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Characterization of a novel CYP19A1 (aromatase) R192H mutation causing virilization of a 46,XX newborn, undervirilization of the 46,XY brother, but no virilization of the mother during pregnancies

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ABSTRACT

Background: P450 aromatase (CYP19A1) is essential for the biosynthesis of estrogens from androgen precursors. Mutations in the coding region of CYP19A1 lead to autosomal recessive aromatase deficiency. To date over 20 subjects have been reported with aromatase deficiency which may manifest during fetal life with maternal virilization and virilization of the external genitalia of a female fetus due to low aromatase activity in the steroid metabolizing fetal–placental unit and thus high androgen levels. During infancy, girls often have ovarian cysts and thereafter fail to enter puberty showing signs of variable degree of androgen excess. Moreover, impact on growth, skeletal maturation and other metabolic parameters is seen in both sexes.

Objective and hypothesis: We found a novel homozygous CYP19A1 mutation in a 46,XX girl who was born at term to consanguineous parents. Although the mother did not virilize during pregnancy, the baby was found to have a complex genital anomaly at birth (enlarged genital tubercle, fusion of labioscrotal folds) with elevated androgens at birth, normalizing thereafter. Presence of 46,XX karyotype and female internal genital organs (uterus, vagina) together with biochemical findings and follow-up showing regression of clitoral hypertrophy, as well as elevated FSH suggested aromatase deficiency. Interestingly, her older brother presented with mild hypospadias and bilateral cryptorchidism and was found to carry the same homozygous CYP19A1 mutation. To confirm the clinical diagnosis, genetic, functional and computational studies were performed.

Methods and results: Genetic analysis revealed a homozygous R192H mutation in the CYP19A1 gene. This novel mutation was characterized for its enzymatic activity (Km, Vmax) in a cell model and found to have markedly reduced catalytic activity when compared to wild-type aromatase; thus explaining the phenotype. Computational studies suggest that R192H disrupts the substrate access channel in CYP19A1 that may affect binding of substrates and exit of catalytic products.

Conclusion: R192H is a novel CYP19A1 mutation which causes a severe phenotype of aromatase deficiency in a 46,XX newborn and maybe hypospadias and cryptorchidism in a 46,XY, but no maternal androgen excess during pregnancy.

1. Introduction

P450 aromatase (CYP19A1; OMIM 107910; GeneID 1588) is a member of the cytochrome P450 superfamily of enzymes. These membrane bound proteins are involved in the metabolism of drugs and xenobiotics including the biosynthesis of cholesterol and

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steroid hormones. Within this group, P450 aromatase belongs to the subgroup of steroid hydroxylases in the steroid biosynthetic pathway (Miller, 2005; Santen et al., 2009; Conley and Hinshelwood, 2001). P450 aromatase is required for the synthesis of estrogens (C18 steroids) from androgen precursors (C19 steroid) (Fig. 1), and compared to other steroid hydroxylases is highly specific for androgens as substrates (Grumbach and Auchus, 1999; Simpson, 1994, 2004). It catalyzes the conversion of androstenedione to estrone, testosterone to estradiol and 16α-hydroxytestosterone to estriol (Fig. 1) Miller, 2005; Meinhardt and Mullis, 2002. Among these precursors, androstenedione and testosterone are recognized as the most common physiological substrates for P450 aromatase (Conley and Hinshelwood, 2001). Aromatase activity, similar to other microsomal cytochrome P450s requires cytochrome P450 oxidoreductase (POR) Pandey and Flück, 2013; Pandey et al., 2007 and the co-factor reduced nicotinamide adenine dinucleotide phosphate (NADPH) and is located in membranes of endoplasmic reticulum (Conley and Hinshelwood, 2001). Mutations in POR also affect aromatase activity (Pandey et al., 2007; Flück et al., 2011). P450 aromatase is highly conserved among all vertebrates (Fig. 2 and Suppl Fig. 1) (Czajka-Oraniec and Simpson, 2010).

Aromatase is encoded by CYP19A1 that belongs to the family 19 of the cytochrome P450 proteins (Nelson et al., 2004) and is expressed in several tissues including the ovaries, testes, placenta, adipose tissue, and bone osteoblasts (Simpson, 2004). The human *CYP19A1* gene (RefSeq NM_000103.3, 15q21.1) spans about 123 kb and consists of a coding region of 9 exons (~30 kb, exons 2–10). A large number of alternative first exons and nine different transcriptional start sites with individual promoters for *CYP19A1* are known (Grumbach and Auchus, 1999; Meinhardt and Mullis, 2002). Various tissues have their own promoters allowing for a very complex, tissue specific regulation of CYP19A1 expression and thus estrogen biosynthesis. However, there is only one aromatase protein expressed in the various human tissues (NP_000094) (brain, gonads, placenta, adipose tissue, bone, etc.) and its function is the same although availability of different substrates may vary between tissues (Meinhardt and Mullis, 2002; Lin et al., 2007).

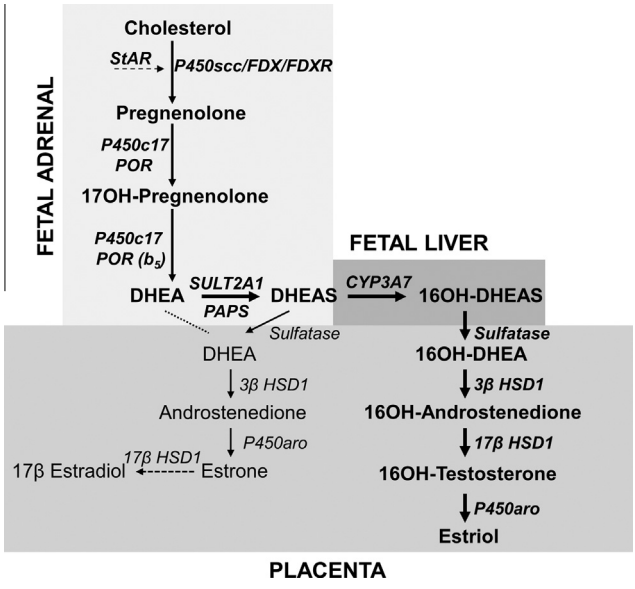

Fig. 1. The fetal–placental unit. Steroid biosynthesis and metabolism of the fetal adrenal in humans showing the role of CYP19A1 in the production of estrogens. CYP17A1 converts pregnenolone to 17OH-pregnenolone and dehydroepiandrosterone (DHEA) which is then metabolized to estrogens either directly in the placenta or through intermediates formed in the fetal liver and then sent to the placenta. All estrogen formation requires CYP19A1 activity.

The catalytic process leading to the aromatization of androgens is quite complex and consists of three steps involving transfer of three pairs of electrons and oxygen consumption. The first two steps of aromatase reaction involve hydroxylations of the C19 methyl group while the third step, aromatization of the A-ring of steroid substrate, is specific for aromatase. Recently, several X-ray crystal structures of CYP19A1 bound to androstenedione and inhibitors have been described (Lo et al., 2013; Ghosh et al., 2012, 2010, 2009).

Aromatase deficiency (OMIM 613546) is a very rare autosomal recessive disorder which was described in about 24 subjects to date (Lin et al., 2007; Bilezikian et al., 1998; Conte et al., 1994; Harada et al., 1992; Ito et al., 1993; Morishima et al., 1995; Shozu et al., 1991; Bouillon et al., 2004; Carani et al., 1997; Deladoey et al., 1999; Ludwig et al., 1998; Mullis et al., 1997; Portrait-Doyen et al., 1996; Belgorosky et al., 2003; Hauri-Hohl et al., 2011; Herrmann et al., 2002; Lanfranco et al., 2008; Maffei et al., 2004, 2007; Pepe et al., 2007; Pura et al., 2003; Richter-Unruh, 2008). The first case of aromatase deficiency has been reported in 1991 (Shozu et al., 1991). Generally, mutations in the coding region of the *CYP19A1* gene lead to a decrease or loss of aromatase activity and therefore estrogen deficiency (Czajka-Oraniec and Simpson, 2010). Mostly, single base changes are found in exons V, IX and X; however, point mutations are also described in other exons (Fig. 3) Belgorosky et al., 2009. In addition, deletions as well as insertions and several splice site mutations have been described to cause aromatase deficiency (Belgorosky et al., 2009). Moreover, a −41 bp CYP19A1 promoter I.1 variation which was shown to lower gene transcription has been identified recently in a patient with aromatase deficiency (Hauri-Hohl et al., 2011). By contrast, no activating CYP19A1 mutations due to changes in the coding region of the gene have been described so far, but aromatase excess (OMIM 139300) may result from enhanced gene expression caused by genomic rearrangements at 15q21. These rearrangements include duplications, deletions and inversions that create chimeric genes consisting of the coding exons of CYP19A1 and promoter-associated exons of neighbouring genes (Demura et al., 2007; Fukami et al., 2011, 2013; Shihara et al., 2013; Shozu et al., 2003). Furthermore, in addition to these disease-causing genetic mutations, more than 80 genetic polymorphisms have been described in the CYP19A1 gene (Ma et al., 2005). P450 aromatase deficiency manifests during fetal life in both sexes. Mothers carrying an affected fetus suffer from progressive virilization during pregnancy due to their inability to aromatize androgens derived from fetal adrenal in the placenta (Fig. 1). As a consequence of low aromatase activity in the fetal–placental unit, elevated androgen concentrations in the female fetus lead to various degrees of virilization of the female external genitalia at birth while the male external genitalia remains normal. During infancy and childhood there are either no symptoms of aromatase deficiency (mostly boys), although girls may manifest with abdominal symptoms of ovarian cysts due to subtle changes in the hypothalamic–pituitary–gonadal axis because of missing feedback regulation of low levels of estrogens that are obviously needed during prepubertal years (Janner et al., 2012). At puberty, lack of estrogens results in hypergonadotropic hypogonadism with failure of spontaneous pubertal development necessitating supplementation therapy. The impact of aromatase deficiency on growth, skeletal maturation and bone homeostasis, as well as changes in insulin sensitivity and lipid profile has been described in previous studies (Czajka-Oraniec and Simpson, 2010; Belgorosky et al., 2009). As a result of variable degree of androgen excess, acne and hirsutism may ensue and these signs of virilization mostly worsen with age. Bone age is typically delayed because estrogens are crucial for epiphyseal maturation and closure, and decreased bone mineral density can be observed (Ito et al., 1993; Mullis et al., 1997; Belgorosky et al., 2003).