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In-silico gene-protein analysis and clinical phenotype characterisation of four novel NR5A1/SF1 gene mutations presenting with 46,XY DSD

Rieko Tadokoro Cuccaro[1], Ajay Thankamony[1], A Emile J Hendriks[1], Sabah Alvi[2], Ruth Armstrong[3], Jonathan Bruty [3], Ieuan A Hughes[1] and Carlo L Acerini[1]
1.Department of Paediatrics, University of Cambridge 2. Paediatric & Adolescent Endocrine Clinic Unit, The General Infirmary, Leeds, UK 3. East Anglian Medical Genetics Service, Cambridge

UNIVERSITY OF CAMBRIDGE

Background

Disorders of sex development (DSD) due to mutations in the *NR5A1* (SF1) gene result in a highly variable phenotype.

Objective

To report the clinical phenotype and the molecular / structural characteristics of the gene-protein product arising from four novel mutations of the NR5A1 (SF1) gene found in patients presenting with 46,XY DSD.

Methods

Phenotype determined from interrogation of clinical case notes. Interpretation of DNA-protein molecular interactions were modelled *in-silico* using PyMOL Molecular Graphic System. Mutation effect and structural analyses verified using *PROVEAN (SIFT), PolyPhen2; MutationTaster, FATHMM and SAAP* software programmes.

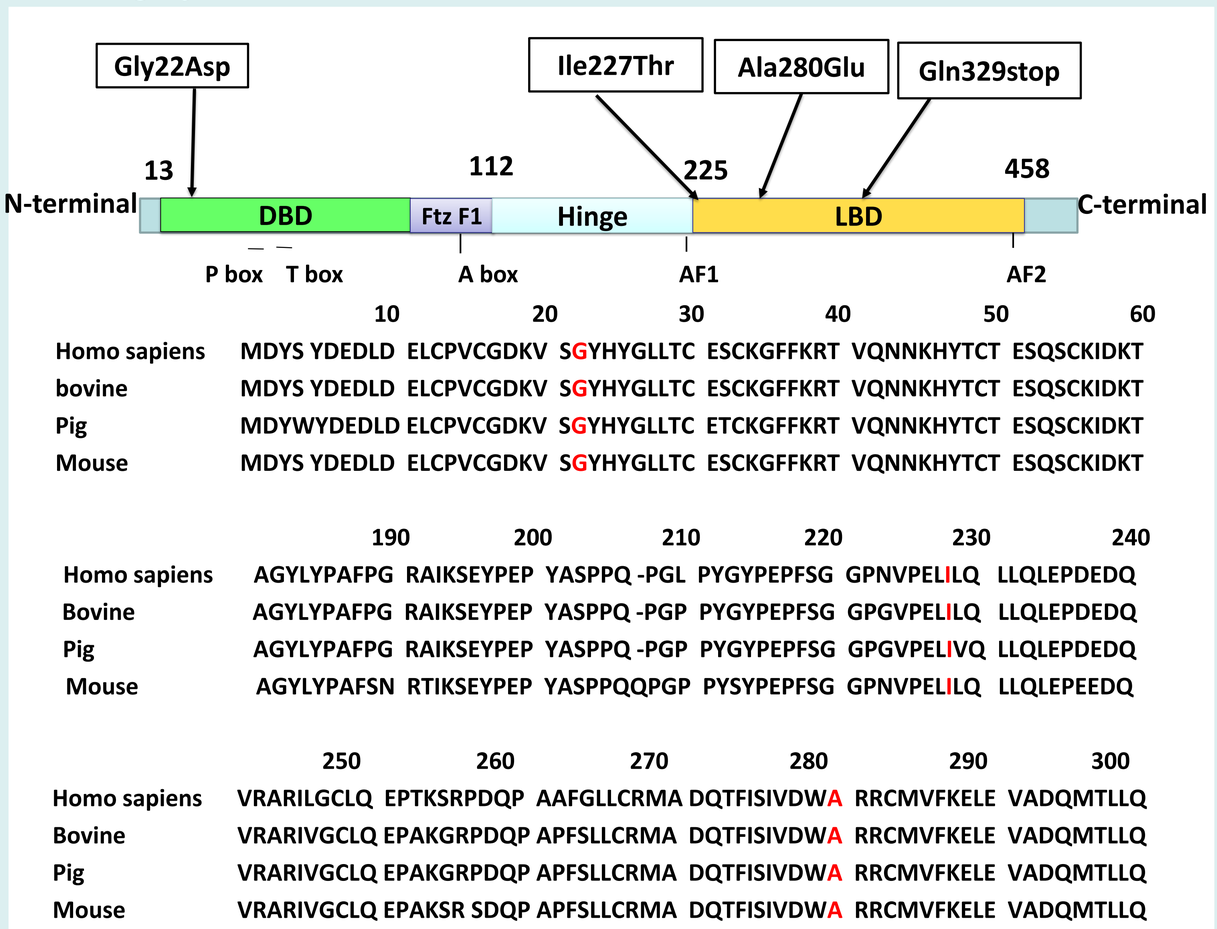
Results

Patient phenotype

	Case 1	Case 2	Case 3	Case 4
Mutation	Gln329Stop	Gly22Asp	Ala280Glu	Ile227Thr carrier of PORD
Age at first presentation	14 yrs	birth	14 yrs	14 yrs
Presentation	hirsutism	mismatch between prenatal karyotype and phenotype	delayed puberty	virilisation and delayed puberty
External genitalia	clitoromegaly, rugous labia majora	normal female	clitoromegaly	mild clitoromegaly
Internal genitalia	absent uterus	absent uterus	rudimentary uterus	absent uterus
Gonads	inguinal	labia	intra-abdominal (past history of blt. inguinal hernia)	inguinal
Testosterone (basal / stimulated nmol/l)	5.8 / 22.6	0.3 / 1.8	4.8 / -	14.9 / 17.0

Mutations

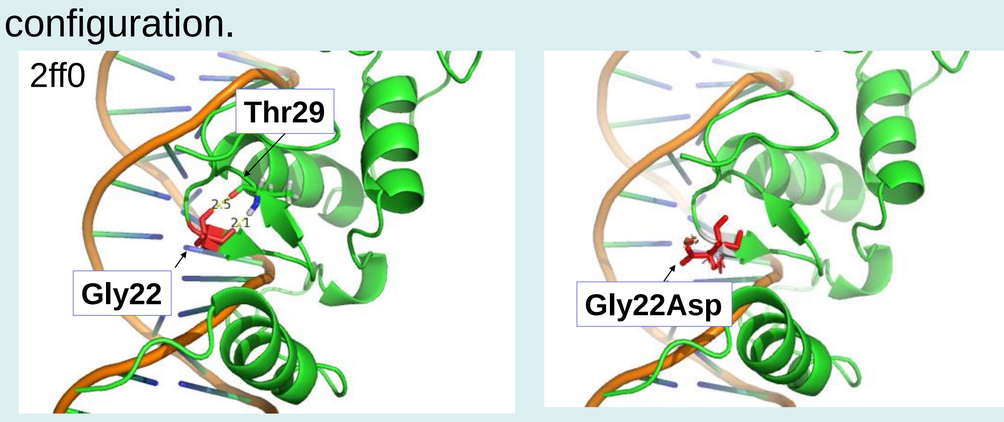
G22D is located in the DNA binding domain and Ile227Thr, A280E and Q329X in the ligand binding domain. The 3 missense mutations alter highly conserved amino acids.



Structure analysis of two missense mutations using Pymol

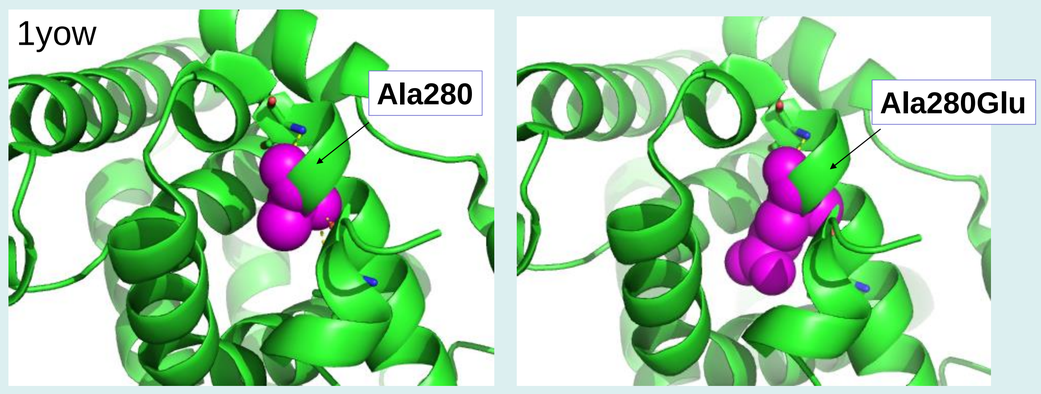
❑Gly22Asp

Substitution of Gly to Asp results in loss of hydrogen bonds with threonine at position 29 and alters DNA binding domain configuration.



❑Ala280Glu

Ala280 is located within the central protein core and substitution to bulky glutamic acid is likely to alter folding.



❑Ile227Thr

The mutation introduces a hydrophilic residue into the core of the protein.

Bioinformatic analyses

	Gly22Asp	Ala280Glu	Ile227Thr
PROVEAN	Deleterious (score -5.760)	Deleterious (score -4.291)	Deleterious (score -3.463)
PolyPhen-2	Probably damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00)	Probably damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00)	Probably damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00)
MutationTaster	Disease causing	Disease causing	Disease causing
FATHMM	Damaging (score: -5.08)	Damaging (score: -6.22)	Damaging (score: -5.22)
SAAP	Pathogenic. Native residue is involved in interface and binding.	Pathogenic. The mutation introduces a hydrophilic residue into the core of the protein.	Neutral. The mutation introduces a hydrophilic residue into the core of the protein.

Discussions

We report 4 novel NR5A1 mutations presenting with 46XY DSD and variable degrees of virilisation. *In-silico* molecular and structural analyses confirm the mutations to be highly pathogenic. Case 3 also highlights a sibling with a 46XX karyotype with the same mutation. The clinical significance of this for the sibling is uncertain, and raises specific challenges with respect to provision of counselling, predicting clinical prognosis and future management.

Acknowledgement

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