Structural analysis of the impact of a novel androgen receptor gene mutation in two unrelated adult patients with mild androgen insensitivity syndrome

Mirella Hage [1], Delphine Drui [2], Bruno Francou [3], Sandra Mercier [4], Anne Guiochon-Mantel [3,5], Joelle Belaisch-Allart [6], Yann Péréon [7], Laure Cazabat [1,8], Philippe De Mazancourt [9], Marie Laure Raffin-Sanson [1,8]

[1]Centre Hospitalier Universitaire Ambroise Paré, Service d'Endocrinologie Diabétologie et Nutrition, Assistance Publique-Hôpitaux de Paris, F- 92100 Boulogne Billancourt, France
[2] Centre Hospitalier Universitaire Nantes, Service d'Endocrinologie, L'institut du Thorax, F-44000 Nantes, France
[3]Centre Hospitalier Universitaire Bicêtre, Service de Génétique Moléculaire, Pharmacogénétique et Hormonologie, Assistance Publique-Hôpitaux de Paris, F-94270 Le Kremlin-Bicêtre, France
[4]Centre Hospitalier Universitaire Nantes, Service de Génétique Médicale, L'institut du Thorax, F-44000 Nantes, France
[5]INSERM UMR1185, Faculté de médecine Paris Saclay, Université Paris-Saclay
[6] Centre Hospitalier des Quatre Villes, Service de Gynécologie-Obstétrique et Médecine de la Reproduction, 141, Grande Rue, 92318 Sèvres, France
[7] Centre Hospitalier Universitaire Nantes, Centre de Référence Maladies Neuromusculaires Nantes-Angers, Hôtel-Dieu, Filnemus, F-44093, Nantes, France
[8]EA4340, Université de Versailles Saint-Quentin-en-Yvelines, UFR des sciences de la santé Simone Veil, F- 78423, Montigny-le-Bretonneux, France
[9]Centre Hospitalier Universitaire Ambroise Paré, Laboratoire de Biochimie-Génétique Moléculaire, UMR1179, F-92100 Boulogne Billancourt, France

Context

Androgen insensitivity syndrome (AIS)
- ✓ Rare X-linked recessive disorder
- ✓ Caused by androgen receptor (*AR*) gene mutations
- ✓ Spectrum of androgen dysfunction
  - gynecomastia and/or infertility in mild AIS (MAIS)
  - ambiguous or undermasculinized genitalia in partial AIS
  - complete testicular feminization in complete AIS
- ✓ More than 800 different mutations in the *AR* gene identified

Objective

- ✓ To report a novel mutation in the *AR* gene associated with MAIS in two unrelated adult patients presenting for infertility and a decrease in physical athletic performance.
- ✓ To characterize the functional impact of this mutation using 3D modeling studies

Patients and Methods

- ✓ **Patient 1** was referred at the age of 38 years for infertility.
- He had gynecomastia, bilateral testicular hypotrophy and mild gynecomastia
- His semen analysis showed oligoasthenoteratospermia.
- Lab results revealed ↗testosterone levels, ↗FSH, ↗androgen sensitivity index (ASI) suggesting AIS.
- The couple underwent successful in vitro fertilization and intracytoplasmic sperm injection resulting in a twin pregnancy.

- ✓ **Patient 2** was referred at the age of 45 years for evaluation of a fatigue and a decrease in physical athletic performance.
- He had a history of gynecomastia, surgically treated during adolescence but normal external genitalia.
- He also presented with oligoasthenoteratospermia, ↗testosterone plasma levels and an ↗ ASI.
- Despite his impaired semen analysis, he fathered two children without assisted reproductive technology. Because of his persistent fatigue, the patient was offered a trial of high dose dihydrotestosterone therapy which improved his symptoms and his quality of life.

- ✓ Family history for infertility or gynecomastia was negative in both patients.
- ✓ AR gene analysis was performed from peripheral blood by Sanger sequencing.
- ✓ Structural analysis and molecular 3D modeling of the mutated *AR* was performed

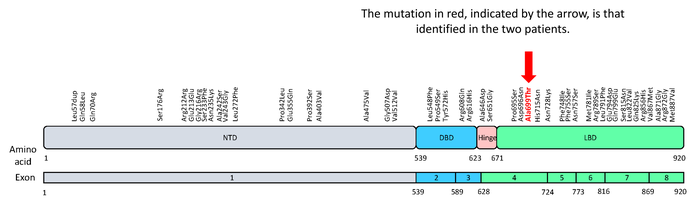
Hormone levels in the two patients with MAIS

Hormone	Normal range for men	Patient 1	Patient 2
Basal FSH (IU/L)	1.5-12.4	7.6	1.6
Basal LH (IU/L)	1.7-8.6	14.6	7.2
Total Testosterone (nmol/L)	11.8-34.5	50	38,5
SHBG (nmol/L)	10-50	59	122.7
Inhibine B	80-270	92	NA
AMH (ng/mL)	2-13	5.8	NA
Estradiol (pmol/L)	36-220	132	139
ASI (IU x nmol/L^2)	6.7–138.7	730	477

Semen analysis results in the two patients

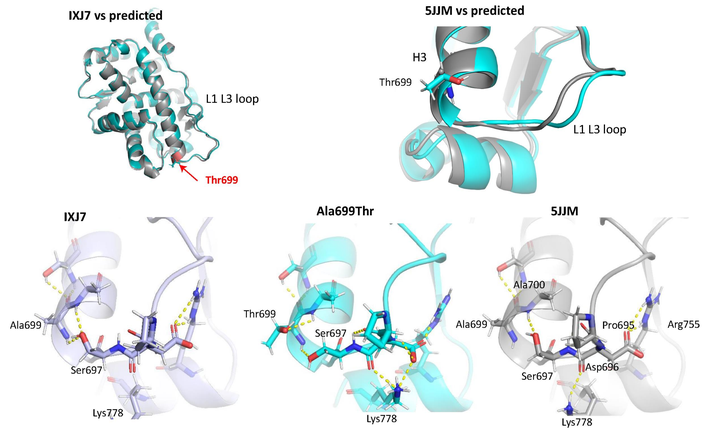
Parameters	WHO 2010 criteria	Patient 1	Patient 2
Semen volume	>1.5 ml	10	2.5
Total sperm count	>39 million	5.2	22
Sperm concentration	>15 million/ml	0.52	8.8
Total Motility	>40 %	35	20
Progressive Motility	>32 %	5	NA
Vitality	>58 % of vital sperm	48	NA
Sperm Morphology	>4 % of normal sperm morphology	36	12

AR gene sequencing in the two patients revealed a common novel missense mutation, Ala699Thr, in exon 4 within the ligand binding domain



Less than 50 different mutations in *AR* gene have been associated with MAIS

Structural analysis showed that this mutation may impact dimer stability upon ligand binding or may affect allosteric changes upon dimerization



Conclusions

- ✓ The structural modifications induced by the p.Ala699Thr mutation very likely account for the mild androgen insensitivity syndrome seen in these two patients.
- ✓ Complementary functional studies will be required to confirm the pathogenicity of this mutation.
- ✓ This study highlights the usefulness of structural studies in providing a greater understanding of the functional consequences of a mutation and expands the database of *AR* gene mutations.
- ✓ The proper diagnosis of adult patients with MAIS may be helpful for the adequate counseling of infertile male patient undergoing assisted reproductive techniques.

The authors declare no conflict of interest