Human Aromatase Deficiency

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Abbreviations

ArKO Aromatase knockout
BMD Bone mineral density
CRE cAMP response element
CREB cAMP response element binding protein
CYP19A1 Aromatase gene
DHEAS Dehydroepiandrosterone sulfate
E2 Estrogen
ERT Estrogen replacement therapy
GH/IGF1 axis Somatotropic axis
GnRH Gonadotropin releasing hormone
ND Not determined
SF1 Steroidogenic factor 1
WT Wild type

Several molecular CYP19A1 gene alterations associated with cytochrome P450 aromatase (cP450arom) deficiency have been described in both females and males. This has contributed considerably to the understanding of cP450arom activity in different tissues, influencing sexual differentiation, patterns of gonadotropin secretion by the hypothalamic–pituitary axis, reproductive capacity, lipid metabolism, insulin sensitivity, and skeletal maturation and growth. Studies of aromatase deficiency in humans were complemented with studies in mouse knockout models that demonstrated the role of estrogens in several tissues. In this article, molecular studies, the clinical phenotypic variations throughout life in both sexes, gonadal function, fertility, and gender identity are addressed.

Characteristics of cP450arom

cP450arom is the enzyme that catalyzes the synthesis of estrogens from androgens. Therefore, the activity of this enzyme complex affects both androgen metabolism and estrogen synthesis. The biological importance of the aromatase complex is related not only to its role in the synthesis of estrogens, but also to its potential influence on the balance of the androgen–estrogen ratio in different tissues. In the 1980s, the human aromatase protein was purified from placental microsome and the aromatase activity was demonstrated by conversion of androstenedione to estrone (**Pasanen and Pelkonen, 1981**; **Mendelson et al., 1985**; **Kellis and Vickery, 1987**; **Osawa et al., 1987**; **Hall et al., 1987**). The aromatase complex is expressed in the endoplasmic reticulum of cells and consists of two components: cP450arom and

a ubiquitous flavoprotein, NADPH-cytochrome P450 reductase. Although androstenedione and testosterone are the most common and the most physiologically important substrates, 16OH-dehydroepiandrosterone sulfate (DHEAS) arising from fetal liver hydroxylation of fetal adrenal DHEAS is an important substrate for placental estriol synthesis during pregnancy, in both humans and higher primates (**Belgorosky and Rivarola, 2004**). In humans, cP450arom appears to be the product of a single gene—CYP19A1—located on chromosome 15q21.1. The size of the aromatase gene is larger than 123 kb; however, the protein-coding sequence is contained within 9 exons (2 − 10) spanning approximately 35 kb of DNA (**Means et al., 1989**). Several promoters are found within a 90-kb region upstream of the coding region associated with multiple first exons that are involved in tissue-specific expression. These exons, which are not translated, generate alternative splicing in such a fashion that the coding region, and hence the protein sequence, is conserved in every tissue (**Fig. 1**).

The three-dimensional crystal structure of human aromatase was identified with purified aromatase protein extracted from placental microsome (**Ghosh et al., 2009**). The human aromatase consists of a heme group and a polypeptide chain of 503 amino-acid residues and exhibits high substrate specificity in catalyzing the synthesis of estrogens from androgen precursors. The most highly conserved region consists essentially of a four-helix bundle, two sheets, and the heme-binding region (**Graham-Lorence et al., 1995**).

cP450arom is expressed in a number of tissues, including the syncytiotrophoblast layer of the placenta, gonads, adipose tissue, bone, brain (including the hypothalamus, hippocampus, and amygdala), coronary arteries, and various fetal tissues, such as liver, skin, intestine, testis, and ovary (**Graham-Lorence et al., 1995**). CYP19A1 gene expression is subjected to multifactor regulation by a diverse group of hormones and factors that differ markedly between tissues. Thus, a strict control over tissue-specific expression is necessary for proper regulation of estrogen synthesis during fetal development, as well as during postnatal life.

The human placenta (syncytiotrophoblast layer) is capable of aromatizing large amounts of androgen precursors produced by the fetal and maternal adrenal glands into estrogens. At term, approximately equal amounts of estrogens are produced from circulating maternal DHEAS and fetal DHEAS. The rate of estrogen production and the level of circulating estrogens increase markedly during pregnancy. Following fertilization, concentrations of 17β-estradiol increase gradually to a range of 6–30 ng/mL at term (Tal, 2000). This enzyme protects the fetus from virilization even in the presence of large amounts of aromatizable androgens. One of the clinical signs of aromatase deficiency may manifest during pregnancy, as a pregnant mother of an aromatase-deficient fetus becomes virilized (see later).

Reported Human Mutations

Since aromatase deficiency was first described by **Shozu et al. (1991)**, 37 cases (26 females and 11 males) have been published (PubMed indexed). These cases are listed in **Tables 1** and **2**.

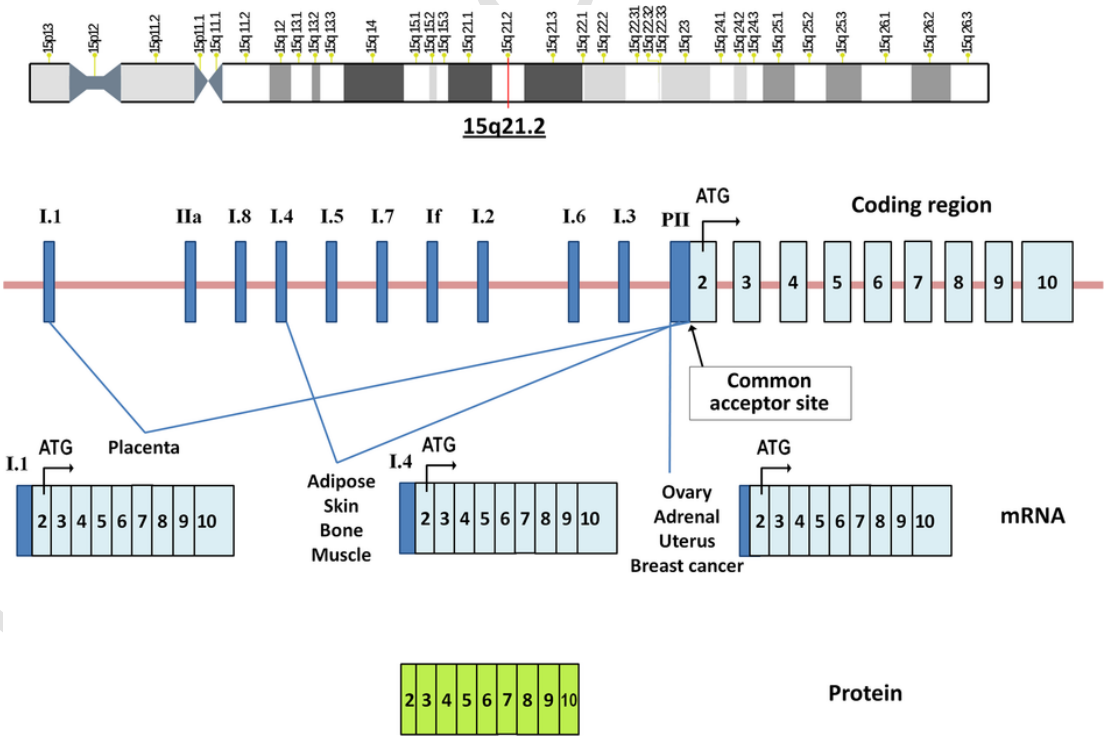

Fig. 1 Representation of the CYP19A1 splicing mechanism.

Table 1 Molecular defects of the *CYP19* gene, in vitro aromatase activity of mutants, and clinical phenotype in published female deficient subjects

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Shozu et al. (1991), **Harada et al. (1992)**	c.[743 + 2T > C];[743 + 2T > C]	IVS6 + 2T > C Mutation at consensus 5′splice site (5′ss) in intron 6 which results in the use of a cryptic site downstream and in the in-frame incorporation of 87 nucleotides. Then, a protein with additional highly hydrophobic 29 amino acids will be generated	Less than 0.3% of wild type (WT) control. The extra amino acids might induce conformational changes and reduction of activity	Ambiguous genitalia at birth
Ito et al. (1993), **Conte et al. (1994)**	c.[1303C > T];[1310G > A]	p.Arg435Cys: Arg435 is highly conserved within the heme-binding region among cP450s p.Cys437Tyr: The cysteine 437 is the critical cysteine in the heme-binding domain and is the most conserved residue in all cP450 proteins	p.Arg435Cys mutant protein showed approximately 1.1% of WT activity p.Cys437Tyr mutant protein was inactive	Ambiguous genitalia at birth Delayed bone age Absent puberty and pubertal growth spurt. Ovarian cyst and virilizing signs at puberty
Morishima et al. (1995)	c.[1123C > T];[1123C > T]	p.Arg375Cys	0.2% of WT activity Protein modeling studies suggest that the affected region may affect the substrate access channel to the membrane	Ambiguous genitalia at birth Absent puberty and pubertal growth spurt According bone age Ovarian cyst and virilizing signs at puberty
Mullis et al. (1997), **Janner et al. (2012)**, **Burckhardt et al. (2015)**	c.[1224delC];[296 + 1G > A]	p.Lys409AsnfsTer36: A base pair deletion (C) in codon 408 (exon 9). A frameshift occurs resulting in a nonsense codon IVS3 + 1G > A: G > A change at the canonical donor splice site in intron 3. The splice site is ignored, and a stop codon arises 3 bp downstream. No active transcript was found	ND. However, no aromatase activity is expected	Ambiguous genitalia at birth Ovarian cysts during infancy and childhood Low BMD at 3.5 years of age Follow-up from 3.5 to 15 years under ERT. Normalization of growth and bone maturation with different threshold for estradiol action on FSH regulation

Table 1 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Ludwig et al. (1998)	c.[1108G > A];[1108G > A]	p.Val370Met	ND	Ambiguous genitalia at birth Lipid abnormalities early in life
Belgorosky et al. (2003), **Pepe et al. (2007)**, **Guercio et al. (2009)**	c.[1236delA]; [628G > A]	p.Glu412AspfsTer33: a bp deletion (A) in glu 412 (exon 9) causing a frame-shift generating a stop codon 98 bp downstream. Then, a truncated protein with an altered heme-binding domain might be generated c.628G > A: G to A at the consensus donor splice site in exon 5–intron 5 junction causing in-frame exon 5 skipping	The peptide expressed from p.Glu412AspfsTer33 is expected to be completely inactive as the frame shift altered the highly conserved heme-binding domain The protein without amino acids 151–209 encoded by exon 5 does not have aromatase activity As the –E5 mRNA might be expressed as a splice variant in normal tissues, some + E5 transcripts from the c.628G > A allele might explain the partial Aromatase deficiency phenotype (**Lin et al., 2007**; **Pepe et al., 2007**)	Ambiguous genitalia at birth Delayed bone age during childhood Ovarian cysts from infancy to puberty Spontaneous breast development at 7.7 years Virilizing signs at puberty Normal BMD during follow-up Insulin resistance since 7.2 years of age and Type 2 diabetes during follow-up
Lin et al. (2007)	c.[1303C > T];[1303C > T]	p.Arg435Cys	0.7%–1.5% of WT activity The arginine at position 435 forms part of the absolutely conserved heme-binding motif and is absolutely conserved This mutation destabilizes the active site of the aromatase enzyme, which disrupts the efficient functioning of the enzyme complex	Ambiguous genitalia at birth Delayed bone age Ovarian cysts Spontaneous but nonprogressive breast development
Lin et al. (2007) (siblings)	c.[700_702delTTC];[700_702delTTC]	p.Phe234del: Deletion of one of two phenylalanine at position 234–235 in exon 6. This pair of amino acids is highly conserved in cP450 aromatase of other species	16%–19% of WT activity. Substrate binding seems to be largely unaffected but maximal enzymatic activity is reduced consistent with partial aromatase activity	Sibling 1: Ambiguous genitalia at birth. Low androgen levels in infancy Sibling 2: Pubertal subject raised male. Male gender identity and male gender role behavior. Spontaneous puberty and gynecomastia. Gender dysphoria delayed bone age and ovarian cysts

Table 1 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Lin et al. (2007)	c.[452-621_628 + 803del]; [452-621_628 + 803del]	1600 bp deletion at genomic DNA. This deletion is predicted to remove exon 5 resulting in the in-frame deletion 59 amino acids	Complete loss of function	Ambiguous genitalia at birth Absent puberty and delayed bone age Small ovaries Low androgen levels Moderate dyslipidemia
Hauri-Hohl et al. (2011)	c.[1374A > G]; c.[− 41C > T]	De novo p.Asn411Ser Paternally inherited C > T mutation in the placenta promoter 41 base pairs upstream of exon 1	Functional studies demonstrated that p.Asn411Ser is a loss-of-function mutation. Vmax, Km and Kcat demonstrated null catalytic activity Cell-culture system for placenta promoter functional studies demonstrated a 50% significant reduction in the presence of c.-41C > T mutation and the mutant promoter has no dominant negative effect on WT	Clitoromegaly at birth Normal ovaries Normal serum prepubertal androgens and gonadotropin levels
Verma et al. (2012)	c.[1303C > T];[1303C > T]	p.Arg435Cys	0.7%–1.5% of WT activity The arginine at position 435 forms part of the absolutely conserved heme-binding motif and is absolutely conserved This mutation destabilizes the active site of the aromatase enzyme, which disrupts the efficient functioning of the enzyme complex	Ambiguous genitalia at birth High FSH levels during infancy At 13.5 years hirsutism Spontaneous telarche Obesity, acanthosis nigricans, and tall stature. High gonadotropin levels and bilateral cystic ovaries. Persistent hyperinsulinemia despite ERT and metformin supplementation. Normal lipid profile. Low BMD at 16 years

Table 1 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Ludwikowski et al. (2013)	c.[422G > A]; [422G > A]	p.Trp141Ter	ND	Ambiguous genitalia at birth Elevated serum LH and FSH along with low estradiol and androgen levels, and normal ovaries in infancy (20 months)
Ludwikowski et al. (2013)	c.[422G > A]; [422G > A]	p.Trp141Ter	ND	Ambiguous genitalia at birth Elevated serum LH and FSH along with low estradiol and androgen levels, and normal ovaries in childhood (5 years)
Gagliardi et al. (2014)	c.[915_941dup] Homozygous or hemizygous	Duplication of nine amino acids (p.Ala306_Ser314dup) in the protein This duplication occurs within the aromatase α-helix Prediction tools of protein secondary structure suggest a break of the α-helix around the middle, turning a continuous helix into the structure of helix coil-helix. This would be likely to disrupt substrate and cofactor binding resulting in a lack of estrogen synthesis	ND	Ambiguous genitalia at birth Absent puberty Hypoplastic uterus and bilateral streak ovaries At 32 years tall stature, eunuchoidal proportions, abdominal obesity, impaired fasting glucose, dyslipidemia and insulin resistance, moderate hepatic steatosis Osteopenia/ osteoporosis Bone fracture at 25 years

Table 1 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Bouchoucha et al. (2014)	c.[575G > A]; [575G > A]	p.Arg192His	p.Arg192His mutant was found to have markedly reduced aromatase activity. Both the km and the Vmax were adversely affected. The catalytic efficiency of metabolizing androstenedione was reduced to 19%. Modeling of the structure of the novel p.Arg192His variant of the CYP19A1 protein revealed a crucial role of the arginine 192 residue in substrate binding as well as catalysis	Ambiguous genitalia with elevated serum androgen levels at birth, normalizing thereafter Persistently elevated serum FSH levels
Marino et al. (2015)	c.[628G > A]; [628G > A]	G to A at the consensus donor splice site in exon 5–intron 5 junction causing in-frame exon 5 skipping	The protein without amino acids 151–209 encoded by exon 5 does not have aromatase activity (**Lin et al., 2007**; **Pepe et al., 2007**)	Ambiguous genitalia at birth Spontaneous breast development at 11 years. Spontaneous menarche at 12 years with regular menses. Clinical and biochemical hyperandrogenism, high basal serum gonadotropin levels, and multiple ovarian cysts at puberty
Marino et al. (2015)	c.[628G > A]; [242A > G]	c.628G > A: G to A at the consensus donor splice site in exon 5–intron 5 junction causing in frame exon 5 skipping p.Tyr81Cys. Residue highly conserved	c.628G > A: The protein without amino acids 151–209 encoded by exon 5 does not have aromatase activity (**Lin et al., 2007**; **Pepe et al., 2007**) p.Tyr81Cys: Predicted to be "probably damaging" on the basis of SIFT scores of 0.00 and PolyPhen2 scores of 1.0 indicating that it is most likely to be deleterious The p.Tyr81Cys mutant was found to have 14.3% aromatase activity and showed lower Vmax, higher Km, and lower catalytic efficiency (Vmax/Km) as compared with wild-type aromatase (**Chen et al., 2015**)	Ambiguous genitalia at birth At 7.7 years multiple ovarian cysts and bone age delay, normal serum androgen and high basal serum gonadotropin levels

Table 1 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Marino et al. (2015)	c.[628G > A]; [628G > A]	G to A at the consensus donor splice site in exon 5–intron 5 junction causing in-frame exon 5 skipping	The protein without amino acids 151–209 encoded by exon 5 does not have aromatase activity (**Lin et al., 2007**; **Pepe et al., 2007**)	Ambiguous genitalia at birth Bone age delay Spontaneous breast development at 9 years with large ovarian cysts high basal gonadotropin and testosterone levels at puberty. Normal OGTT
Marino et al. (2015)	c.[574C > T]; [574C > T]	p.Arg192Cys: residue highly conserved	ND SIFT tool predicted this variant to affect protein function with a highly deleterious tolerance index score of 0.00 The mutation was predicted to be probably damaging with a score of 1.000 (sensitivity 0.00; specificity 1.00) using the structure based approach PolyPhen-2	Ambiguous genitalia at birth Normal serum androgens and high basal gonadotropin levels at 3 years Normal OGTT
Marino et al. (2015)	c.[574C > T]; [1369C > T]	p.Arg192Cys: residue highly conserved p.Arg457Ter	ND	Ambiguous genitalia at birth Spontaneous breast development at 10 years with enlarged ovaries. Normal serum basal gonadotropin and androgens levels at puberty Normal OGTT
Saraco et al. (2015)	c.[1263 + 5G > A];[1263 + 5G > A]	IVS9 + 5G > A The mutation is localized five bases from the 5′ beginning of intron 9 Several splicing prediction programs confirmed that the splicing donor site disappears in the presence of the mutation, resulting in retention of intron 9	Abnormal spliced mRNA that includes intron 9 was confirmed by RT-PCR of total RNA from patient's lymphocytes and with Splicing Assays The presence of an in-frame stop codon 18 bp downstream of the splice junction in intron 9 results in the synthesis of a truncated (46-kDa) and inactive aromatase protein	Ambiguous genitalia Normal ovaries and uterus at 4 years At 13.5 years delayed bone age, small uterus and bilateral ovarian cysts, primary amenorrhea, elevated basal serum LH and FSH levels. Low BMD under ERT at 16.4 years

Table 1 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Akçurin et al. (2016)	c.[568insC]; [568insC]	p. Leu190ProfsTer9	ND	Clitoromegaly, partial labial fusion, and hyperandrogenism at birth. Severe neonatal hypoxic-ischemic encephalopathy Normal serum androgens and gonadotropins levels at 7 years. Hypoplastic ovaries
Akçurin et al. (2016)	c.[568insC]; [568insC]	p.Leu190ProfsTer9	ND	Ambiguous genitalia and hyperandrogenism at birth At 5 years mild clitoromegaly, normal serum androgens and high basal gonadotropin levels Hypoplastic ovaries
Akçurin et al. (2016)	c.[568insC]; [568insC]	p.Leu190ProfsTer9	ND	Ambiguous genitalia and hyperandrogenism at birth At 2.2 years clitoromegaly, normal serum androgens and high basal gonadotropin levels Hypoplastic ovaries.
Zhu et al. (2016)	c.[264delG]; [1036_1037insTTCTGTTTCCACAGGTGAGAGAG]	p.Trp88Ter; p.Lys346IlefsTer21	Modeling studies based on reported structures indicated that both mutations lead to a loss of aromatase catalytic residues that form the active site and access channel, as well as the heme-binding region of the enzyme encoded by exon 10 Aromatase enzyme activity revealed nearly complete abolishment of enzyme activity with very low estrone conversion levels from androstenedione substrate	Ambiguous genitalia at birth

ND, not determined; *ERT*, estrogen replacement therapy; *BMD*, bone mineral density; *OGTT*, oral glucose tolerance test.

Table 2 Molecular defects of the *CYP19* gene, in vitro aromatase activity of mutants and clinical phenotype in published male deficient subjects

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Morishima et al. (1995), **Bilezikian et al. (1998)**	c.[1123C > T];[1123C > T]	p.Arg375Cys in a highly conserved region	0.2% of WT activity Protein modeling studies suggest that the affected region may be important in anchoring the region of the protein proximal to the substrate access channel to the membrane	Continuous linear growth, delayed bone maturation, tall stature, eunuchoid body proportions Cis gender, heterosexual, modest libido Macroorchidism Increased basal gonadotropins and testosterone Hypertension, obesity Dyslipidemia, insulin resistance Osteopenia
Carani et al. (1997)	c.[1094G > A];[1094G > A]	p.Arg365Gln	0.4% of WT activity	Continuous linear growth, delayed bone maturation, tall stature, eunuchoid body proportions Moderate bone pain. Genu valgum Cisgender identity and sexual orientation. Normal libido Infertility Microorchidism Normal basal testosterone, slightly elevated FSH and LH in the upper normal range Overweight. Dyslipidemia Normal OGTT
Deladoëy et al. (1999), **Bouillon et al. (2004)**	c.[469delC];[469delC]	p.Val158PhefsTer20 C base deletion in exon 5 causing a frame shift and a premature stop codon after 21 codons	Not determined (ND) However, the resultant peptide does not contain the substrate-binding pocket (I helix), the electron-accepting site and the heme-binding site. An inactive protein would be expected	At birth: Normal genitalia, and serum AMH levels Normal serum basal and GnRH stimulated FSH levels at 2 months of age At 16 years: Tall stature, delayed bone maturation Normal virilization and testicular volume High serum basal testosterone, and normal serum basal gonadotropin levels Osteoporosis

Table 2 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Herrmann et al. (2002)	c.[628-3C > A];[628-3C > A]	IVS5-3C > A C to A transition at bp − 3 at the splicing acceptor site in exon 6. Exon 6 is completely excised leading to a frame shift and premature stop codon 8 nucleotides downstream the end of exon 5	ND The resulting peptide most likely will not be processed, but even if it were, it would not result in a functional aromatase because it lacks the substrate-binding pocket, the electron-accepting site and the heme-binding site (**Herrmann et al. 2002**)	Continuous linear growth, tall stature, eunuchoid body proportion Genu valgum, kyphoscoliosis, pectus carinatus Cisgender, heterosexual, and normal libido Normal genitalia and testicular volume Increased serum basal testosterone and FSH, normal serum LH levels. Azoospermia Obesity. Insulin resistance. Osteoporosis
Maffei et al. (2004)	c.[628G > A];[628G > A]	G to A transition in the last nucleotide in exon 5 The mutated DNA will generate an mRNA that includes the intron 5 sequence which contains an in-frame stop codon 30 bp downstream the splice junction	ND A truncated and inactive protein lacking the heme-binding domain would be expected	Continuous linear growth, delayed bone maturation, tall stature, eunuchoid body proportions Diffuse bone pain, genu valgum Cisgender, heterosexual, referred normal libido Bilateral cryptorchidism (surgery unsuccessful at 6 years). Small inguinal testes, total germ depletion (biopsy) Normal serum basal LH and testosterone, and increased FSH levels Overweight, dyslipidemia. Type 2 diabetes Osteoporosis

Table 2 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Maffei et al. (2007)	c.[380T > G];[1124G > A]	p.Met127Arg; p.Arg375His	In vitro analysis demonstrated a reduction of aromatase activity when the two mutations were expressed separately or coupled.p.Arg375His showed aromatase activity of 7%. Aromatase activity decreased to 0% when the two mutations were coupled	Continuous linear growth, delayed bone maturation, tall stature Diffuse bone pain, genu valgum Cisgender, heterosexual, normal libido Normal testicular volume Normal serum LH and testosterone, increased FSH levels Focal hypospermatogenesis on testicular biopsy Obesity, acanthosis nigricans, hepatomegaly. Moderate dyslipidemia, insulin resistance Osteoporosis
Lanfranco et al. (2008)	c.[312_334del]; [1263 + 1G > A]	p.Phe312LeufsTer49: 23 bp deletion in exon 4 that would be expected to cause a frame shift with a premature stop codon at nucleotide 361 in exon 4 IVS9 + 1G > T: point mutation in the first nucleotide of intron 9 that would lead to an aberrant splicing of the mRNA	A truncated and inactive protein lacking the heme-binding domain would be expected from the c.312_334del allele	Continuous linear growth, delayed bone maturation, tall stature, eunuchoid body proportions, genu valgum Cisgender, normal sexual orientation Normal testicular volume. Right cryptorchidism surgery at 3 years Mild asteno-teratozoospermia. Increased serum basal FSH with normal LH and testosterone levels Overweight, insulin resistance. Mild dyslipidemia Osteoporosis

Table 2 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Baykan et al. (2013)	c.[1124G > A];[1124G > A]	p.Arg375His Previously reported (**Maffei et al., 2007**)		Continuous linear growth, delayed bone maturation, tall stature, eunuchoid body proportions Bone pain, fractures Macroorchidism Normal serum gonadotropin with increased testosterone levels Normal sperm count Overweight. Dyslipidemia Normal OGTT. Hepatosteatosis. Osteoporosis
Bouchoucha et al. (2014)	c.[575G > A];[575G > A]	p.Arg192His Amino acid conserved across species and involved in substrate access and catalysis	p.Arg192His mutant was found to have markedly reduced aromatase activity. Both the km and the Vmax were adversely affected. The catalytic efficiency of metabolizing androstenedione was reduced to 19%. Modeling of the structure of the novel p.Arg192His variant of the CYP19A1 protein revealed a crucial role of the arginine 192 residue in substrate binding as well as catalysis	Mild hypospadias (glans), normal-length phallus, bilateral inguinal testes Normal serum FSH, LH, testosterone, AMH, and inhibin B levels
Chen et al. (2015)	c.[384A > G];[1494T > C]	p.Tyr81Cys; p.Leu451Pro Both the Tyr81 and Leu451 residues were highly conserved in vertebrate aromatase orthologs, and were also conserved in human aromatase paralogs	Three-dimensional modeling predicted that the p.Tyr81Cys and p.Leu451Pro mutations would probably result in loss of aromatase function In-cell aromatase activity assay: p.Tyr81Cys mutant was found to have 14.3% wild-type activity, whereas the p.Leu451Pro mutant was found to have 3.1% wild-type activity p.Tyr81Cys mutant showed lower Vmax, higher Km, and lower catalytic efficiency p.Leu451Pro mutant had lower Vmax, Km and catalytic efficiency	Continuous linear growth, delayed bone maturation, tall stature, eunuchoid body proportions, genu valgum Normal libido Normal testicular volume Normal spermogram Increased serum basal FSH, with normal LH and testosterone levels Overweight, acanthosis nigricans, dyslipidemia and severe steatohepatitis Impaired glucose tolerance and hyperinsulinemia Osteopenia

Table 2 (Continued)

Report	*Gene mutations*	*Description*	*Aromatase activity*	*Phenotype*
Miedlich et al. (2016)	c.[628G > A];[628G > A]	Previously reported (**Pepe et al., 2007**)		Continuous linear growth, delayed bone maturation, unfused growth plate, arachnodactyly Normal libido Normal testicular volume Normal serum FSH and LH levels with upper normal basal testosterone Low BMI. Normal OGTT Osteoporosis

ND, not determined; *BMI*, body mass index; *OGTT*, oral glucose tolerance test.

Several deleterious mutations in the coding region of the CYP19A1 gene were reported in aromatase deficient patients, comprising point mutations, deletions, insertions, splice site mutations, as well as a placental promoter I.1 variation that reduced gene transcription.

Tables 1 and **2** describe the molecular defects, in-vitro aromatase activity of mutants, and clinical phenotype in female and male aromatase-deficient subjects, respectively. Variations in phenotype are compared with in-vitro functional derangements of mutants. Data reported suggest some genotype–phenotype correlation as lower cP450arom activity was associated with a more severe phenotype. However, phenotype is also dependent on sex and age.

Pregnancy, the Fetus, and Newborns

The active human placental aromatization of androgens protects the fetus against the virilizing action of fetal androgens. In congenital aromatase deficiency, the overload of androgens may cause signs of maternal virilization (acne, deep voice, clitoris enlargement) during pregnancy, and this might alert obstetricians to the possibility of this diagnosis. After delivery these symptoms usually disappear gradually (Shozu; **Morishima et al., 1995**; **Mullis et al., 1997**; **Ludwig et al., 1998**; **Deladoëy et al., 1999**; **Herrmann et al., 2002**; **Belgorosky et al., 2003**; **Lin et al., 2007**; Richter-Unruh, 2008; **Hauri-Hohl et al., 2011**; **Verma et al., 2012**; **Ludwikowski et al., 2013**; **Marino et al., 2015**; **Akçurin et al., 2016**; **Zhu et al., 2016**; **Miedlich et al., 2016**). Nevertheless, this finding is not always present, since about 1% of normal placental aromatase activity seems enough to prevent virilization of the mother. Therefore, CYP19A1 mutations that retained partial activity did not lead to maternal virilization during pregnancy (**Mullis et al., 1997**; **Grumbach and Auchus, 1999**). Patient data suggest that estrogen synthesis in the blastocyst, fetus, and placenta is not essential either for normal embryonic and fetal development and survival or in the physiology of the pregnant woman (**Conte et al., 1994**; **Morishima et al., 1995**). Onset of labor has been described as spontaneous (Shozu; **Mullis et al., 1997**; **Deladoëy et al., 1999**; **Belgorosky et al., 2003**) and newborns were born full term with adequate weight for gestational age (Shozu; **Conte et al., 1994**; **Morishima et al., 1995**; **Mullis et al., 1997**; **Deladoëy et al., 1999**; **Belgorosky et al., 2003**; **Bouchoucha et al., 2014**; **Zhu et al., 2016**).

In the female fetus, placental aromatization of androgens is particularly important to avoid an effect of androgens on the differentiation of the external genitalia. In a newborn with 46,XX ambiguous genitalia, aromatase deficiency should be considered among other entities after ruling out the diagnosis of congenital adrenal hyperplasia (CAH) because of its high incidence. Moreover, before the definitive diagnosis of aromatase deficiency was made, some patients were assumed to have CAH and treated as such (**Verma et al., 2012**; **Saraco et al., 2015**).

In most female cases of aromatase deficiency described in the literature, ambiguous genitalia, with various degrees of masculinization of the external genitalia, were reported. As expected, in all these cases, gonads were nonpalpable and differentiation of the female internal genitalia was not affected. Milder genital manifestations, such as clitoral hypertrophy or partial fusion of the labia, have been described in four females (**Lin et al., 2007**; **Hauri-Hohl et al., 2011**; **Marino et al., 2015**; **Akçurin et al., 2016**). The discordant presentation of mild androgenization with the complete lack of enzyme activity described by Lin is difficult to explain and it is a matter of speculation. **Hauri-Hohl et al. (2011)** described a newborn with transient mild hypertrophy of the clitoris having a loss-of-function missense mutation in CYP19A1 combined with the first-described variant of the placenta promoter with a significant reduction in function. This phenotype might represent a placenta-specific, prenatally limited component of aromatase deficiency occurring in utero only.

Even though aromatase deficiency manifests during fetal life in both sexes, external genitalia in 46,XY newborns remain normal and there are no symptoms of aromatase deficiency during infancy and childhood in most boys (**Deladoëy et al., 1999**). In only one boy

reported by **Bouchoucha et al. (2014)** was the presence of glandular hypospadias and bilateral cryptorchidism with inguinal testes described; however, causality between the CYP19A1 mutation and the genital anomaly in this patient remains elusive.

An endocrinological profile has been described in some female cases with aromatase deficiency during the first month of postnatal life. Low estrogen levels along with high androgen levels were found in the cord serum and serum androgen levels returned rapidly to normal after delivery in some cases (**Shozu et al., 1991**; **Deladoëy et al., 1999**). Extremely high serum LH and FSH levels resulting in high serum androgen levels were reported in two aromatase-deficient newborn girls (**Belgorosky et al., 2003**; **Akçurin et al., 2016**). This abnormal serum gonadotropin pattern might reflect a central change in the activity of the gonadotropin releasing hormone (GnRH) pulse generator and/or an effect at the pituitary level, presumably induced by increased androgens and aromatase deficiency during fetal and neonatal life. **Bouchoucha et al. (2014)** described a female newborn who presented with elevated serum testosterone and androstenedione levels at birth that normalized thereafter. Normal serum testosterone levels were reported in an affected girl of 3 days (**Conte et al., 1994**). No data exist on gonadotropin levels during the newborn period in affected boys. Very high serum-free testosterone and androstenedione levels at 2 weeks of postnatal life followed by a decrease to the normal range during the first month of life was reported in only one affected newborn boy (**Deladoëy et al., 1999**). At 4 weeks after birth, the estradiol levels were low; the androstenedione level was decreasing toward the normal level for age and sex, whereas the free testosterone level had already dropped to within the normal range.

Follow-Up

In almost all cases of aromatase deficiency the clinical phenotype was reported only once, when initial diagnosis was made. Only a few reports describe longitudinal clinical and biochemical findings of the affected subjects of both sexes (**Conte et al., 1994**; **Bilezikian et al., 1998**; **Bouillon et al., 2004**; **Guercio et al., 2009**; **Verma et al., 2012**; **Janner et al., 2012**; **Burckhardt et al., 2015**; **Marino et al., 2015**; **Saraco et al., 2015**). Fortunately, a growing number of reports has broadened the spectra of phenotypes and genetic mutations underlying this rare disorder and increased the knowledge on aromatase physiology.

The clinical phenotype of aromatase deficiency includes changes in the hypothalamic–pituitary–gonadal axis, ovarian cyst formation, alterations of growth, skeletal maturation, and bone homeostasis, as well as changes in insulin sensitivity and lipid profile. As previously mentioned, the phenotype depends on sex and age, and may vary according to the level of enzyme activity.

Hypothalamic–Pituitary–Gonadal Axis

Biochemical findings in aromatase-deficient patients have further clarified the role of estrogens on the sex steroid-gonadotropin feedback system in humans. Aromatase and estrogen receptor alpha are predominantly expressed in the pituitary (**Scully et al., 1997**; **Shughrue et al., 1998**) and the hypothalamus.

In patients with aromatase deficiency, the elevated levels of androgens fail to suppress gonadotropins into the normal range in the absence of estrogen supporting the primary role of estrogen in the feedback mechanism of gonadotropin secretion within the hypothalamic–pituitary–gonadal axis in both males and females (**Conte et al., 1994**; **Morishima et al., 1995**; **Ludwig et al., 1998**; **Belgorosky et al., 2003**; **Lin et al., 2007**; Ritcher-Urun, 2008; **Guercio et al., 2009**; **Verma et al., 2012**; **Janner et al., 2012**; **Bouchoucha et al., 2014**; **Marino et al., 2015**; **Zhu et al., 2016**; **Morishima et al., 1995**; **Carani et al., 1997**; **Herrmann et al., 2002**; Maffei et al., 2004, 2007; **Lanfranco et al., 2008**; **Baykan et al., 2013**).

In girls with aromatase deficiency, during infancy and childhood the hypothalamic–pituitary–gonadal axis showed persistently high basal and stimulated serum FSH levels (**Conte et al., 1994**; **Mullis et al., 1997**; **Ludwig et al., 1998**; **Belgorosky et al., 2003**; **Lin et al., 2007**; **Verma et al., 2012**; **Ludwikowski et al., 2013**; **Bouchoucha et al., 2014**; **Marino et al., 2015**; **Akçurin et al., 2016**; **Zhu et al., 2016**), suggesting that during infancy and childhood, minimal amounts of estrogens are required to restrain pituitary FSH in normal prepubertal females. Basal serum LH levels were quite variable, ranging from normal (**Conte et al., 1994**; **Mullis et al., 1997**; **Belgorosky et al., 2003**; **Lin et al., 2007**; **Hauri-Hohl et al., 2011**; **Verma et al., 2012**; **Bouchoucha et al., 2014**; **Akçurin et al., 2016**) to high (**Ludwig et al., 1998**; **Ludwikowski et al., 2013**; **Marino et al., 2015**; **Akçurin et al., 2016**). A serum LH hyper-response after LHRH stimulation was observed in two girls (**Mullis et al., 1997**; **Belgorosky et al., 2003**). Two patients showed initial high basal serum LH levels that normalized thereafter (**Marino et al., 2015**; **Zhu et al., 2016**). In two affected girls studied longitudinally (**Belgorosky et al., 2003**; **Janner et al., 2012**), different patterns of serum LH levels were found in response to GnRH during prepuberty. LH amplitude was reported to be normal by **Janner et al. (2012)** but high by **Belgorosky et al. (2003)**. Belgorosky et al. also showed that in early prepuberty, along with normal serum androgen levels, peak serum LH response was 10 times lower than in late prepuberty. It has been known for many years that sex hormones of extragonadal origin (adrenal) or administrated exogenously produce not only the development of secondary sexual characteristics, but also an advance in the induction of the onset of GnRH-dependent puberty in boys and in girls. Therefore, in the study by Belgorosky et al., it was speculated that the pubertal serum LH response to acute GnRH stimulation detected in this aromatase-deficient girl might be secondary to a prolonged effect of androgens on the central nervous system, which includes, among other effects, the maturation of the GH/IGF1 axis, resulting, in turn, in an irreversible maturation of the GnRH pulse generator. In the patient described by **Hauri-Hohl et al. (2011)**, accordingly to the clinical phenotype, serum gonadotropin levels measured several times between the ages 4 and 10.5 were always normal. Interestingly, the patient presented with a placenta-specific, prenatally limited component of aromatase deficiency as mentioned earlier.

During puberty, basal and post-GnRH LH and FSH levels were elevated in almost all affected females (**Conte et al., 1994**; **Morishima et al., 1995**; **Belgorosky et al., 2003**; **Lin et al., 2007**; Richter-Unruh, 2008; **Guercio et al., 2009**; **Janner et al., 2012**; **Verma et al., 2012**; **Gagliardi et al., 2014**; **Marino et al., 2015**; **Saraco et al., 2015**). Serum androgens (testosterone and androstenedione) levels were clearly high compared with normal values for age and stage of sexual development mirrored elevated LH levels, whereas serum estradiol levels were extremely low. However, normal serum gonadotropin and androgen levels were found at 10 years of age in an affected patient with a partial form of aromatase deficiency (**Marino et al., 2015**). This hormonal pattern supports the concept of a primary role of estrogen in the negative feedback mechanism of gonadotropin secretion within the hypothalamic–pituitary–gonadal axis in females. Moreover, levels of serum gonadotropins and testosterone decreased after low-dose estrogen replacement in an aromatase-deficient man (**Carani et al., 1997**). A different threshold for estradiol action in aromatase-deficient adolescents under estrogen therapy was reported by **Guercio et al. (2009)**, **Janner et al. (2012)**, **Marino et al. (2015)**, and **Saraco et al. (2015)**. Even though during late prepuberty and puberty, systemic estrogen administration was followed by clinical signs of estrogen response, such as normalization of growth and skeletal maturation, this was not able to normalize the pituitary–gonadal axis, particularly serum FSH levels and ovarian cysts. It was speculated that this atypical response may have been secondary to lower aromatase activity and poor local estrogen synthesis from androgen precursors in peripheral tissues not only at ovarian levels, but also at local hypothalamic–pituitary levels (**Marino et al., 2015**; **Saraco et al., 2015**). It seems unlikely that the lack of serum FSH level suppression was related to a low level of serum inhibin B since in an aromatase-deficient patient reported by **Burckhardt et al. (2015)**, the upper normal serum inhibin B levels detected were unable to suppress serum FSH levels under low-dose E2 treatment.

In contrast to affected infant girls, hormonal studies in a 2-month-old boy reported by **Deladoëy et al. (1999)** revealed that basal and GnRH-stimulated FSH levels were normal despite low serum estrogen concentrations, suggesting that estrogens are not involved in the regulation of FSH secretion during infancy. Moreover, in this boy normal serum inhibin B levels for sex and age were found. Hence, it had been proposed that inhibin B might be a major contributor to the regulation of serum FSH secretion in normal infant males. The fact that in the affected boy, free testosterone and androstenedione levels were very high at 2 weeks of postnatal life, followed by a decrease to the normal range during the first month of life, suggests a role for cP450arom in fetal and newborn testicular function. Indeed, it has been described that cP450arom is highly expressed in fetal and neonatal human testes, compared with testes of individuals of older prepubertal ages (**Berensztein et al., 2006**). This finding suggests that aromatase activity might play a role in modulating testosterone secretion: the high aromatase expression of human neonatal Leydig cells decreased at 2–4 months of age, while peak testosterone secretion occurred during the postnatal testicular activation period. A similar normal hormonal profile, compatible with normal testicular function and gonadotropin secretion, was reported in an affected prepubertal boy at 4 and 6 years of age (**Bouchoucha et al., 2014**). Therefore, a gender-specific negative feedback mechanism could be suggested, at least during infancy and early childhood.

Data from early and midpubertal boys receiving estrogens or aromatase inhibitors suggest that once puberty starts, estrogens become involved in the negative feedback control of gonadotropin secretion (**Kletter et al., 1997**; **Wickman et al., 2001**).

Different studies have evaluated the role of estradiol in the control of gonadotropin secretion in males and have demonstrated that estrogens have a direct modulatory and inhibitory effect on the pituitary release and/or synthesis of LH and FSH (**Finkelstein et al., 1991**). In adult men with aromatase deficiency slight increments of basal serum FSH were consistently found despite normal inhibin B levels (**Morishima et al., 1995**; **Carani et al., 1997**; **Herrmann et al., 2002**; Maffei et al., 2004, 2007; **Lanfranco et al., 2008**; **Baykan et al., 2013**; Chen, 2016). Only two male patients with normal FSH levels together with increased basal testosterone levels were reported (**Bouillon et al., 2004**; **Miedlich et al., 2016**). It could be suggested that high serum testosterone might be responsible for a complete negative feedback on gonadotropins even in the absence of estradiol. However, in other affected individuals, basal serum FSH levels were increased even when testosterone was elevated above the reference values (**Morishima et al., 1995**; **Rochira et al., 2006**). We can also speculate that in the presence of high serum testosterone levels, minimal residual aromatase activity could already be enough for gonadotropin suppression (**Miedlich et al., 2016**). Nonetheless, normal serum FSH levels were also found in an aromatase-deficient man with null mutations within the CYP19A1 gene (**Bouillon et al., 2004**).

Basal serum LH levels are within the normal range in most affected aromatase-deficient men, and in only two cases mild increases in serum LH levels were reported (**Morishima et al., 1995**; **Baykan et al., 2013**). Nevertheless, when assessed by dynamic tests, increases in LH pulsatility and pulse amplitude were demonstrated in affected men (**Rochira et al., 2006**; **Hayes et al., 2000**).

Puberty

Classically, in complete aromatase deficiency at puberty, lack of estrogens results in hypergonadotropic hypogonadism with failure of spontaneous pubertal development and primary amenorrhea but excessive virilization (**Conte et al., 1994**; **Morishima et al., 1995**; **Lin et al., 2007**; Richter-Unruh, 2008; **Gagliardi et al., 2014**). The pubertal spurt is absent and bone age delayed.

Since the first description of partial forms of aromatase deficiency by Lin et al. in 2007, variable or nonclassic phenotypes have been reported (**Lin et al., 2007**; **Pepe et al., 2007**; **Verma et al., 2012**; **Marino et al., 2015**; **Saraco et al., 2015**), illustrating that phenotypic variability can occur in aromatase insufficiency in humans. Affected females showed spontaneous breast development that in some cases progressed to Tanner stage IV (**Lin et al., 2007**; **Marino et al., 2015**), but with primary amenorrhea. In almost all, these findings were accompanied by the presence of a pubertal size uterus, enlarged ovarian cysts, high basal serum gonadotropin and androgen (testosterone and androstenedione) levels, and virilizing signs. Hence, low levels of residual aromatase activity can be associated with breast development and estrogen biosynthesis, particularly when circulating androgenic substrate concentrations (androstenedione, testosterone) are elevated. Functional studies of reported cP450arom mutants showed variable residual aromatase activity that could explain the partial

phenotype found (**Table 1**). Interestingly, two described mutations associated with splicing events resulted in transcript variants that were also found in normal human steroidogenic tissues, suggesting that a misbalance between normal and alternative-inactive splicing variants might explain the partial deficiency phenotype.

Detailed information on pubertal milestones in aromatase-deficient males is scarce; however, in most of them pubertal development was normal and persistent linear growth was observed after reaching Tanner stage 5 (**Morishima et al., 1995**; **Carani et al., 1997**; **Bilezikian et al., 1998**; **Bouillon et al., 2004**; Maffei et al., 2004, 2007; **Baykan et al., 2013**; **Chen et al., 2015**; **Miedlich et al., 2016**). The use of aromatase inhibitors to improve adult height in pubertal boys with idiopathic short stature was associated with an increase in serum gonadotropin levels and a consequent rise in testosterone concentrations, raising concerns about pubertal progression (Hero, 2005). It could be speculated that affected boys may show an accelerated pubertal tempo consistent with supraphysiological levels of testosterone.

Gonadal Development

The expression of aromatase in the ovary plays an important role in the regulation of the reproductive cycle in females. Aromatase is also expressed in the male gonad; however, in contrast to its key role as an endocrine coordinator in females, in males, the paracrine effects of aromatase products are essential for normal spermatogenesis (Stocco, 2012).

Ovaries

In the ovaries, cP450arom is expressed in two different stages of differentiation of a unique cell, the granulosa cell: the pre-ovulatory follicles and corpora lutea of ovulatory women. Aromatase expression in the granulosa cells of the ovary is regulated primarily by promoter II under the control of the gonadotropin FSH, whose action is mediated by cAMP. Transcriptionally, regulation is by a hexameric sequence binding SF-1 and an imperfect CRE binding CREB and other factors (**Simpson et al., 2002**).

The well-timed and cell-specific expression of aromatase in the ovary is crucial for the autocrine regulation of folliculogenesis, the endocrine control of the female reproductive tract, and the coordination of gonadotropin secretion. Estrogen modulation of the structure and function of oviducts, the uterus, and the vagina are essential for oocyte survival, fertilization, and implantation (Stocco, 2012).

Multiple ovarian cysts have been described in human aromatase-deficient patients in infancy and childhood (**Mullis et al., 1997**; **Belgorosky et al., 2009**; **Marino et al., 2015**) and particularly during puberty (**Conte et al., 1994**; **Morishima et al., 1995**; **Lin et al., 2007**; **Belgorosky et al., 2009**; **Verma et al., 2012**; **Janner et al., 2012**; **Eklioglu et al., 2014**; **Burckhardt et al., 2015**; **Marino et al., 2015**; **Saraco et al., 2015**), as well as in female aromatase knockout (ArKO) mice (**Britt et al., 2000**). It was assumed that an amplification of FSH signaling that occurred in the presence of high intraovarian androgens could be involved in the development of ovarian follicular cysts already in infancy (**Belgorosky et al., 2009**; **Janner et al., 2012**). Accordingly, mutations in the FSHB subunit as well as mutations in the FSH receptor result in a phenotype of small ovaries containing only primordial follicles (**Themmen and Huhtaniemi, 2000**). It is remarkable that histopathological analysis was consistent with that of the polycystic ovary syndrome (**Morishima et al., 1995**).

Despite the classical picture of bilateral polycystic ovaries described in the natural development from infancy to adulthood in these patients, the ovarian phenotype is not consistent in some affected females. In 2007, Lin et al. reported a pubertal female who had severe aromatase deficiency showed mild postnatal androgenization of the genitalia, moderate elevation of androgen levels at puberty, and small ovaries at adolescence. Functional studies indicated that aromatase activity was severely disrupted, but there was no explanation for her mild excess androgen production. Later, in 2014, Gagliardi et al. reported the natural history of aromatase deficiency in a female who was not treated until adulthood. At the age of 25 years, the patient showed the classical phenotype, but bilateral streak ovaries were found on laparoscopy and excised. Histological examination revealed atretic and primordial follicles, but no evidence of ovulation. Streak ovaries are consistent with the phenotype of the ArKO mouse followed through adulthood. An age-dependent phenotype was observed in the ArKO ovaries, with a progressive deterioration or degeneration of the ovaries (**Britt et al., 2000**). The ovarian morphology of ArKO mice (**Simpson et al., 2002**) showed an early block in follicular development at the antral stage with absent corpora lutea, followed by hemorrhagic cysts and subsequently, absent secondary and antral follicles and atresia of the primary follicles with increased collagen deposition. This model might explain the phenotype of this adult patient considering that the longer life span of the human may permit more complete follicular atresia and collagen deposition mimicking the classical streak ovaries seen in Turner's syndrome (**Gagliardi et al., 2014**). Nevertheless, **Akçurin et al. (2016)** have recently described three related Turkish cousins with a severe form of aromatase deficiency secondary to a novel homozygous 568insC mutation. These girls presented with hypoplastic ovaries already in prepubertal years. No functional studies have been performed to understand this complex phenotype. Therefore, the mechanism involved in the ovarian phenotype and outcome is poorly understood.

Testes

In contrast to the restricted expression of aromatase in the ovary, the enzyme is widely expressed in the testis and accessory glands. The wide distribution of aromatase in the male gonads is essential to maintain the high levels of estradiol needed for normal spermiogenesis, sperm maturation, sperm motility, and possibly acrosomal reaction.

In ArKO mice, testicular histology and morphology showed age- and diet-related changes. From 4.5 months of life, dysmorphic seminiferous tubules and disrupted spermatogenesis become noticeable (Robertson et al., 1999, 2002). At 1 year of age, ArKO mice consistently show reduced testicular volume, spermiogenesis failure, and Leydig cell hyperplasia, probably as a consequence of increased serum LH levels.

In aromatase-deficient adult males, testicular volume has been reported to be variable, ranging from macroorchidism (**Morishima et al., 1995**) to normal (**Bouillon et al., 2004**; **Lanfranco et al., 2008**; **Miedlich et al., 2016**) or even microorchidism (**Carani et al., 1997**; **Maffei et al., 2004**). Information on testicular histology and morphology is very scarce, with data from only two affected male adults. One of the patients had a history of bilateral cryptorchidism and surgery at the age of 6, making the interpretation of the findings even more difficult.

Fertility

Several factors may affect reproduction in women with aromatase deficiency, mostly in complete forms, including hypergonadotropic hypogonadism, hyperandrogenism, ovarian cysts, and surgical consequences of genital reconstruction of ambiguous genitalia. Although variable phenotypes have been described and some affected females have partial forms of aromatase deficiency with spontaneous breast development and uterine growth despite androgen excess and virilization (**Lin et al., 2007**; **Pepe et al., 2007**; **Verma et al., 2012**; **Marino et al., 2015**; **Saraco et al., 2015**), disease course in adulthood and long-term consequences on fertility are unknown. Data on the long-term follow-up of these patients might clarify our understanding of the reproductive outcomes (**Guercio et al., 2015**).

Fertility is uncertain in aromatase-deficient males, but may be impaired considering the role of estrogens in human testicular function, spermatogenesis, and fertility. In normospermic adult males, lower sperm concentration and reduced sperm motility were associated with aromatase polymorphisms that decrease enzyme activity (Lazarus, 2011). Data on sperm analysis or testicular biopsies of adult aromatase-deficient men are scarce and inconsistent. Pre-existing conditions, such as cryptorchidism, further complicate the interpretation of the results. Total germ-cell depletion, focal hypospermatogenesis, low sperm counts, and decreased motility have been reported (**Carani et al., 1997**; **Herrmann et al., 2002**; Maffei et al., 2004, 2007; **Baykan et al., 2013**).

Gender Identity/Psychosexual Development

In mammals, sex differences in brain structure and function are programmed by exposure to testosterone during a critical period in perinatal development (**Cooke et al., 1998**). In the female, fetal androgen action might have consequences on brain programming of future sexual identity and behavior (**Bao and Swaab, 2011**). However, changes in gender identity, sex role behavior, and gender dysphoria are unusual in 46,XX aromatase-deficient patients, although reported in detail in a few cases (**Conte et al., 1994**; **Morishima et al., 1995**; **Sudeep et al., 2013**). Male gender identity, male gender role behavior, and gender dysphoria were reported in only one 46,XX subject raised as male (**Lin et al., 2007**). Whether prenatal and postnatal androgen exposure coupled with relative estrogen insufficiency—a unique feature of aromatase insufficiency—is important, or whether social and cultural influences are predominant, remains unclear.

In the affected males, adult male cisgender identity, heterosexual orientation, and normal libido have been reported (**Morishima et al., 1995**; **Carani et al., 1997**; **Herrmann et al., 2002**; **Maffei et al., 2007**; **Lanfranco et al., 2008**; **Chen et al., 2015**). Only a modest decreased libido was reported in one subject without change after high-dose estrogen treatment (**Morishima et al., 1995**; **Bilezikian et al., 1998**).

Insulin Sensitivity and Lipid Profile

In adipose tissue, aromatase is expressed primarily in the stromal mesenchymal cells or preadipocytes where the majority of aromatase transcripts contain exon I.4 followed by exon I.3 and II (**Simpson et al., 2002**). Estrogens regulate glucose homeostasis in the skeletal muscle and liver by modulating insulin production in pancreatic β-cells and regulating energy balance in the hypothalamus. In addition, an antilipogenic and pro-lipolytic effect acting directly on adipose tissue has been reported (**Foryst-Ludwig and Kintscher, 2010**).

Data obtained from ArKO mice of both sexes showed an adipose phenotype together with insulin resistance (IR), elevated serum lipid, and fatty liver, suggesting an association between aromatase deficiency and metabolic alterations (**Jones et al., 2000**; **Nemoto et al., 2000**; **Takeda et al., 2003**).

IR, an abnormal lipid profile, as well as clinical features similar to the metabolic syndrome, have been well described in aromatase-deficient adult men. In these patients, estrogen therapy has been suggested to play an important role in lipid regulation, fatty acid homeostasis, and glucose metabolism (**Morishima et al., 1995**; **Carani et al., 1997**; **Herrmann et al., 2005**; Maffei et al., 2004, 2007; **Lanfranco et al., 2008**; **Baykan et al., 2013**; **Chen et al., 2015**). Recently, however, an aromatase-deficient man harboring the c.628 G > A mutation without biochemical features of IR, dyslipidemia, or overweight/obesity has been reported. Although this mutation has been related to the metabolic phenotype in other affected subjects (**Maffei et al., 2004**; **Guercio et al., 2009**), the capacity of the specific mutation c.628 G > A to translate into expression of differentially functioning proteins (**Pepe et al., 2007**) might explain the variable phenotypes.

In female aromatase-deficient patients, the relationship between carbohydrate metabolism, lipid profile, and estrogen deficiency has not been clearly defined. Lipid abnormalities were found in a Vietnamese affected girl of 18 months of age (**Ludwig et al., 1998**) and in two adolescents reported by **Lin et al. (2007)**. In one of the latter patients the metabolic profile normalized under estrogen therapy. IR and glucose intolerance but a normal lipid profile was reported in two affected females with partial forms of aromatase deficiency at 9 and 13.5 years of age (**Belgorosky et al., 2003**; **Guercio et al., 2009**; **Verma et al., 2012**). Both presented spontaneous thelarche along with high serum FSH levels, clinical and biochemical hyperandrogenism, and ovarian cysts. In two other pubertal affected females with partial aromatase deficiency reported by **Marino et al. (2015)**, normal glucose tolerance along with normal androgen levels was noticed. This finding might reflect that high androgen levels contribute to the development of IR in women as previously proposed (**Peiris et al., 1989**). A more classic or prominent metabolic picture of abdominal obesity, hepatic steatosis, dyslipemia, and IR was reported in a 25-year-old affected woman in whom estrogen treatment was delayed until adulthood. Since in ArKO mice models, metabolic abnormalities were age dependent, prolonged estrogen deficiency could be required in women to develop a full metabolic phenotype. Long-term studies would be necessary to draw more solid conclusions in this regard.

Even though metabolic abnormalities were resolved by estrogen treatment in ARKO mice (**Takeda et al., 2003**), in humans, the effect of estrogen therapy on glucose homeostasis and IR is complex and controversial (**Jones et al., 2006**). Failure to improve IR in some aromatase-deficient patients of both sexes was reported (**Herrmann et al., 2002**; **Maffei et al., 2004**; **Guercio et al., 2009**; **Verma et al., 2012**; Chen, 2016). Moreover, the optimal dose and route of estrogen replacement therapy in such cases are still unclear due to the scarcity of the disease (Chen, 2016).

Growth, Skeletal Maturation, and Bone Homeostasis

Androgens and estrogens are important for the maintenance of the skeleton in both sexes (**Grumbach and Auchus, 1999**; **Simpson et al., 2002**). The important role of aromatase in bone physiology was clearly shown in ArKO mice (**Oz et al., 2000**), in which reduced bone density and skeletal abnormalities were observed.

The male aromatase-deficient phenotype is characterized by tall stature with eunuchoid body proportions and continued linear growth into adulthood due to unfused epiphysis, genu valgum, and osteopenia/osteoporosis that remarkable improves with estrogen replacement treatment. This aspect has been addressed in detail in several reports (**Bulun, 2014**; **Grumbach and Auchus, 1999**; **Bilezikian et al., 1998**; **Bouillon et al., 2004**; **Lanfranco et al., 2008**; **Zirilli et al., 2008**; Rochira et al., 2007, 2015).

In affected females bone age is delayed, particularly in late prepuberty, and absence of the growth spurt is observed. Estrogen therapy has been shown to induce a pubertal growth spurt and epiphyseal fusion (**Belgorosky et al., 2009**; **Janner et al., 2012**; **Verma et al., 2012**; **Marino et al., 2015**; **Saraco et al., 2015**). Since aromatase-deficient women are treated earlier to induce pubertal development, it may be possible that these subjects, if untreated, will develop the same phenotype as that observed in the male counterpart. Concordantly with this, a nontreated 32-year-old Indian woman with tall stature, eunuchoidal proportions, osteopenia, and fractures was reported (**Gagliardi et al., 2014**).

Data on the role of estrogens in bone mineralization during childhood are scarce. In the patient reported by **Mullis et al. (1997)**, low bone mineral density (BMD) was found. Moreover, BMD improved after treatment with low-dose estrogens, suggesting that minimal quantities of estrogens are also required to preserve mineral bone acquisition in early childhood. Nevertheless, in the case reported by **Belgorosky et al. (2003)**, BMD remained normal throughout the follow-up. The explanation could be that in this particular case, partial cP450arom deficiency was found, secondary to a mutation at the consensus donor splice site in the exon 5/intron 5 junction. Therefore, some expression of the cP450arom protein at the growth plate might be expected, which may have been enough to maintain normal mineral bone acquisition, at least during childhood.

Estrogen Replacement Therapy

The clinical consequences of estrogen deficiency in aromatase-deficient patients reinforce the importance of clinical management and estrogen replacement. Recently, this aspect has been carefully addressed by **Bulun (2014)** and **Zhu et al. (2016)**. To date there is no consensus regarding the age of initiation and the dosage of estrogen to use to guide this indication. Thus, such treatment should be individualized at this time.

Conclusions

Patients with cP450arom deficiency represent a source of knowledge on estrogen physiology and pathology, in particular on the role of estrogens in bone maturation, gonadotropin modulation in both sexes, gonadal development and function, as well on glucose and lipid metabolism. Recently, data on new molecular mechanisms have expanded the clinical and biochemical phenotype of affected individuals. At this time, long-term studies are needed to establish a therapeutic strategy to prevent the devastating consequences of prolonged estrogen deficiency.

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Glossary

Acanthosis nigricans Skin pigmentation disorder in which dark thick poorly defined areas of velvety texture appear especially in the neck, armpit, and body folds. Related to hyperinsulinism.
Aromatization The last step in estrogen synthesis. The reaction includes three hydroxylations of the 19 methyl group of the androgen molecule with the simultaneous elimination of the methyl group that results in the formation of a benzene ring.
Astenospermia Less than 50% of motile spermatozoa.
Azoospermia Absence of spermatozoa in the ejaculate.
Congenital adrenal hyperplasia Group of steroidogenic disorders that impair cortisol biosynthesis.
Disorders of sexual development Congenital conditions in which chromosomal, gonadal, or anatomical sex is atypical.
GnRH pulse generation Hypothalamic neuronal network that control the pulsatile secretion of gonadotropins.
Hypospadias Disorder of the anterior urethral and penile development in which the urethral opening is ectopically located on the ventral aspect of the penis.
Osteopenia A skeletal condition characterized by a decreased bone mineral density when compared with the reference standard (between − 1 and − 2.5 SD).
Osteoporosis A skeletal condition characterized by a decreased bone mineral density when compared with the reference standard (under − 2.5 SD).
Polycystic ovary syndrome Disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology.
Teratospermia Less than 30% of morphologically normal spermatozoa.