nonpolar side-chain and is hydrophobic, tending to cluster with hydrophobic residues inside the proteins. Also, proline has significant effect upon the geometry of the backbone chain. On the other hand, glutamine and threonine have uncharged polar side chains and are relatively hydrophilic, clustering on the outside of proteins. To obtain valuable information concerning structure-function relationship of the 3B-HSD2 mutant enzymes we attempted to characterize the functional significance of Pro222Gln mutation on the enzyme activity by molecular modeling the P222Q enzyme. Homology modeling of 3B-HSD2 has already been achieved by other authors. Human 3B-HSD2 is a member of the short-chain dehydrogenase (SDR) superfamily which has 30% overall sequence identity with *E. coli* UDP-galactose-4-epimerase, another SDR for which the X-ray crystal structure determination has been reported. Based on this sequence identity, a 3D ribbon model of human 3B-HSD2 has been constructed. The 3D models of the native 3B-HSD2 and Gln222 mutant enzymes allowed visualization in comparison of both proline (native enzyme) and glutamine (mutant enzyme) on residue position 222. The Pro222 residue hydrogen-bonds with amino acids Val218 and Ser228 and makes a hydrophobic interaction with Val225. Besides interactions with the above amino acids, the mutant residue Gln222 makes additional hydrophobic interactions with Asn227 and Val228 and additional hydrogen-bonds with Thr219, Val220 and Asn227. These five additional residues interactions introduced by the mutated residue Glu222 may result on new residues interactions that appear to prevent the appropriate access and/or binding of the substrate to its domain. Hence, the protein is stable but the lack of enzymatic activity may be due to the additional interactions that would disrupt the substrate binding domain. On the other hand, the mutant Thr222 residue lacks hydrophobic interactions and shows hydrogen-bonds with Val225 and Gly226. The replacement of a hydrophobic residue (proline) to a hydrophilic (threonine) plus the lack of hydrophobic interactions on codon 222 may introduce conformational rearrangements on 3B-HSD2 that seems to compromise the folding pattern of the enzyme, which would explain the Thr222 enzyme being a totally unstable protein. In summary, the molecular homology modeling of 3B-HSD2 has provided insights concerning structure-function relationship of 3B-HSD2 mutant enzymes. The proposed models emphasize the probable critical role of residue Pro222 for the folding pattern and catalytic activity of the enzyme. Indeed, residue Pro222 appears to be essential for the hydrophobic surfaces on that particular position of the enzyme and seems to perform specific residue

interaction that would be critical for the 3B-HSD2 enzyme to achieve the appropriate conformation for its catalytic activity. The findings allowed us to correlate the proposed models with the experimental and clinical data previously reported.