Novel *CYP11A1* mutations in 15 patients (13 families) with variable clinical presentations

Claire Goursaud[1],Florence Roucher-Boulez[1], Delphine Mallet-Motak[1], Raja Brauner[2], Claire-Lise Gay[3], Ane Mercedes Garcia[4], Anne Lienhardt-Roussie[5], Farida Jennane[6], Maryam Razzaghy Azar[7], Yves Morel[1]

[1]Hospices Civils de Lyon, Laboratoire d'Endocrinologie Moléculaire et Maladies Rares, Lyon-Bron, France; [2]Fondation ophtalmologique Adolphe de Rothschild and Université Paris Descartes, Paris, France; [3]CHU Hospices Civils de Lyon, Service d'Endocrinologie Pédiatrique, Lyon-Bron, France; [4]Centro medico Orinoco, Endocrinologo Pediatra, Ciudad Guyana, Bolivar, Venezuela; [5]Hôpital de la mère et de l'enfant, Département de pédiatrie médicale, Limoges, France; [6]CHU Ibn Rochd, Hôpital d'enfants, unité d'Endocrinologie/Diabétologie et Gynécologie de l'Enfant et de l'adolescent, Casablanca, Morocco; [7]Metabolic Disorders Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute and Hazrat Aliasghar Children's Hospital, Iran University of Medical Sciences, Tehran, Iran

BACKGROUND

The side chain cleavage enzyme (CYP11A1) catalyzes the conversion of cholesterol to pregnenolone, the first rate-limiting step of steroidogenesis. CYP11A1 mutations are associated with primary adrenal insufficiency (PAI) and, in 46,XY patients, Disorders of Sex Development (DSD). 35 patients (27 families) have been previously reported in the literature including 15 intermediate forms documented:

- Six 46,XY patients with normal male external genitalia, including 5 homozygous for p.R451W
- Five 46,XY patients with partial DSD (micropenis, hypospadias…)
- Four 46,XX patients with late onset of PAI (≥ 18 months)

We report 15 patients (13 families) with 15 *CYP11A1* mutations (10 new ones) and variable clinical presentations.

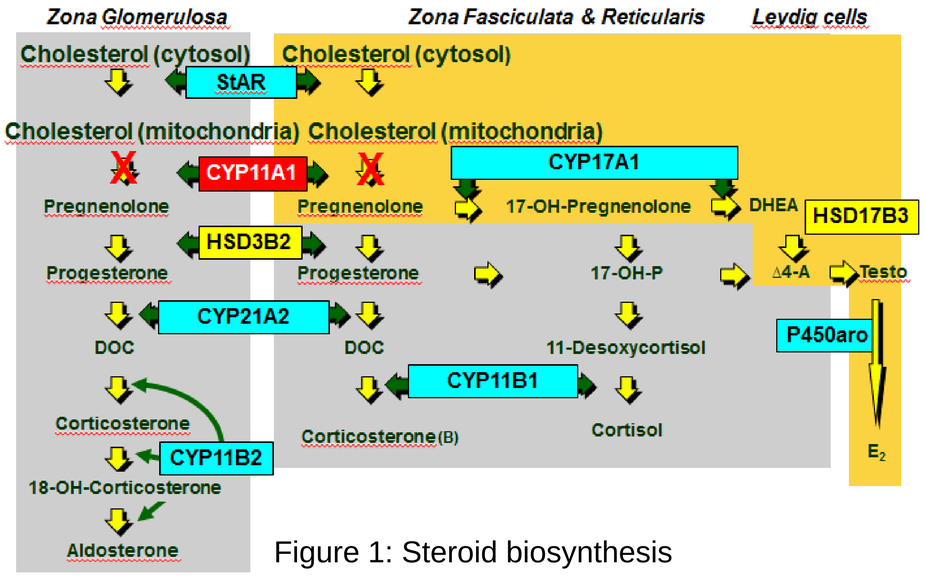

Figure 1: Steroid biosynthesis

METHODS

- Sanger sequencing: selective amplification by PCR followed by conventional dideoxy sequencing of exons and the exon-intron boundaries on a ABI-3730XL and compared to the human genome (GRCh37/hg19) using SeqScape® software v3 (Life Technologies, CA, USA)
- Massive Parallel Sequencing (MPS): Design Ampliseq (exons +/- 50 pb). Library preparation : Ion AmpliSeq™ Library Kit. Séquencing: Ion Torrent Proton™. Informatic analysis: Torrent Suite™ software v5.1
  57 genes analyzed including *AAAS, AIRE, Cited2, CYP11A1, MC2R, MCM4, MRAP, NNT, NR0B1, NR5A1, PBX1, PRDX3, StAR, TXNRD2*
- *In silico* studies : predictive software, sequences alignment (between species and steroidogenesis CYP) using Clustal omega and Genedoc, molecular modelisation using Swiss-PdbViewver

RESULTS

Diagnosis based on:

– SW (10 patients):
- 5 patients 46,XY (3, 4, 6a, 12, 13) with complete female phenotype homozygous or compound heterozygous for 7 mutations: p.G94D, p.P104L, p.A277S, p.D329G, p.R465W, p.L170Vfs*30 and p.R120X
- 1 patient 46,XY (2) without DSD homozygous for a mutation reported in similar cases: p. R451W
- 2 patients 46,XX (8, 11) with PAI diagnosed in the first year of life (one day and 7 months) compound heterozygous for p.G138R/p.L170Vfs*30 and p.R396G/p.R465Q
- 2 patients 46,XX (5, 9) with late onset of PAI (> 3 years) with p.R120Q, p.E314K, p.R465W mutations

– DSD: 2 patients 46,XY (7a, 10) with p.E314K, p.R465W and p.G454D mutations (For the patient 7a, US failed to reveal uterus)

– Familial history (3 patients)
- 2 patients 46,XY with DSD (1, 7b) carrying p.A269V, p.E314K and p.R465W mutations
- 1 patient 46,XY DSD with complete female phenotype (6b) compound heterozygous for p.R120X and p.A277S

Family	c.DNA	protein	Activity	Karyotype	diagnosis of PAI	DSD	circumstances of diagnosis
1	c.806C>T/c.806C>T	p.A269V/p.A269V	11%	46,XY	3 months	buried penis, cryptorchidism	sister's history
2	c.1351C>T/c.1351C>T	p.R451W/p.R451W	32%	46,XY	13 months	None	SW
3	c.1393C>T/c.1393C>T	p.R465W/p.R465W	?	46,XY	9 days	female external genitalia	SW
4	c.281G>A/c.281G>A	p.G94D/p.G94D	?	46,XY	15 months	female external genitalia	SW
5	c.359G>A/c.940G>A	p.R120Q/p.E314K	?/?	46,XX	3 years and 8 months	NA	SW
6a	c.358C>T/c.829G>T	p.R120X/p.A277S	?/?	46,XY	2 years and 6 months	female external genitalia	SW
6b	c.358C>T/c.829G>T	p.R120X/p.A277S	?/?	46,XY	None (neonatal diagnosis due to familial history)	female external genitalia	sister's history
7a	c.940G>A/c.1393C>T	p.E314K/p.R465W	?/?	46,XY	3 years	female external genitalia	DSD
7b	c.940G>A/c.1393C>T	p.E314K/p.R465W	?/?	46,XY	None	Hypospadias	sister's history
8	c.412G>A / c.508_509delCT	p.G138R/ p.L170Vfs*30	?/?	46,XX	1 day	NA	SW
9	c.940G>A/c.1393C>T	p.E314K/p.R465W	?/?	46,XX	4 years	NA	SW
10	c.1361G>A/c.1361G>A	p.G454D/p.G454D	?	46,XY	12 days*	micropenis, hypospadias, cryptorchidism	DSD
11	c.1186A>G/c.1394G>A	p.R396G/p.R465Q	?/?	46,XX	7 months	NA	SW
12	c.508_509delCT/ c.311C>T	p.L170Vfs*30/ p.P104L	?/?	46,XY	10 months	female external genitalia	SW
13	c.986A>G/c.986A>G	p.D329G/p.D329G	?	46,XY	3 months	female external genitalia	SW

NA: not applicable; SW: Salt wasting * PAI detected by biological salt loss following the exploration of DSD

Table 1:Description of patients with *CYP11A1* mutations

Nucleotide change	Protein change	Grantham	AA Conservation: Steroido-genesis CYP	AA Conservation: Species	Protein consequence	Location	Predictive software: SIFT	Predictive software: Polyphen-2	Predictive software: Mutation Taster	dbSNP ID	Allele count: ESP	Allele count: ExAC	Family number
c.281G>A	p.G94D	94	-	+++		β 1 strand	d	dc	pb	NA	NA	NA	4
c.311C>T	p.P104L	98	-	+++		B helix	d	dc	pb	NA	NA	NA	12
c.359G>A	R120Q	43	-	+++	Missense mutation at an AA involved in heme binding and in recognition substrat region	B-B' loop	d	dc	pb	NA	NA	NA	5
c.412G>A	p.G138R	125	+	+++		B'-C loop	d	dc	pb	NA	NA	NA	8
c.829G>T	p.A277S	99	-	+++	exon 4 skipping	G helix	d	dc	pb	NA	NA	NA	6
c.940G>A	p.E314K	56	-	+	Missense mutation in I helix (helix involved in heme binding)	I helix	t	b	pb	rs6161	37/12949	294/121388	7-9
c.986A>G	p.D329G	94	+	+++		I helix	d	dc	pb	rs748120824	NA	1/121218	13
c.1186A>G	p.R396G	125	+	+++	Missense mutation at an AA involved in heme binding and in recognition substrat region	β1-4 strand	d	dc	pb	NA	NA	NA	11
c.1361G>A	p.G454D	94	-	+++		K''-L loop	d	dc	pb	rs773652136	NA	1/121410	10
c.1393C>T	p.R465W	101	+	+++	Missense mutation at an AA involved in recognition of Adx	L helix	d	dc	pb	rs141235847	1/12989	2/121412	3-7
c.1394G>A	p.R465Q	43	+	+++	Missense mutation at an AA involved in recognition of Adx	L helix	d	dc	pb	NA	NA	NA	11

Table 2: *In silico* studies for missense mutations (except p.A269V and p.R451W already studied)

d: deleterious; dc: disease causing; pb: probably damaging; NA: not applicable

Mutations that seem to be less pathogen

Mutations that seem to be severe

CONCLUSION

The incidence of *CYP11A1* mutations (33%) is high in our cohort of patients with first step of steroidogenesis deficiency (*STAR* and *CYP11A1* gene). Diagnosis is based on SW in approximately 67% of cases. For some mutations, *in silico* studies seem to predict good genotype-phenotype correlation. Our patient without DSD is homozygous for p.R451W, mutation found in 5 patients 46,XY with the same phenotype. Intermediate forms are at risk to be misdiagnosed because the phenotype overlaps with other causes of PAI. This emphasizes the utility of MPS allowing the study of many causative genes simultaneously. Further studies should be done to explore these dissociated forms.

55th Annual ESPE Meeting, September 2016, Paris, France

C. Goursaud *et al.*

With the support of the *« Association des Internes et des Anciens Internes en Pharmacie des Hôpitaux de Lyon »*

55ESPE 6--P1

Adrenal

Claire Goursaud

DOI: 10.3252/pso.eu.55ESPE.2016

Poster presented at: ESPE 2016 PARIS