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Aromatase Activity is Disrupted by Mutations in P450 Oxidoreductase

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Background: The steroidogenic enzyme aromatase (CYP19A1) is a protein located in endoplasmic reticulum (ER) that catalyzes the conversion of androgens to estrogens. Both deficiency and excess of aromatase activity lead to disease states implicating its role in human biology. Cytochrome P450 (CYP) enzymes in ER use reduced nicotinamide adenine dinucleotide phosphate through cytochrome P450 oxidoreductase (POR) for their metabolic activities. Mutations in POR cause disorders of sexual development due to the deficiencies in several steroid metabolizing enzymes like CYP17A1, CYP21A2 and CYP19A1. The effect of POR mutations on different P450 activities depends on individual partner proteins. So each P450-POR mutant combination should be studied individually. **Objective:** Study the impact of mutations in the flavin binding domain of POR (A115V, T142A, P284L, P284T and Q153R) on CYP19A1 activity, which can potentially influence the estrogen metabolism. **Method:** The WT and mutant human POR proteins were expressed in bacteria and membranes were isolated. Human CYP19A1 was produced as His-tag recombinant protein and purified by Ni^{2+} metal chelate chromatography. POR variants were characterized by standard cytochrome *c* reduction assay and flavin content of proteins was analyzed. Bacterial membranes containing WT or mutant POR along with CYP19A1 were reconstituted into liposomes and the aromatase activity was determined by tritiated water release assay using radiolabeled androstenedione as substrate. Kinetic parameters (Km, Vmax) were calculated for each mutant and compared with WT POR. **Results:** Mutations in the flavin binding domain of POR alter the cytochrome c reduction rate. We found severe effect of POR mutation on CYP19A1 enzyme activity. The POR mutants P284L, P284T, A115V and T142A showed less than 20% activity in supporting CYP19A1 reactions. Interestingly, the POR variant Q153R showed 50% higher activity than WT. **Conclusion:** Our study suggests that alteration in aromatase activity may have an impact on estrogen metabolism. Lower aromatase activities due to POR mutation might affect the fetal androgen metabolism, especially in pregnant women with a male child.

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Mosaic Xq Partial Duplication Leading to Virilisation of an Adolescent Female

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Background: We present a 17-year-old female who presented with a 1 year history of hirsutism, male pattern baldness, marked cystic acne and mild cliteromegaly. She had her menarche at the age of 15 years and continued thereon to have a regular menstrual cycle. She was pubertal on examination (B3, P5, A5) with no neurological deficit. **Objective and hypotheses:** This female presented with marked clinical hyperandrogenism. We initially suspected polycystic ovarian syndrome (PCOS) given her phenotype. Through investigation and characterisation she has been found to have an unusual genetic mutation which we hypothesise is causative of her symptoms. **Method:** Our case had blood taken for baseline endocrinology, genetic testing, provocation testing, 24 h urine for steroid profiling, and an ultrasound scan of her abdomen/pelvis. She was commenced on treatment for PCOS with Yasmin which she has not shown an early response to. **Results:** She was found to have a normal pubertal ultrasound scan, normal baseline endocrinology, normal provocation testing and a normal 24 hour urinary steroid profile. Her microarray revealed she carries a 46XX/47XX+ mosaic karyotype with a supernumerary marker chromosome which was shown to be derived from the long arm of the X chromosome. This additional genetic material contains the androgen receptor gene but does not include the XIST gene and therefore genes present in this marker chromosome would not be subject to x-inactivation. This was found to be a de novo mutation. **Conclusion:** We present a novel case of a de novo genetic mutation that we hypothesise has led to overexpression of the androgen receptor leading to her having increased sensitivity to normal levels of circulating androgens. The resulting severe phenotype mimics PCOS in appearance but with normal blood biochemistry, urinary steroid profile and ultrasound.

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Genotyping Patients with Differences of Sex Development: 25 Years of Investigation of an Italian Population of 308 Cases (194 46,XY and 114 46,XX)

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Background: Differences of sex development (DSDs) (conditions with atypical development of chromosomal, gonadal or anatomic sex) are classified into three groups: sex chromosome DSD, 46,XYDSD and 46,XX DSD. Around 1 newborn in 5000 presents ambiguous genitalia with a major challenge for male or female assignment. The identification of a genetic cause can contribute to a correct diagnosis and to optimize both management and genetic counselling. **Objective and hypotheses:** To describe the results of the diagnostic activity on a large cohort of cases (chromosomal DSD excluded), mostly from the Nord-Est Italian regions referring to our centre in the period 1991–2016. **Method:** Hundred and ninety-four cases with 46, XY DSD and 114 cases of 46,XX DSD where analysed by Sanger sequencing and/or MLPA for the major candidate genes/regions for the specific DSD condition. **Results 46, XY DSD:** A genetic cause was identified in 14 out of the 27 Gonadal dysgenesis **(A)**; in 85 out of