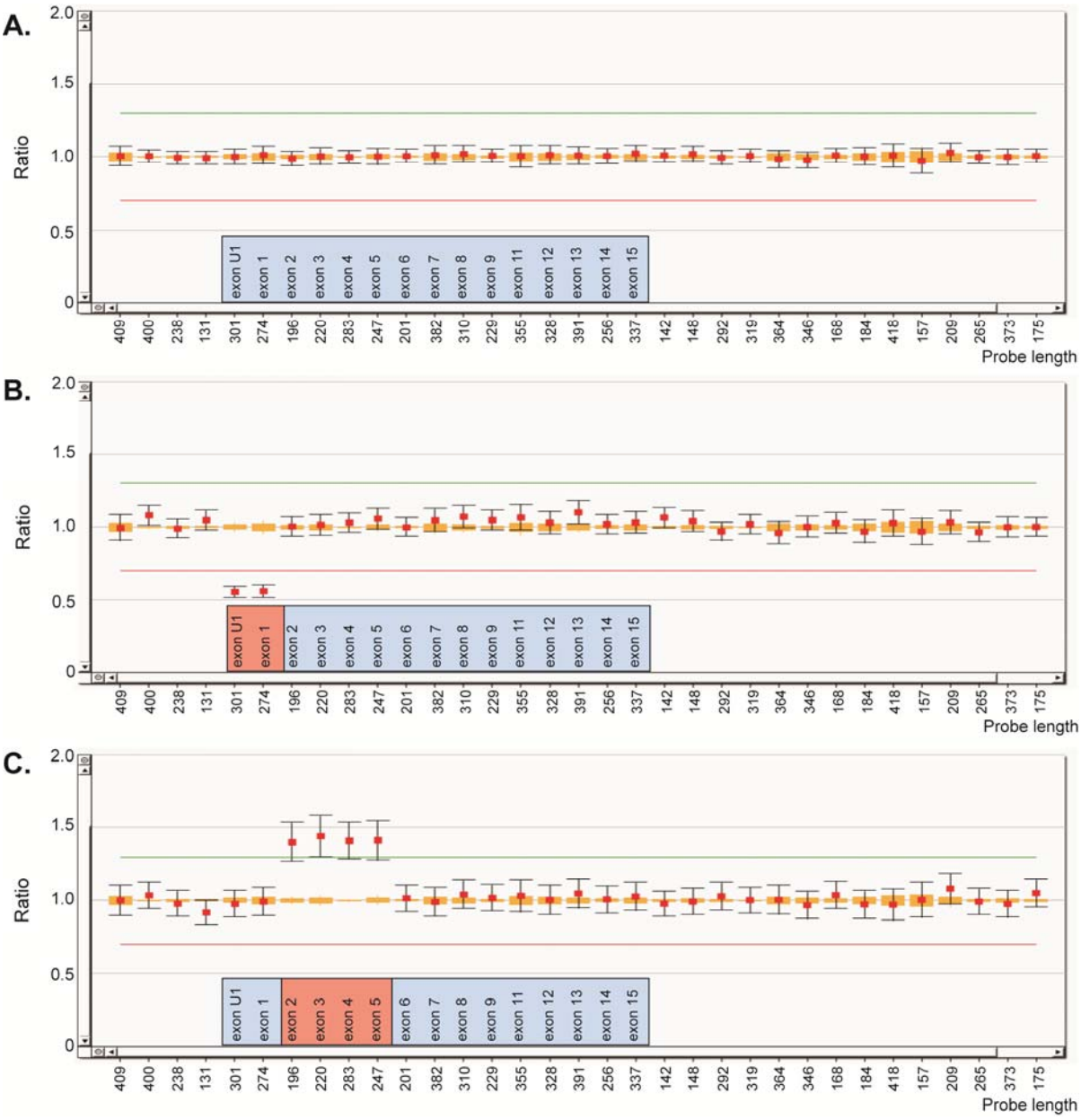
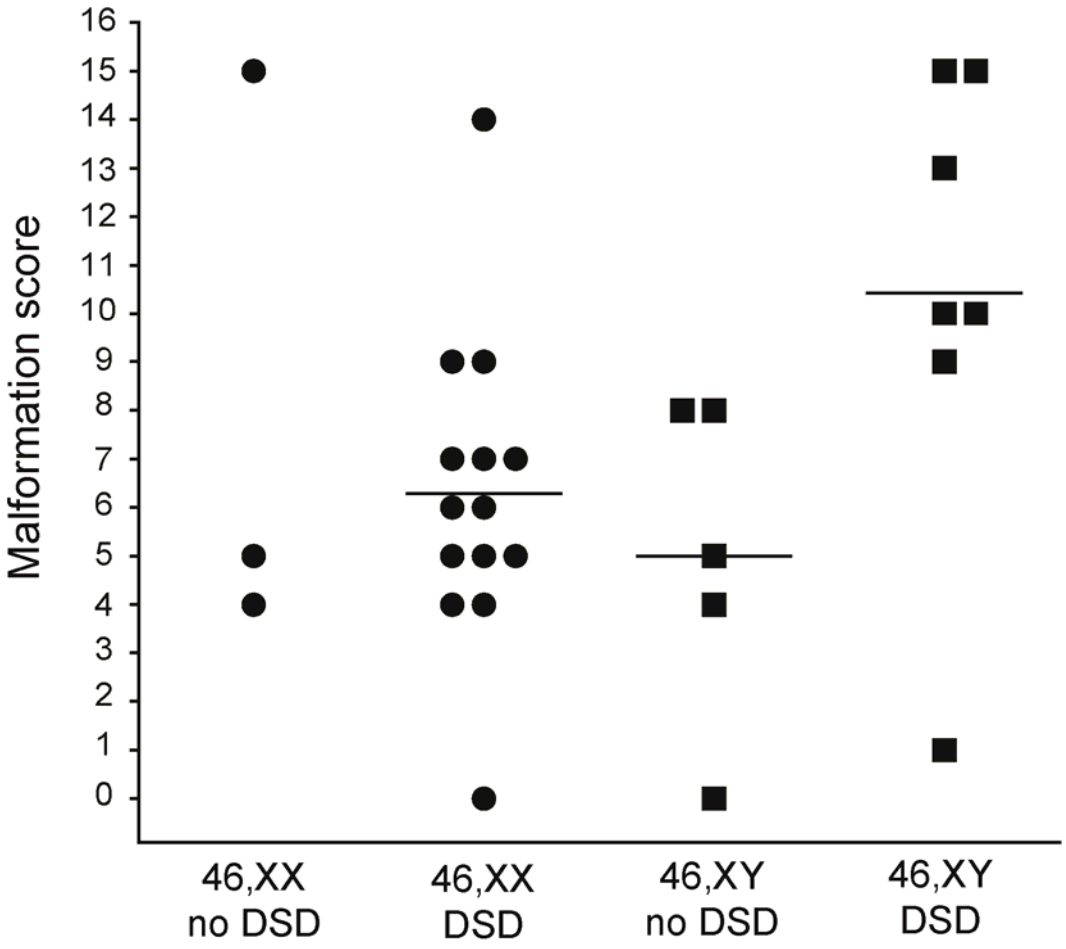


Suppl. Fig. 1 Graphical representation of the results of multiplex ligation-dependent probe amplification (MLPA) in an unaffected control patient (**Panel A**), patient 13 with deletion of untranslated exon 1 and exon 1 of the *POR* gene (**Panel B**), and patient 30 with duplications of exons 2-5 of the *POR* gene (**Panel C**). The yellow boxes represent the variation of the reference samples. Red dots show the ratio of the analyzed sample with whiskers representing the variation of the probe ratio of the sample compared to the reference runs. The red horizontal line indicates the threshold for deletions (below 0.75), whereas the green horizontal line marks the threshold for duplications (above 1.3). The blue box indicates the probes for the POR gene. Probe lengths outside the blue box indicate reference probes that are used to create a normalization constant for data-normalization. Red boxes indicate the positions of genetic alterations.



Suppl. Fig. 2 Comparison of PORD malformation score and DSD phenotype. 46,XY individuals presenting with DSD have higher malformation scores than 46,XY individuals with normal sex development (p=0.034) and 46,XX DSD patients (p= 0.022).

Suppl. Table 1. Urinary steroid hormone metabolites measured by gas chromatography/mass spectrometry (GC/MS).

Full trivial name	Abbreviation	Metabolite of
Androsterone	An	androstenedione, testosterone, 5α-dihydrotestosterone
Etiocholanolone	Et	androstenedione, testosterone
Tetra-11-dehydrocorticosterone	THA	11-dehydrocorticosterone,corticosterone
5α-Tetra-11-dehydrocorticosterone	5αTHA	11-dehydrocorticosterone, corticosterone
Tetrahydrocorticosterone	THB	corticosterone
5α-Tetrahydrocorticosterone	5αTHB	corticosterone
Pregnanediol	PD	progesterone
17-hydroxypregnanolone	17HP	17-hydroxyprogesterone
Pregnanetriol	PT	17-hydroxyprogesterone
Pregnanetriolone	PT'ONE	21-deoxycortisol*
Tetrahydrocortisol	THF	cortisol
5α-Tetrahydrocortisol	5αTHF	cortisol
Tetrahydrocortisone	THE	cortisone, cortisol

* 21-deoxycortisol will be synthesized in lieu of 11-deoxycortisol if 21-hydroxylase activity is impaired and thus its accumulation represents the most specific indicator of 21-hydroxylase deficiency.

pl. Table 2. Impact of identified *POR* gene mutations on protein function. CYP17A1 17α-hydroxylase and 17,20 lyase activities ssed in yeast co-expressing human CYP17A1 and human wild-type or mutant POR (this study, (21), (30)) or with yeast microsomes expr an CYP17A1 co-incubated with bacterially expressed POR wildtype or mutant protein (7). The effects of the POR mutants were expres centages of the catalytic efficiencies (V_{max}/K_m) observed for the two enzymatic reactions in comparison to enzyme kinetics obtained with e POR.

otein position	Location	Coding effect	Functional effect
onic deletions and duplications identified by MLPA analysis			
l ex U1-1	Ex U1, 1	deletion	Exonic deletion – predicted to yield a non-functional protein and abolish activity
p ex 2-5	Ex 2,3,4,5	duplication	Exonic duplication – predicted to yield a non-functional protein and abolish activity
lice site mutations			
S6 -2A>T	IVS6	Splice site	Predicted to result in loss of splice acceptor site using NetGene2
S7+2dupT	IVS7	Splice site	Predicted to result in loss of splice donor site using NetGene2
S8 +1G>A	IVS8	Splice site	Predicted to result in loss of splice donor site using NetGene2
otein truncating mutations			
Y87X	Ex3	Nonsense	Early N-terminal truncation – predicted to abolish activity
R223X	Ex6	Nonsense	Early N-terminal truncation – predicted to abolish activity
Y376L*fsX74*	Ex10	Frameshift	Early truncation yielding a 450 aa protein including a 74 aa out-of-frame sequence nonsense sequence – predicted to abolish activity
444H*fsX6*	Ex11	Frameshift	Early truncation yielding a 450 aa protein including a 6 aa out-of-frame sequence – predicted to abolish activity; shown to undergo nonsense-mediated mRNA decay (14)
Q455R*fsX90*	Ex11	Frameshift	Early truncation yielding a 545 aa protein including 90 aa out-of-frame sequence – predicted to abolish activity
V472A*fsX102*	Ex12	Frameshift	Early truncation yielding a 574 aa protein including 102 aa out-of-frame sequence - predicted to abolish activity
Y576X	Ex13	Nonsense	C-terminal truncation resulting in 576 aa protein; expected to render a non-functional protein
E601S*fsX12*	Ex13	Frameshift	C-terminal truncation resulting in 613 aa protein including 12 aa out-of-frame sequence; expected to render a non-functional protein

R616X	Ex14	Nonsense	C-terminal truncation resulting in 616 aa protein; CYP17A1 17α-hydroxylase 0% of WT activity (7) CYP17A1 17,20 lyase activity 0% of WT activity (7)
ssense mutations and insertion/duplication			
T142A	Ex4	Missense	CYP17A1 17α-hydroxylase activity 60% of WT POR (7) CYP17A1 17,20 lyase activity 54% of WT POR (7)
Y181D	Ex5	Missense	CYP17A1 17α-hydroxylase activity 0% of WT POR (21) CYP17A1 17,20 lyase activity 0% of WT POR (21)
G188_V191dup	Ex5	Duplication	CYP17A1 17α-hydroxylase activity 37% of WT POR CYP17A1 17,20 lyase activity 70% of WT POR
A287P	Ex8	Missense	CYP17A1 17α-hydroxylase activity 36% of WT POR (21) CYP17A1 17,20 lyase activity 23% of WT POR (21)
R457H	Ex11	Missense	CYP17A1 17α-hydroxylase activity 40% of WT POR (7) CYP17A1 17,20 lyase activity 21% of WT POR (7)
R498P	Ex12	Missense	CYP17A1 17α-hydroxylase activity 66% of WT POR CYP17A1 17,20 lyase activity 58% of WT POR
C569Y	Ex13	Missense	CYP17A1 17α-hydroxylase activity 28% of WT POR (7) CYP17A1 17,20 lyase activity 13% of WT POR (7)
Y607C	Ex14	Missense	CYP17A1 17α-hydroxylase activity 56% of WT POR (32) CYP17A1 17,20 lyase activity 44% of WT POR (32)
H628P	Ex14	Missense	CYP17A1 17α-hydroxylase activity 35% of WT POR (21) CYP17A1 17,20 lyase activity 21% of WT POR (21)

designates exonic location, IVS (intervening sequence) designates intronic location of the respective *POR* mutation;
POR, wildtype P450 oxidoreductase

Suppl. Table 3. Kinetic parameters (±SEM) of human CYP17A1 17a-hydroxylase and 17,20 lyase activities after yeast co-expression of wild type human CYP17A1 with wild type human P450 oxidoreductase (POR) and POR mutants p.R498P and G188_V191dup, respectively.

	Wild type	**p.R498P**	**p.G188_V191dup**
17α-hydroxylase activity			
V_{max} (pmol/µg * min)	0.224±0.015	0.125±0.013	0.082±0.053
K_m (µM)	0.973±0.189	0.819±0.148	0.960±1.376
Catalytic efficiency (V_{max}/K_m)	0.230	0.153	0.086
% wild type	100	67	37
17,20 lyase activity			
V_{max} (pmol/µg * min)	0.078±0.002	0.066±0.005	0.055±0.004
K_m (µM)	0.922±0.067	1.347±0.217	0.926±0.194
Catalytic efficiency (V_{max}/K_m)	0.085	0.049	0.060
% wild type	100	58	70

Suppl. Table 4. Manifestation of disordered sex development (DSD) according to chromosomal sex and *POR* mutation in patients with PORD (n=30).

DSD (virilization/ undermasulinization)	Karyotype	Mutation 1	Mutation 2	PatNo
N	XX	T142A	Y376L*fsX74*	P26
N	XX	A287P	Del ex U1-1	P13
N	XX	A287P	IVS6 -2A>T	P06
Y	XX	A287P	A287P	P12
Y	XX	A287P	A287P	P15
Y	XX	A287P	A287P	P18
Y	XX	A287P	A287P	P20
Y	XX	A287P	R223X	P29
Y	XX	A287P	H628P	P11
Y	XX	A287P	IVS8 +1G>A	P14
Y	XX	A287P	—	P05
Y	XX	A287P	Dup ex 2-5	P30
Y	XX	C569Y	Y181D	P03
Y	XX	—	—	P02
Y	XX	—	—	P22
Y	XX	R457H	A287P	P01
Y	XX	R498P	R498P	P25
Y	XX	Y87X	—	P21
N	XY	A287P	A287P	P08
N	XY	A287P	A287P	P16
N	XY	A287P	IVS7+2dupT	P28
N	XY	A287P	R616X	P27
N	XY	C569Y	Y181D	P04
Y	XY	A287P	G188_V191dup	P17
Y	XY	A287P	I444H*fsX6*	P19
Y	XY	A287P	IVS6 -2A>T	P07
Y	XY	A287P	V472A*fsX102*	P09
Y	XY	Q455R*fsX544*	IVS7+2dupT	P10
Y	XY	R457H	Y576X	P23
Y	XY	Y607C	E601S*fsX12*	P24

N, no; Y, yes.