

## SUPPLEMENTARY MATERIAL AND METHODS

### Table of contents

<b>1. METHODS</b> .....	2
1.1. <i>Genetic studies</i> .....	2
<b>2. SUPPLEMENTARY TABLES AND FIGURES</b> .....	3
2.1. <i>Supplemental Table S1: List of genes tested by Next Generation Sequencing</i> .....	3
2.2. <i>Supplemental Figure S1: Illustration of the POR variants mapping in P1 in relation to the length of next-generation sequencing reads</i> .....	7
2.3. <i>Supplemental Table S2: Summary of additional genetic findings in PORD probands who underwent a panel of 84 genes as detailed above</i> .....	7
2.4. <i>Supplemental Table S3: Steroid precursors levels before and after ACTH stimulation</i>	8

## 1. **METHODS**

### 1.1. *Genetic studies*

Genomic DNA (gDNA) was extracted from whole blood cells using an automated QIA Symphony platform (Qiagen, Venlo, The Netherlands). Briefly, for each patient, 500 ng gDNA was sonicated using a Covaris M220 (Covaris Inc., MS, Woburn, MA, USA). Illumina® adapters and other oligonucleotides were provided by Eurofins Genomics (Ebersberg, Germany). Whole genome Illumina® libraries were prepared using NEBNext® DNA Library Prep Master Mix Set for Illumina® (NEB Inc., Ipswich, MA, USA) robotized on a Biomek Span 8 workstation (Beckman, Villepinte, France). Enrichment was processed using Sureselect XT kit (Agilent, Santa Clara, CA, USA) robotized on a Biomek 4000 workstation (Beckman, Villepinte, France). Paired end 2 x 150bp sequencing was performed by batch of 23 patients on a MiSeq® Illumina™ sequencer. *CYP21A2* gene (NM\_000500.6) was initially analyzed in all patients using both bidirectional Sanger sequencing and Multiplex ligation-dependent probe amplification [MLPA] (SALSA MLPA P050 CAH probemix, MRC Holland, Amsterdam, NL). Subsequently, a panel of genes involved in disorders of steroidogenesis and reproductive function, including *POR* (NM\_000941.3), was analyzed. The full list of the 84 genes is described in Supplementary Table S1.

## 2. SUPPLEMENTARY TABLES AND FIGURES

### 2.1. *Supplemental Table S1: List of genes tested by Next Generation Sequencing*

<b>HGNC ID</b>	<b>Genes (HGNC)</b>	<b>Full name</b>	<b>Locus</b>	<b>Chr</b>	<b>Strand</b>	<b>#MIM</b>	<b>Refseq selection</b>	<b>Category</b>
<b>385</b>	AKR1C2	aldo-keto reductase family 1 member C2	10p15.1	chr10	-	600450	NM_001354.5	DSD
<b>387</b>	AKR1C4	aldo-keto reductase family 1 member C4	10p15.1	chr10	+	600451	NM_001818.3	DSD
<b>464</b>	AMH	anti-Mullerian hormone	19p13.3	chr19	+	600957	NM_000479.3	DSD
<b>465</b>	AMHR2	anti-Mullerian hormone receptor type 2	12q13.13	chr12	+	600956	NM_020547.3	DSD
<b>644</b>	AR	androgen receptor	Xq12	chrX	+	313700	NM_000044.3	DSD
<b>795</b>	ATM	ATM serine/threonine kinase	11q22.3	chr11	+	607585	NM_000051.3	POF
<b>886</b>	ATRX	ATRX, chromatin remodeler	Xq21.1	chrX	-	300032	NM_000489.4	POF
<b>29353</b>	BHLHB9	basic helix-loop-helix family member b9	Xq22.1	chrX	+	300921	NM_001142524.1	POF
<b>1068</b>	BMP15	bone morphogenetic protein 15	Xp11.22	chrX	+	300247	NM_005448.2	POF
<b>1552</b>	CBX2	chromobox 2	17q25.3	chr17	+	602770	NM_005189.2	DSD
<b>2590</b>	CYP11A1	cytochrome P450 family 11 subfamily A member 1	15q24.1	chr15	-	118485	NM_000781.2	DSD
<b>2593</b>	CYP17A1	cytochrome P450 family 17 subfamily A member 1	10q24.32	chr10	-	609300	NM_000102.3	DSD
<b>2600</b>	CYP21A2	cytochrome P450 family 21 subfamily A member 2	6p21.33	chr6	+	613815	NM_000500.7	DSD
<b>2865</b>	DHH	desert hedgehog	12q13.12	chr12	-	605423	NM_021044.3	DSD
<b>2877</b>	DIAPH2	diaphanous related formin 2	Xq21.33	chrX	+	300108	NM_006729.4	POF
<b>2927</b>	DMC1	DNA meiotic recombinase 1	22q13.1	chr22	-	602721	NM_007068.3	POF
<b>2934</b>	DMRT1	doublesex and mab-3 related transcription factor 1	9p24.3	chr9	+	602424	NM_021951.2	DSD
<b>2935</b>	DMRT2	doublesex and mab-3 related transcription factor 2	9p24.3	chr9			NM_006557.6	DSD

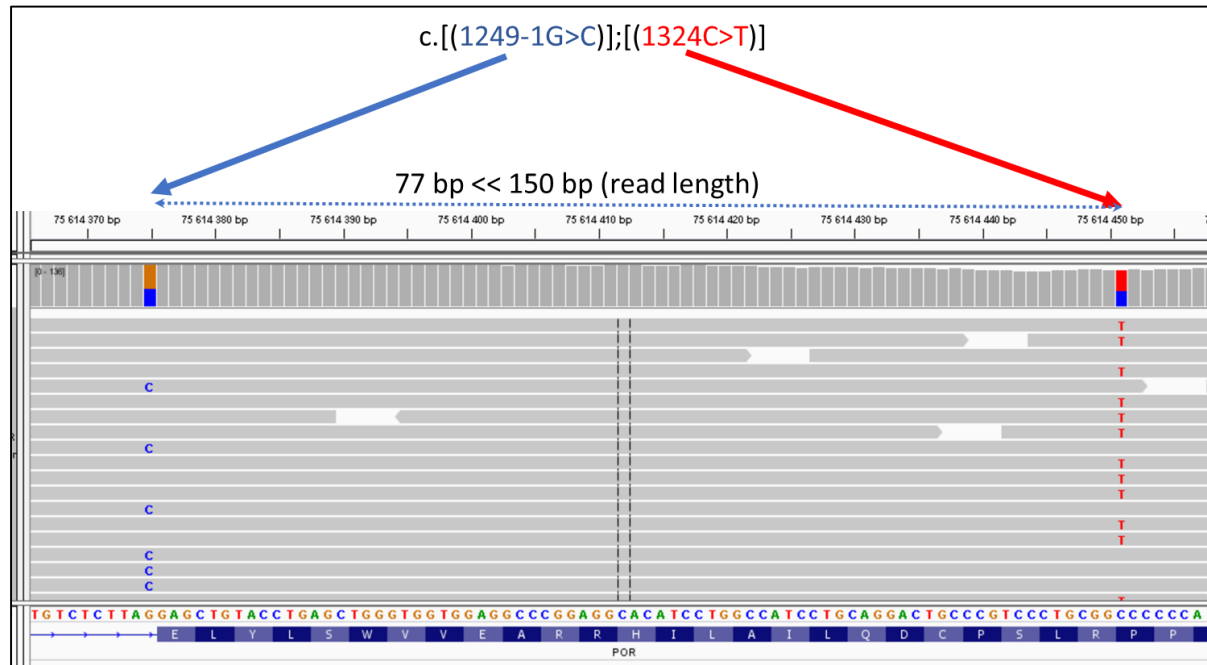
<b>3266</b>	EIF2S2	eukaryotic translation initiation factor 2 subunit beta	20q11.22	chr20					DSD
<b>3341</b>	EMX2	empty spiracles homeobox 2	10q26.11	chr10	+	600035	NM_001165924.1		DSD
<b>3467</b>	ESR1	estrogen receptor 1	6q25.1	chr6	+	133430	NM_000125.3		STEROID
<b>3468</b>	ESR2	estrogen receptor 2	14q23.3	chr14	-	601663	NM_001437.2		STEROID
<b>24669</b>	FIGLA	folliculogenesis specific bHLH transcription factor	2p13.3	chr2	-	608697	NM_001004311.3		POF
<b>3775</b>	FMR1	fragile X mental retardation 1	Xq27.3	chrX	+	309550	NM_002024.5		POF
<b>5021</b>	FOXA1	forkhead box A1	14q21.1	chr14	-	602294	NM_004496.3		POF
<b>5023</b>	FOXA3	forkhead box A3	19q13.32	chr19	+	602295	NM_004497.2		POF
<b>3800</b>	FOXC1	forkhead box C1	6p25.3	chr6	+	601090	NM_001453.2		DSD
<b>1092</b>	FOXL2	forkhead box L2	3q22.3	chr3	-	605597	NM_023067.3		DSD
<b>3964</b>	FSHB	follicle stimulating hormone beta subunit	11p14.1	chr11	+	136530	NM_000510.2		POF
<b>3969</b>	FSHR	follicle stimulating hormone receptor	2p16.3	chr2	-	136435	NM_000145.3		POF
<b>4135</b>	GALT	galactose-1-phosphate uridylyltransferase	9p13.3	chr9	+	606999	NM_000155.3		DSD
<b>4173</b>	GATA4	GATA binding protein 4	8p23.1	chr8	+	600576	NM_001308093.1		DSD
<b>4224</b>	GDF9	growth differentiation factor 9	5q31.1	chr5	-	601918	NM_005260.4		POF
<b>4278</b>	GJA4	gap junction protein alpha 4	1p34.3	chr1	+	121012	NM_002060.2		POF
<b>4484</b>	GPR3	G protein-coupled receptor 3	1p36.11	chr1	+	600241	NM_005281.3		DSD
<b>20193</b>	HFM1	HFM1, ATP dependent DNA helicase homolog	1p22.2	chr1	-	615684	NM_001017975.4		POF
<b>5212</b>	HSD17B3	hydroxysteroid 17-beta dehydrogenase 3	9q22.32	chr9	-	605573	NM_000197.1		DSD
<b>5218</b>	HSD3B2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	1p12	chr1	+	613890	NM_000198.3		DSD
<b>6065</b>	INHA	inhibin alpha subunit	2q35	chr2	+	147380	NM_002191.3		POF
<b>6067</b>	INHBB	inhibin beta B subunit	2q14.2	chr2	+	147390	NM_002193.2		POF
<b>6514</b>	LATS1	large tumor suppressor kinase 1	6q25.1	chr6	-	603473	NM_004690.3		DSD

<b>6584</b>	LHB	luteinizing hormone beta polypeptide	19q13.33	chr19	-	152780	NM_000894.2	POF
<b>6585</b>	LHCGR	luteinizing hormone/choriogonadotropin receptor	2p16.3	chr2	-	152790	NM_000233.3	POF
<b>28838</b>	LHX8	LIM homeobox 8	1p31.1	chr1	+	604425	NM_001001933.1	DSD
<b>14222</b>	LHX9	LIM homeobox 9	1q31.3	chr1	+	606066	NM_020204.2	DSD
<b>2568</b>	MAMLD1	mastermind like domain containing 1	Xq28	chrX	+	300120	NM_001177465.2	DSD
<b>6848</b>	MAP3K1	mitogen-activated protein kinase kinase kinase 1	5q11.2	chr5	+	600982	NM_005921.1	DSD
<b>16147</b>	MCM8	minichromosome maintenance 8 homologous recombination repair factor	20p12.3	chr20	+	608187	NM_032485.5	POF
<b>21484</b>	MCM9	minichromosome maintenance 9 homologous recombination repair factor	6q22.31	chr6	-	610098	NM_017696.2	POF
<b>7095</b>	MID1	midline 1	Xp22.2	chrX	-	300552	NM_000381.3	DSD
<b>7328</b>	MSH5	mutS homolog 5	6p21.33	chr6	+	603382	NM_002441.4	POF
<b>7652</b>	NBN	nibrin	8q21.3	chr8	-	602667	NM_002485.4	POF
<b>7668</b>	NCOA1	nuclear receptor coactivator 1	2p23.3	chr2	+	602691	NM_003743.4	STEROID
<b>7669</b>	NCOA2	nuclear receptor coactivator 2	8q13.3	chr8	-	601993	NM_001321703.1	STEROID
<b>7670</b>	NCOA3	nuclear receptor coactivator 3	20q13.12	chr20	+	601937	NM_181659.2	STEROID
<b>7672</b>	NCOR1	nuclear receptor corepressor 1	17p11.2	chr17	-	600849	NM_006311.3	STEROID
<b>7673</b>	NCOR2	nuclear receptor corepressor 2	12q24.31	chr12	-	600848	NM_006312.5	STEROID
<b>22448</b>	NOBOX	NOBOX oogenesis homeobox	7q35	chr7	-	610934	NM_001080413.3	POF
<b>7960</b>	NR0B1	nuclear receptor subfamily 0 group B member 1	Xp21.2	chrX	-	300473	NM_000475.4	DSD
<b>7978</b>	NR3C1	nuclear receptor subfamily 3 group C member 1	5q31.3	chr5	-	138040	NM_000176.2	STEROID
<b>7979</b>	NR3C2	nuclear receptor subfamily 3 group C member 2	4q31.23	chr4	-	600983	NM_000901.4	STEROID
<b>7983</b>	NR5A1	nuclear receptor subfamily 5 group A member 1	9q33.3	chr9	-	184757	NM_004959.4	DSD
<b>8620</b>	PAX6	paired box 6	11p13	chr11	-	607108	NM_001604.5	DSD

<b>8910</b>	PGR	progesterone receptor	11q22.1	chr11	-	607311	NM_000926.4	STEROID
<b>16090</b>	PGRMC1	progesterone receptor membrane component 1	Xq24	chrX	+	300435	NM_006667.4	POF
<b>9005</b>	PITX2	paired like homeodomain 2	4q25	chr4	-	601542	NM_153426.2	DSD
<b>13711</b>	POF1B	premature ovarian failure, 1B	Xq21.1	chrX	-	300603	NM_024921.3	POF
<b>9208</b>	POR	cytochrome p450 oxidoreductase	7q11.23	chr7	+	124015	NM_000941.2	DSD
<b>28611</b>	RICTOR	RPTOR independent companion of MTOR complex 2	5p13.1	chr5	-	609022	NM_152756.4	POF
<b>30287</b>	RPTOR	regulatory associated protein of MTOR complex 1	17q25.3	chr17	+	607130	NM_020761.2	POF
<b>21679</b>	RSPO1	R-spondin 1	1p34.3	chr1	-	609595	NM_001038633.3	DSD
<b>26026</b>	SOHLH2	spermatogenesis and oogenesis specific basic helix-loop-helix 2	13q13.3	chr13	-	616066	NM_017826.2	POF
<b>11199</b>	SOX3	SRY-box 3	Xq27.1	chrX	-	313430	NM_005634.2	DSD
<b>11204</b>	SOX9	SRY-box 9	17q24.3	chr17	+	608160	NM_000346.3	DSD
<b>11284</b>	SRD5A1	steroid 5 alpha-reductase 1	5p15.31	chr5	+	184753	NM_001047.3	DSD
<b>11285</b>	SRD5A2	steroid 5 alpha-reductase 2	2p23.1	chr2	-	607306	NM_000348.3	DSD
<b>11311</b>	SRY	sex determining region Y	Yp11.2	chrY	-	480000	NM_003140.2	DSD
<b>11356</b>	STAG3	stromal antigen 3	7q22.1	chr7	+	608489	NM_012447.3	POF
<b>11359</b>	STAR	steroidogenic acute regulatory protein	8p11.23	chr8	-	600617	NM_000349.2	DSD
<b>12382</b>	TSPYL1	TSPY like 1	6q22.1	chr6	-	604714	NM_003309.3	DSD
<b>12783</b>	WNT4	Wnt family member 4	1p36.12	chr1	-	603490	NM_030761.4	DSD
<b>12796</b>	WT1	Wilms tumor 1	11p13	chr11	-	607102	NM_024426.4	DSD
<b>12799</b>	WWOX	WW domain containing oxidoreductase	16q23.1	chr16	+	605131	NM_016373.3	DSD
<b>12823</b>	XPNPEP2	X-prolyl aminopeptidase 2	Xq26.1	chrX	+	300145	NM_003399.5	DSD

Genes are classified in several categories: steroid signaling (STEROID), disorder of sexual development (DSD), premature ovarian failure (POF), other

2.2. Supplemental Figure S1: Illustration of the POR variants mapping in P1 in relation to the length of next-generation sequencing reads



2.3. Supplemental Table S2: Summary of additional genetic findings in PORD probands who underwent a panel of 84 genes as detailed above

Proband	POR event	Approach	Number of variants	Number of Variants, MAF ≤ 2%	Class 4,5 variants	Class 2 variants (all heterozygous)
P1	c.[(1249-1G>C)];[(1324C>T)]	NGS	351	24	None	CYP17A1 NM_000102 c.970-18A>G, p.(?)
P2	c.[(1825C>T)];[(1859G>C)]	NGS	323	15	None	CYP11A1 NM_001099773 exon5:c.466G>A, p.E156K
P4	c.[(1648C>T)];[(1826_1849del)]	NGS	337	15	None	MAP3K1 NM_005921 exon10:c.1846G>A, p.G616R
P5	c.[(859G>C)];[(859G>C)]	Sanger	N/A	N/A	N/A	N/A

N/A, not assessed; NSG, Next generation sequencing; Nb, Number

## 2.4. Supplemental Table S3: Steroid precursors levels before and after ACTH stimulation

	Patient 1	Patient 2	Patient 4	Patient 5
11-desoxycortisol (nmol/l)				
<i>Basal</i>	1.9	0.5	1.3	0.5
<i>60 min after ACTH</i>	2.4	2.4	-	0.7
21-desoxycortisol (nmol/l)				
<i>Basal</i>	1.7	0.5	-	1.9
<i>60 min after ACTH</i>	8.3	34.9	-	11
Desoxycortisterone (nmol/l)				
<i>Basal</i>	-	0.2	-	0.6
<i>60 min after ACTH</i>	-	0.9	-	1.7
Corticosterone (nmol/l)				
<i>Basal</i>	36.9	5.7	-	36.9
<i>60 min after ACTH</i>	97	80.3	-	137.7

To convert values for 11-desoxycortisol to ng/ml, multiply by 0.35. To convert values for 21-desoxycortisol to ng/ml, multiply by 0.35. To convert values for desoxycorticosterone to ng/ml, multiply by 0.33. To convert values for corticosterone to ng/ml, multiply by 0.35.