INSELSPITAL
UNIVERSITÄTSSPITAL BERN
HÔPITAL UNIVERSITAIRE DE BERNE

KINDERKLINIKEN Bern

u^b UNIVERSITÄT BERN

gcb Graduate School for Cellular and Biomedical Sciences

Bioinformatic and functional studies of new variants in *SRD5A2* to explain androgen excess reveal new loss of function variants

Efstathios Katharopoulos[1,2,3], Amit Pandey[1,2], Kay Sara Sauter[1], Christa E.Flück[1,2]

[1] Dept. Of Pediatrics, Division of Endocrinology, Diabetology & Metabolism, and [2]Dept. of Biomedical Research, [3]Graduate School Bern, Bern Universiy Hospital and University of Bern, Switzerland

Background

Androgens are steroid hormones necessary for human sex development. Testosterone (T), dihydrotestosterone (DHT), 11-keto-T and 11-keto-DHT are active androgens and exert their effect by activating the androgen receptor (AR)[1] (Fig.1). Steroid reductases 5α (SRD5As) catalyse the conversion of T to DHT in the classic androgen production pathway or 17OH-progesterone to 17OH-dihydroprogesterone and androstenedione to 5α-dione in alternate pathways leading to DHT syntheis. SRD5As play a pivotal role for the production of active androgens in all pathways. There are two enzymes with differential expression. SRD5A2 is expressed in reproductive organs and the liver. SRD5A1 is expressed in the periphery and the liver. Human loss-of-function mutations of *SRD5A2* are known, and cause severe 46,XY undervirilization, while gain-of-function variants have been suggested in androgen excess syndromes such as premature adrenarche, polycystic ovary syndrome or prostate tumours, but they have not been found so far.

Aim: To search for gain-of-function mutations in the human *SRD5A2* gene to explain androgen excess.

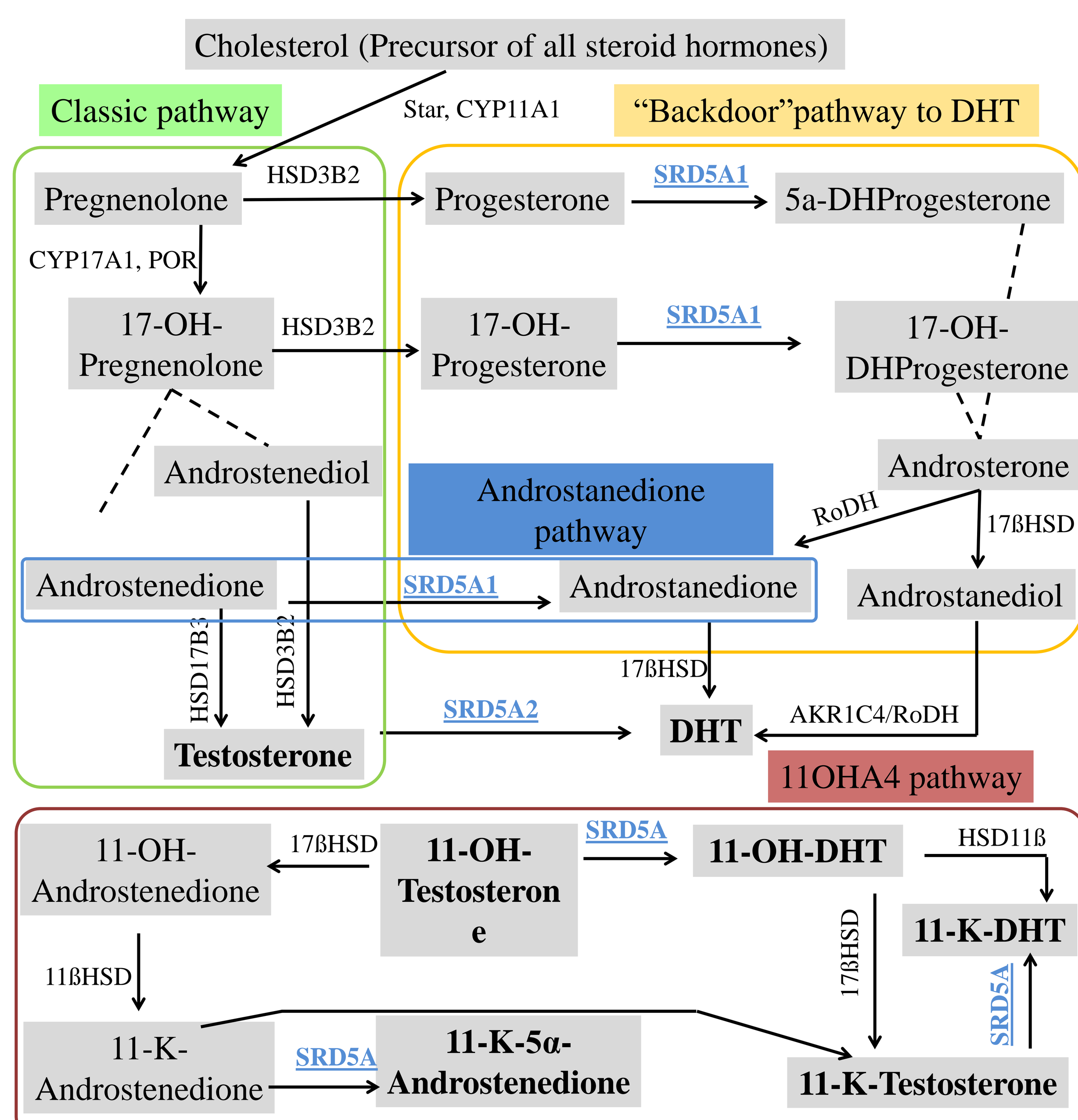

Fig.1: Biosynthetic pathways of androgens. SRD5A enzymes (highlighted in blue) are involved in classic (w/ T) and alternate (w/o T) pathways towards synthesis of active androgens (DHT, 11-keto-DHT etc).

Methods

Bioinformatic studies (Fig.3-4, Table 1)

Coding SNPs for *SRD5A2* were searched in databases (Fig.3). *SRD5A2* sequences were obtained from NCBI. Multiple sequence alignment and conservation analysis were done with CLC Main Workbench and CONSURF. The shift in the enzyme's free energy was predicted for cSNPs with SDM software[2].

As there is no SRD5A crystal structure we constructed a new 3D model based on the bacterial sterol reductase[1] protein using PSI-blast and PDB. Molecular dynamics and docking simulations studies were done with AMBER14 and AUTODOC.

Functional Studies (Fig.5, Table 2)

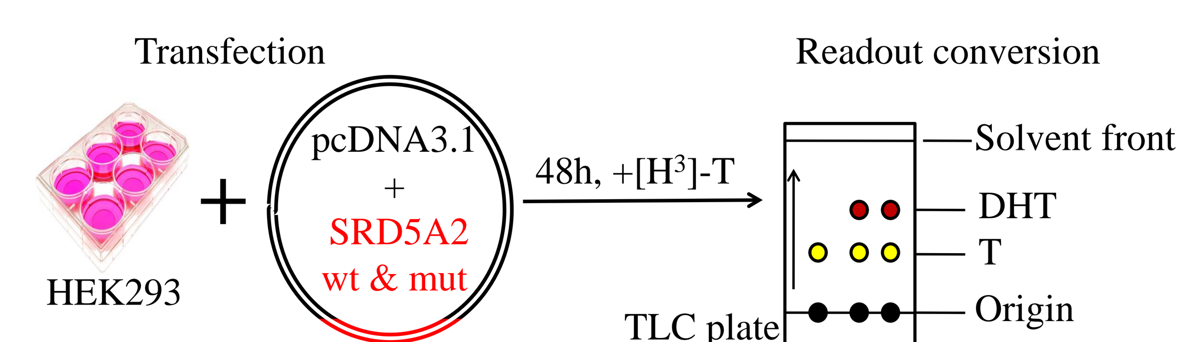

Fig.2: Scheme of experimental setup for functional studies in our cell model

Results

Step 1

Databases

n=357 cSNPS
Pubmed, OMIM, dbSNP, HGMD, Uniprot

- n=122 variants with already known phenotype associated with CaP, PCOS, DSD
- n=25 not in dbSNP
- n=122 nonsense, synonymous, frameshifts mutations
- n=19 variants previously tested

n=79 cSNPs

Step 2

Literature

- A49T gain of function (positive control)
- R227Q loss of function (negative control)

Bioinformatics

n=7 cSNPs

- n=56 physical distance far from active centre of enzyme on the 3D model (Fig.4), or residues located in less conserved regions (Fig.5)
- n=16 predicted low pseudo ΔΔG energy value from amino acid change evaluation (Table 1).
- n=2 R50A, N122A

n=9 cSNPs Functional tests

Fig.3: Novel SNPs of *SRD5A2* selected for testing by our 2-step selection method.

Table 1: Prediction of ΔΔG energy of cSNPs compared with the wt SRD5A2. The protein structure is predicted to be stabilised (positive value) or destabilised (negative value) with each amino acid change.

cSNP	Pseudo ΔΔG energy	cSNP	Pseudo ΔΔG energ
WT	n/a	N122A	+0.58
A49T	-1.27	L167S	-1.53
R50A	-0.09	R168C	-0.65
P106L	-0.58	P173S	+0.1
P106A	-0.36	R227Q	-0.04

Table 2: Apparent catalytic terms for wt and mutant SRD5A2 enzymes

SRD5A2	Km (nM)	Vmax	% of WT
Conversion of T to DHT			
WT	340 ± 36	0.28 ± 0.06	100
R50A	2282 ± 1747	0.85 ± 0.76	45
P173S	1020 ± 192	0.23 ± 0.04	28
Conversion of Prog to DHProg			
WT	164 ± 1.5	0.05 ± 0.005	100
R50A	218 ± 145	0.04 ± 0.024	60
P106L	200 ± 67	0.02 ± 0.010	41
P106A	578 ± 55	0.06 ± 0.017	38
R168C	434 ± 226	0.05 ± 0.014	37
P173S	129 ± 16	0.03 ± 0.001	85
Conversion of Δ4A to 5α-dione			
WT	150 ± 43	0.09 ± 0.017	100
R50A	82 ± 32	0.02 ± 0.009	37
P173S	634 ± 400	0.08 ± 0.04	22

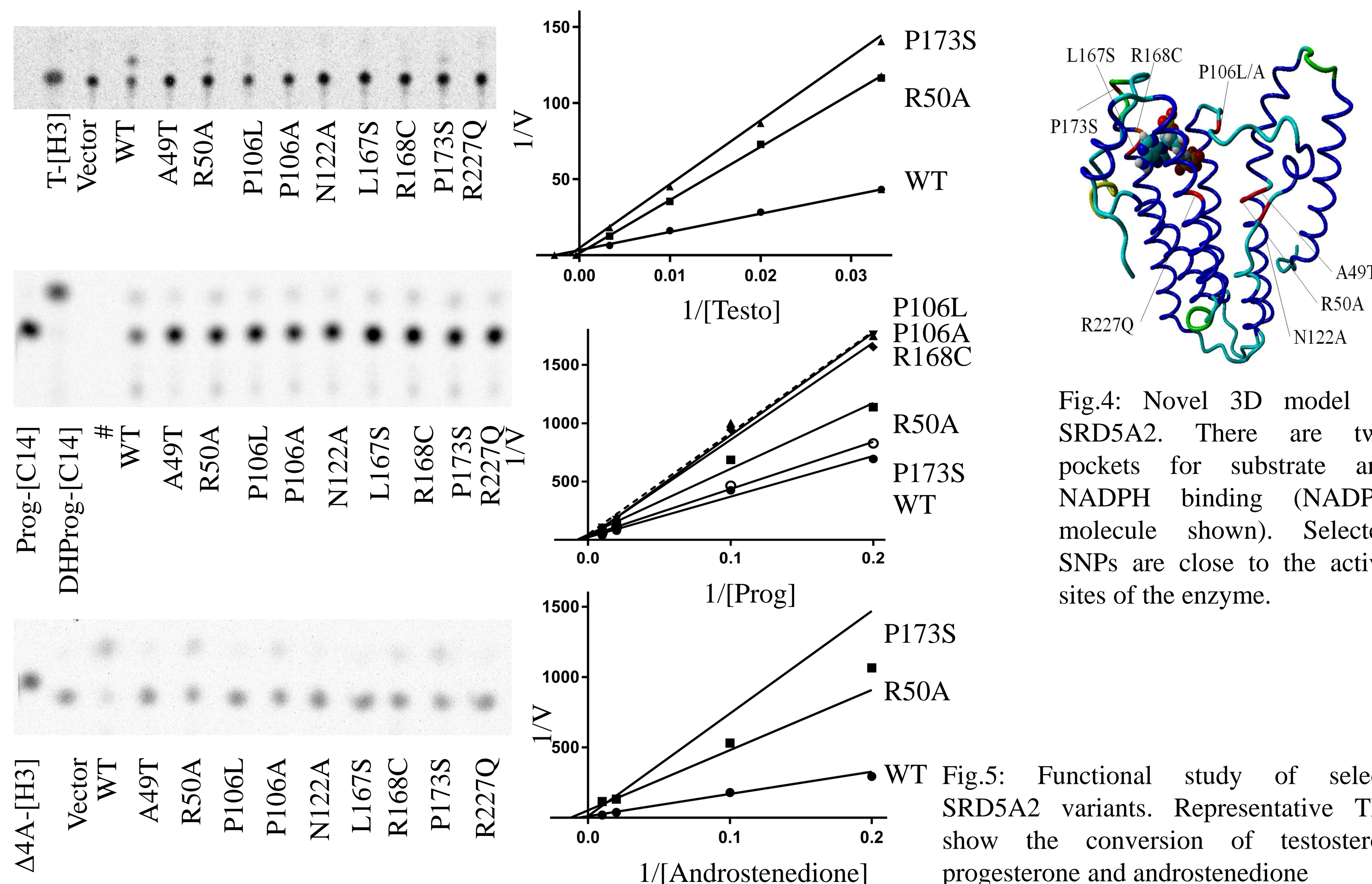

Fig.5: Functional study of selected SRD5A2 variants. Representative TLCs show the conversion of testosterone, progesterone and androstenedione

L167S R168C P106L/A P173S A49T R50A N122A R227Q

Fig.4: Novel 3D model of SRD5A2. There are two pockets for substrate and NADPH binding (NADPH molecule shown). Selected SNPs are close to the active sites of the enzyme.

Conclusions

Our selection method did not detect novel gain-of-function variants in the coding regions of the SRD5A2 gene. Maybe the expression of SRD5A2 is regulated by other means at the transcriptional or posttranslational level leading to excess reductase activity?

However, through this study we found new loss-of-function mutations that had not been characterized before. Individuals with these mutations might show a minor phenotype, which has not been attributed to their SRD5A2 variation yet.

For this work, a novel 3D model of SRD5A2 was developed and it is available for further docking studies

1. Storbeck, K. H., L. M. Bloem, et al. (2013). "11beta-Hydroxydihydrotestosterone and 11-ketodihydrotestosterone, novel C19 steroids with androgenic activity: a putative role in castration resistant prostate cancer?" Molecular and cellular endocrinology **377(1-2): 135-146.**
2.Pandurangan, A. P., Ochoa-Montano, B., Ascher, D. B. & Blundell, T. L. SDM: a server for predicting effects of mutations on protein stability. *Nucleic Acids Res* **45**, W229-W235, doi:10.1093/nar/gkx439 (2017)
3. Li, X., Roberti, R. & Blobel, G. Structure of an integral membrane sterol reductase from Methylomicrobium alcaliphilum. *Nature* **517**, 104-107, doi:10.1038/nature13797 (2015)

Financial disclosure: none

Contact:ekatharo@gmail.com

Poster presented at:

PosterSessionOnline.com