AROMATASE DEFICIENCY IN MEN: A CLINICAL PERSPECTIVE

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Short title: E_2 deficiency in men

Key words: diagnosis, presentation, clinical management, estrogen deprivation, estradiol treatment, male, bone, growth plate, bone mineral density, gonadotropin, testosterone, sex steroids**.**

Word count: 4692

Grant: The work was supported by a grant from the Ministero dell'Università e della Ricerca (MUR, ex-40%-2005).

SUMMARY

Human aromatase deficiency is a very rare disease caused by CYP19A1 aromatase gene mutations resulting in congenital estrogen deprivation. The substantial clinical experience of the presentation, diagnosis and intervention is reviewed in order to describe in a systematic way the clinical aspects of the disease in men and to provide useful clinical advice for its management. At presentation, the clinical features common to all aromatase-deficient men are: tall stature, delayed bone maturation, osteopenia/osteoporosis, and eunuchoid skeleton. The diagnosis is almost always delayed and generally is made during adulthood. An adequate clinical path allows to confirm or reject the clinical diagnosis in all patients suspected with the disease. Unfused epiphyses and undetectable serum estradiol support a clinical diagnosis, the genetic sequencing of the CYP19A1 gene further substantiating the diagnosis. Transdermal estradiol treatment and a dosage of 0.22-0.35 μg/kg should be considered as adequate for replacement therapy and continued lifelong. Serum estradiol, luteinizing-hormone (LH), testosterone and bone mineral density (BMD) should be carefully monitored and considered, in clinical practice, as powerful biochemical markers of adequate estrogen substitution and. The gold standard for starting estrogen treatment seems to be puberty and early diagnosis should be advocated to avoid disease overlook and undermanagement.

REVIEW CRITERIA

We have reviewed the clinical aspects and the clinical management of all the male patients with aromatase deficiency described so far in literature. Four of the total eight cases have been described, diagnosed and clinically managed directly by the Authors (References 6, 9-11 in the reference list). All the additional data has been obtained from medline (PubMed) by searching the literature resources in the U.S. National Library of Medicine and the National Institutes of Health database, using 'aromatase deficiency' and 'estrogen deficiency' separately as keywords and limiting the search to human studies, male gender, and a time period of about 15 years (from 1 January 1993 to 31 December 2008). The reference lists of identified articles were also searched for further papers. Except for one single case-report of a man with aromatase deficiency (Reference 8 in the reference list) and for Reference 48, both reported in English in abstract form, all papers identified were English-language, full-text papers.

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KEY POINT

- Aromatase deficiency is often overlooked in men until adulthood with consequent delayed diagnosis and under management.
- Aromatase deficiency should be strongly suspected in adult men with continuing linear growth and/or documented unfused epiphyses and height increases in adulthood.
- All male infants born from a mother who exhibited signs of progressive virilization during pregnancy as well as all newborns with siblings with documented aromatase deficiency should be screened for the disease.
- X-ray film of the hand and wrist, serum estradiol, testosterone, and LH, are useful for the clinical diagnosis of aromatase deficiency in men, the genetic testing becoming mandatory in children and newborns suspected with the disease.
- A high starting dose of estradiol should be administered to adult men with aromatase deficiency for a quick completion of bone maturation followed by a lifelong lower dosage as replacement therapy.
- A early diagnosis of aromatase deficiency is advocate before irreversible abnormalities develop.

INTRODUCTION

Human aromatase deficiency is a very rare disease caused by naturally occurring mutations in the *CYP19A1* aromatase gene leading to abnormal final protein products, which all result in a non-functioning aromatase enzyme and consequently lead to a complete lack of estrogen synthesis.[1,2] Although present at birth, the disease is often overlooked in men until adulthood: with the single exception of a birth diagnosis followed by early clinical management started already in infancy,[3,4] all the other diagnoses of male aromatase deficiency have been made in adulthood.[5-12] The discovery of human mutations in the *CYP19A1* aromatase gene and the α-estrogen receptor gene,[13] both resulting in male congenital estrogen deficiency, as well as the study of similar gene knock-out animal models have improved our knowledge of the pathophysiological role of estrogen in men.[1,2,14] The understanding of the role of estrogen in the human male has a fascinating and rather long history (for review see Reference 15),[15] but the most important actions of estrogen in men (Box 1) have only recently been characterized in detail, challenging long-held views.[1,2,5,6,13] Until now, however, the papers dealing with the issue have mainly focused on estrogen relevance to male health[14] and pathophysiology,[1,2,12,15] while some clinical aspects have been left in the background and only rarely reviewed.[16] In order to provide useful clinical advice, the Authors approach the clinical aspects of male aromatase deficiency in a systematic way, basing their work on both their own extensive clinical experience with this rare disease[6,9-11] and the data available in literature.

CLINICAL PRESENTATION

Clinical manifestations of the disease (Box2) are believed to occur approximately at puberty - mainly during late adolescence – (Box 3) when the effects of estrogen deprivation on bone become evident. They include the absence of the expected growth arrest, the continuing linear increase in patient height, and the development of eunuchoid body proportions of the skeleton, i.e. the appendicular skeleton (arms and legs) exceeding the axial skeleton in length[1,2,17,18] (Box 2).

The lack of pubertal growth spurt,[1,2,17,19] is believed to be missing in aromatase deficiency,[1,2,17-19] with the height continuing to increase linearly as in children. As yet no appropriate characterization of this event is available for aromatase-deficient men,[5-7,9,10] nevertheless, the lack of any pubertal spurt before the starting of estradiol replacement treatment at the age of 17 in the child with early diagnosis,[4] strongly hints it that direction.

The major symptoms that usually induce the adult patient to seek medical consultation are continuing linear growth,[6,9-11] infertility,[6] and widespread bone pain,[6,9-11] as well as the incidental discovery of unfused epiphyses during radiological examination[6,7,11] (Box 2). At presentation, the clinical features common to all aromatase-deficient men are: tall stature, delayed bone maturation, osteopenia/osteoporosis, eunuchoid skeleton, bone pain and progressive *genu valgum*[12,20] (Box 2). The remaining features described as associated with aromatase deficiency, were not shared by all the patients at presentation,[12,20] but some of them even not specific are very common among these patients[12] (Box 2).

DIAGNOSIS

Diagnosis: general aspects

Due to its paucisymptomatic manifestation during childhood and/or adolescence (Box 3) and its slow progression during adulthood, the disease remains generally overlooked until adulthood,[20] which mostly results in a delayed diagnosis[5-11] (Box 3). Except for the case of a child,[4] the mean age at diagnosis was 27±2.5 years for adult men with aromatase deficiency.[5-11] Similarly, the only man described so far as suffering from estrogen resistance was diagnosed with the condition at the age of 28.[13] Differently from female aromatase deficiency, which manifests itself early at birth (ambiguous genitalia)[21,22] or at puberty (primary amenorrhea),[23] male aromatase deficiency is difficult to diagnose before adulthood. It should be suspected in male newborns if the mother exhibits signs of progressive virilization during the third trimester of pregnancy,[1,2,20] and at puberty when pubertal growth spurt is absent[1,2] (Box 3). However, early signs (absence of a dramatic increase in growth rate at puberty, eunuchoid body proportions of the skeleton and delayed bone maturation) (Box 2) are also very difficult to detect at an early stage of the disease, thus aromatase deficiency appears unlikely to be suspected at puberty. The progressive virilization of the mother (severe acne, voice lowering, hypertrophy of the clitoris, frontal balding, and facial masculinizing changes) during pregnancy has been reported in most of the cases[3,5,7,8] and is also constantly present when the fetus is female.[1,2,12,20,21,22,23] As for the remaining four cases of male aromatase deficiency, the information has been impossible to obtain, since the affected patients were raised by unrelated people[9,10] or no data was available.[6,11] However, the only possible reasons for suspecting aromatase deficiency in an infant are the mother's virilization during the last trimester of pregnancy[1,2,20] or the diagnosis of the disease in a patient's relative (sister or brother)[3,4] (Box 3).

Clinical Diagnosis

An adequate clinical path based on evidence-based information provided by patient's interview, physical examination (Box 4), biochemical and hormonal analyses (Table 1), and radiological investigations is effective in confirming or rejecting the clinical diagnosis (Figure 1) and in selecting candidates to genetic analysis.

Clinical diagnosis: visit and physical examination

The main clinical skills useful at the time of first visit for the diagnosis of aromatase deficiency in men are summarized in Box 4. Relative's information (e.g. the occurrence of the above mentioned mother virilization during pregnancy) is helpful in reinforcing the suspicion of the diagnosis. In three of the eight cases described so far, the aromatase-deficient men were born from consanguineous parents,[3,4,6,7] as for most of the females with aromatase deficiency.[20,23] The family history was unavailable in two other cases,[9,10] and showed no consanguinity in the remaining two.[5,8,11] The parents' height is necessary[5] to calculate patient's target height and to ascertain the presence of tall stature (Box 4). Health status of sister or brothers should be investigated with particular concern to a history of ambiguous genitalia and/or of delayed puberty in sisters.[2,5]

A detailed interview should collect all available data on the patient's growth from birth to adulthood and on the pubertal development and its onset time.[1,2,19] A previous history of cryptorchidism needs to be investigated since abnormalities in the descent of testes have been described in three patients.[8,9,11] Physicians should also ask about the patient's reproductive status, since sex steroids can influence both spermatogenesis and sexual behavior.[1,2,15]

During physical examination, the physicians should look for all the signs summarized in Box 2, comprising the less frequent of them, such as evident skeletal deformities[7] or increased testicular

volume.[5,7] In particular, the recognition of signs of metabolic diseases,[1,2,5,7,9,10,12,20] such as insulin resistance[5,7,9,10] allows to better define the subsequent biochemical analyses.

Differential Diagnosis: biochemical, hormonal and radiological examinations

Aromatase deficiency should be strongly suspected in adult men with continuing linear growth and/or documented unfused epiphyses and height increases in adulthood. The flow chart in Figure 1 has been devised to help physicians in decision-making. A first step in the clinical investigation of aromatase deficiency in adult men (Figure 1) should include the assay of serum estradiol, testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and a standard X-ray film of the hand and wrist in order to characterize the patient's hormonal pattern and identify the presence and the degree of bone maturation delay.[16]

The measurement of serum levels of sex steroids and serum gonadotropins can help in differentiating some clinical conditions from aromatase deficiency (Table 1). Estrogen resistance, a condition with the same clinical features of aromatase deficiency, is easy to distinguish from, since it is characterized by higher than normal serum estradiol (Table 1, Figure 1).[13] Other clinical conditions sharing some of the clinical aspects of aromatase deficiency (tall stature, delayed bone age, osteoporosis, and eunuchoid skeleton) are complete 17α-hydroxylase deficiency, the combined deficiency of 17α-hydroxylase and 17,20-lyase[24] and severe prepubertal male hypogonadism;[25,26] they all display relative estrogen deficiency,[24-27] but also additional clinical features (e.g. underandrogenized genitalia). The absence of very elevated levels of adrenal androgens in serum and the occurrence of normal virilization together with normal-to-high serum testosterone allow to rule out 17α-hydroxylase and 17,20-lyase deficiency[24] and hypogonadism[25,26] respectively as possible causes of delayed bone maturation in aromatase-deficient men (Table 1, Figure 1).

The X-ray film of the hand and wrist is a simple, quick and inexpensive procedure for verifying the completion of bone maturation (fused epiphyses) in an adult man: the initial thinning and finally the disappearance of the growth plate substantiates the growth cessation.[28] In case of incomplete bone maturation (unfused epiphyses), the radiogram can be used to measure in detail the bone age by either of the two methods most widely used in clinical practice: the Greulich and Pyle[29] and the Tanner and Whitehouse (TW2).[30] X-ray films of the hand and wrist showed a pattern of unfused epiphyses in all the adult aromatase-deficient men described till now.[1,2,5-12] As unfused epiphyses are present also in the case of estrogen resistance,[13] 17α-hydroxylase and 17α-hydroxylase-17,20-lyase deficiency,[24] and severe prepubertal male hypogonadism,[25,26] X-ray films alone without the results of hormones evaluation do not differentiate the diagnosis.

The main limitation of the commercially available assays for the detection of serum estradiol is their poor accuracy, especially when serum estradiol is in the lowest quartile or below the lower end of the normal range.[27,31] At present, the gold standard tests for the measurement of very low levels of serum estradiol are the ultrasensitive recombinant cell bioassay[32] and the gas chromatography/tandem mass spectrometry,[33] but they are mainly used for research purposes. However, commercially available kits for the clinical determination of serum estradiol (especially 3rd generation RIA) are improving their sensitivity and specificity[9-11,34] also for measurements within the male range, thus providing useful information for clinical practice, especially if considered together with the other hormonal values of the hypothalamic pituitary gonadal axis.[27,35] The finding of undetectable estradiol, unfused epiphyses, and both gonadotropins and testosterone normal or elevated, together with most of the signs and symptoms listed in Box 2 and Box 3 may be sufficient for a clinical diagnosis of aromatase deficiency in an adult man (Figure 1).[16]

Genetic analysis

Except for the first three to six months after birth, the clinical diagnosis cannot be postulated on the basis of circulating hormones in newborns, in infants, and in prepubertal boys[3,4] due to the irrelevant production of sex steroids during that life periods. Thus, when the disease is suspected in a male subject before puberty, the genetic analysis is mandatory for the diagnosis (Figure 1). The genetic analysis of the *CYP19A1* gene checks for possible point mutations or base pair deletions of the gene. Due to the rarity of the disease, the strength of evidence of a diagnosis based solely on clinical data (signs, symptoms, hormonal and radiological outcomes) is poor, especially if the limits of estradiol assay are considered.[31-33] Thus, in order to further substantiate the diagnosis in adults, the genetic testing should be done if possible, even when the clinical diagnosis has been reached (Figure 1).[1-12,20] In a well-equipped standard molecular biology laboratory the sequencing of the *CYP19A1* is easy to perform, reproducible and it hardly constitutes an insurmountable obstacle (Box 5).

A thorough characterization of the pedigree requires the gene sequencing also of the patient's mother, father and siblings,[5-7,20,22,23] and a standard karyotype may allow to rule out major chromosomal diseases, especially Klinefelter Syndrome, which shares only some clinical features (e.g tall stature, eunuchoidism) with aromatase deficiency, but it is readily distinguished from (Figure 1).

Diagnosis of related disorders

Several metabolic, bone and reproductive abnormalities have been repeatedly described in men with aromatase deficiency.[1-12] The second clinical step in managing a possible case of male aromatase deficiency should include a radiological and a detailed biochemical study (Figure 1), both of which are useful for identifying the ancillary clinical conditions commonly associated with the disease (Box 2 and Box 3).[12] Biochemical analyses should focus on the lipid profile, liver function, and fasting glucose and

insulin in order to evaluate the degree of biochemical abnormalities related to the metabolic syndrome.[9,10,12,20,36,37] Although not yet widely used in the clinical practice, the HOMA score[38] can help to determine the degree of insulin resistance in these patients[9,10,12,20,36,37] (Figure 1). Liver ultrasound may be useful in investigating liver morphology and diagnosing fatty liver disease,[39] which in men is often related to estrogen deficiency.[9-11,40] A full screening of the male reproductive function, which is often impaired in aromatase-deficient men,[1,2,5-11,15] should be based upon hormonal and sperm analysis. Baseline bone status[16,34] can be assessed by investigating the biochemical markers of bone turnover and by measuring bone mineral density (BMD)[41] (Figure 1), the Dual-X-ray-Absorptiometry (DXA) at lumbar spine and femoral neck, representing the gold standard, as in osteoporotic men.[27]

A third clinical investigative step (mainly important in the prepubertal period) may involve the pituitary function, that can be impaired in aromatase-deficient males.[7,11,12,42] When signs of insulin resistance are present or the preliminary results show hyperinsulinism or higher than normal fasting glucose,[8,9,11,36] a clinical assessment of glucose metabolism by standard oral glucose tolerance test becomes mandatory.[43]

TREATMENT

Estrogen replacement treatment should be started as soon as the clinical diagnosis of aromatase deficiency has been reached in an adult man, the genetic diagnosis providing a subsequent viewpoint useful to decide for treatment carrying on. The known effects of estrogen in the human male (Box 1) should constitute the basis of the treatment approach. Estrogen action on growth and growth plate is biphasic: lower amounts of estrogen increase the cartilage growth rate and consequently patient height, while higher amounts of estrogen induce growth arrest[44-46] probably by promoting chondrocyte apoptosis within the epiphyseal cartilage[47] and the subsequent final mineralization of the cartilage.[12,17-19] A prompt start of treatment ensures rapid completion of bone maturation[6,7,10,41] and it hence prevents

a further increase in height and the worsening of eunuchoid body habitus,[18,48] the latter two events being well documented in the case of a long period until estradiol treatment starting[9] and in a case of under-treatment.[11]

Short-mid term estrogen treatment

When a high dose of estrogen is used, the treatment of adult men with aromatase deficiency leads to a rapid, short- to mid-term completion of skeletal maturation.[12,16,17] A dose of estrogen able to keep serum estradiol in the higher quartile of the normal range or slightly above the higher end of the male normal range[6,7,9,16,41] mimics what happens in boys in the final phase of puberty, when serum estradiol levels are higher than in early puberty[32,49] and lead to complete bone maturation[19,46] through a rapid bone elongation and further increases in height followed by quick epiphyseal closure and growth arrest,[1,6,7,9,11,41] within 6-9 months.[1,34,41]

The second main goal in estrogen treatment is BMD normalization. This should mimic the process of peak bone mass acquisition that in these patients would have otherwise happened about eight to ten years before, at the end of puberty.[27,50,51] Estrogen treatment increases BMD and restores normal values within 6-9 months in aromatase-deficient men.[7,9,11,34,41] A longer time may be required, especially at the femoral site[52] or when a lower starting dose of estrogen has being used.[11,50]

A daily dose of transdermal estradiol ranging from 0.36 to 0.52 μg/kg is usually adequate for maintaining normal-to-high levels of serum estradiol and rapidly completing bone maturation and mineralization.[12,17]

Long-term estrogen treatment

Once the epiphyseal closure, bone maturation and mineralization have been achieved, the estrogen treatment should be continued and regarded as lifelong replacement therapy, the main goal being to prevent bone loss and reduce the risk of cardiovascular diseases.

Adequate amounts of exogenous estradiol prevent bone resorption, ensure the maintenance of bone mass and promote long-term bone health[6,7,34,41,50,52] and need to be continued in order to maintain the BMD previously achieved.[27,34,50,52] Lowering the dose of estradiol results in lower than normal serum estradiol, a condition that, precisely as in older normal men,[27,51] leads to a reduction in BMD.[34,52] Also, a normal androgenization proved necessary for the long-term BMD maintenance.[34,50,52,53] Finally, ensuring an adequate estrogen '*milieau*' has been shown to guarantee also optimal cortical mineralization in men,[4,50,53] probably by exerting a priming effect on bone cells and acting as a permissive factor for the anabolic action of androgens.[50]

Other than in bone health, estrogen replacement treatment has led to improvements in other clinical features and/or biochemical abnormalities of adult patients with aromatase deficiency,[6,7,11,41,52] especially those resembling[10] the metabolic syndrome:[43] impaired insulin sensitivity,[5,7,9,10,41] glucose intolerance or diabetes mellitus,[8,9] dyslipidemia[5-11] and visceral adiposity[5-11,52]

A daily dose of transdermal estradiol ranging from 0.22 to 0.35 μg/kg is usually adequate for maintaining serum estradiol within the normal range for adult men.[12]

Effectiveness of estrogen treatment

Estrogen treatment in aromatase deficient men is effective in normalizing gonadotropin secretion,[6,7,9,34,41,42,52] in improving glucose metabolism,[6,7,52] insulin sensitivity,[6,9,36,41] and liver function[9], and in lowering circulating lipids,[6,7,9,41,52] (Box 6). These positive effects of estrogen

treatment mostly lead to an improved metabolic outcome and a reasonable reduction of cardiovascular risk. As male aromatase-deficient men are often reluctant to use 'female' hormones, their adherence to estrogen treatment may be poor, as documented in one striking case,[11] and to a lesser extent before their complete acceptance of the treatment also in the other three patients managed by us (personal observation).[6,9,10] Patient expectations should therefore be managed by providing adequate information on what the expected results are and which goals are not achievable as a way of improving the patient's compliance (Box 6). Some abnormalities associated with aromatase deficiency, such as impaired spermatogenesis, fat distribution and adiposity, and skeletal abnormalities (e.g kyphosis or eunuchoid skeleton), are in fact not modifiable by and do not benefit from estrogen replacement treatment started in adulthood[6,7,9,11,12,15,41,50,54] (Box 6). Physicians should inform the adult patients about those irreversible clinical conditions related to aromatase deficiency that do not benefit from estrogen treatment, the most important one being male infertility.

Estrogen treatment timings

The beginning of estrogen replacement treatment during adulthood is usually due to a delay in diagnosis[5-11,41] (Box 2 and Box 3) and leads to the impossibility of correcting some abnormalities (Box 6), which stem from the prolonged course of the unrecognized disease.

The gold standard timing for starting estrogen treatment in aromatase-deficient males seems to be puberty,[4] but this is subject to a early diagnosis, the difficulties of which have been discussed above (Box 3). When the diagnosis is available at birth or is achieved during infancy, low dosages of exogenous estradiol should be administered at the beginning of puberty (0.8 to 0.12 μg/kg daily)[19,44-46] and then progressively increased at about mid-puberty, mimicking the continuous gradual increases in serum estradiol which characterize the progression of puberty in boys.[32,49] Such treatment proved effective in guarantying a final height within the genetic target in the boy with aromatase deficiency.[4]

A high dose of estradiol should be avoided since it inhibits the pubertal growth spurt from the beginning.[44] Even though no precise age for starting the treatment has been established, the boy has been treated starting from age 17 (final pubertal period) and has not developed the classical skeletal features of adult aromatase-deficient men.[4] This suggests that an appropriate treatment timing is the best choice for normal bone maturation.

Pharmaceutical formulation and safety

Conjugated estrogens, valeriate estradiol, transdermal estradiol patches and gel are all effective. The dosage depends on the type of formulation and the way it is administered. The data on the transdermal patches are the most represented in literature.[6,7,9,11,34,52] A comparison between them becomes therefore possible, with several dosages being suggested for clinical practice. Conjugated estrogens[41] and estradiol valeriate[4] represent an alternative yet valid choice. A formulation containing estradiol should be preferred since it is easy to detect in serum, differently from ethinylestradiol, the latter being probably effective but difficult to monitor during follow-up.

Selective Estrogen Receptor Modulators (SERMs) can also be regarded as a possible alternative to estradiol treatment, but preliminary results in aromatase deficiency treatment are not encouraging.[55]

Estradiol replacement treatment has been well tolerated by aromatase-deficient men. No side effects were reported[4,6-12,16,41] even during the short period of treatment with higher doses of estradiol, which was aimed at bringing serum estradiol shortly above the normal range.[6,12,16,41,50] Moreover, estradiol treatment had no worsening effect on the patients' sexual behaviour. Quite to the contrary, it seems to improve male sexuality, although evidence is still weak.[15,56,57]

The choice of pharmaceutical formulation depends on the physician's experience with estrogen administration and on the patient's compliance and/or preferences[16] and the safety of estrogen

treatment should be monitored, trying in particular to avoid the prolonged use of supraphysiological doses, since to date the safety of high doses has only been established for brief periods of use. [6,12,16,41,50]

Counselling and other treatments

Estrogen replacement treatment should be accompanied by the concomitant administration of calcium plus vitamin D[6,9,41] in order to obtain a synergic effect on bone and prevent or correct vitamin D deficiency.[7,52]

Specific nutritional (hypocaloric diet) and behavioural counselling (lifestyle change) should be offered to treat overweight and the tendency to visceral fat distribution and to prevent overt obesity[10] that have not been modified by estrogen replacement treatment[9,36,52] (Box 6). The counselling is also useful for keeping glucose and lipid metabolism under control.[36] The development of metabolic diseases such as diabetes mellitus or severe dyslipidemia would need specific treatment.

When the diagnosis is reached in adulthood, congenital estrogen deficiency in men is considered linked to an increased cardiovascular risk[1,2,12,58,59] and general recommendations (quitting smoking, blood pressure monitoring and so on) helpful for preventing cardiovascular diseases are needed.

Genu valgum[5-12,41] and other skeletal abnormalities[6,7,50,52,54] require an appropriate specific approach and may necessitate surgical correction. The patient described by Carani et al.[6] underwent a surgical correction of valgism and hip prosthesis surgery to treat bilateral ostenonecrosis of the femoral head.[54] The coexistence of these skeletal abnormalities often limits the patient's ability to move and can contribute to the worsening of overweight and/or obesity as well as of osteopenia.

FOLLOW-UP

The most useful parameters for monitoring the adequacy of the treatment are serum estradiol, followed by serum gonadotropins (especially LH), serum testosterone and BMD.[12,16,17] When all these parameters remain within the normal range it means that optimal dosage has been reached.[16]

In replacement treatment, an adequate dose of estrogen should maintain serum estradiol above the lower quartile but within the upper end of the normal range to ensure normal male estrogenization. The normalization of serum estradiol is generally able to keep serum LH within the normal range.[6,7,9,11] FSH, on the other hand, is more dependent on the fertility pattern and even during treatment it often remains above the normal range, if there is a concomitant impairment of spermatogenesis.[15,42] However, serum gonadotropins and above all LH do represent an accurate parameter for monitoring estrogen replacement therapy, since estradiol, especially circulating estradiol, is the main regulator of gonadotropin secretion both at pituitary[42] and hypothalamic[35] level, which holds also true for non-aromatase-deficient men.[60]

Testosterone is also a good indicator of over-treatment during estrogen therapy, since it decreases significantly when LH is suppressed by excessive exogenous estrogen[6,7,9,12,50] as a consequence of their inhibitory action at both hypothalamic and pituitary levels.[35,60]

DXA allows to monitor bone mineralization, the achievement of peak bone mass,[27] and the relationship of both with the dosage of estrogen. As expected, in an aromatase-deficient man the lowering of transdermal estradiol dose resulted in a decreased BMD,[34] hence BMD does indeed provide information on the adequacy of estradiol treatment for promoting and maintaining bone health.[4,6,7,9,11,16,17,20,34,41,50,52]

Accordingly, serum estradiol, LH, testosterone and BMD should be considered, in clinical practice, as powerful biochemical markers for adequate estrogen substitution, since they are reliable parameters for dose optimization. Indeed, an excessive amount of exogenous estrogen results in LH

suppression and testosterone deficiency,[6,7,9,12,50] while an insufficient amount can result in BMD loss.[11,34] In clinical practice, in order to ensure bone maintenance and physiological estrogenization, dose adjustments are needed in the long-term treatment for identifying the optimal dosage able to keep all the above-mentioned parameters as close as possible to the normal range for adult men.[12,16]

In the short-mid term, the X-ray film of the hand and wrist allows to monitor bone maturation. The completion of bone maturation generally occurs between six to nine months after the start of estrogen treatment, but only if the latter is adequate.[6,7,9,41] An insufficient amount of exogenous estrogen fails to bring about epiphyseal closure as in the patient with estrogen resistance,[61] and lengthen the time course needed for completing bone maturation, thus requiring estrogen dose adjustments.[11]

The possible occurrence and/or worsening as well as the clinical development of associated complications and comorbidities in adult men with aromatase deficiency can either be monitored using several biochemical parameters or by measuring the progress of some signs, such as acanthosis nigricans,[9] and the changes in height and in the other anthropometric parameters useful for monitoring adiposity (weight, waist and hip circumference).[9,10] The monitoring of the latter ones, when accompanied by biochemical tests, is useful for establishing the presence and degree of all the abnormalities related to the metabolic syndrome[9,10,37] and they are also easy to record at each visit. The metabolic patterns,[9,10,37] including NAFLD can improve, at least in part, during estrogen replacement treatment.[6,7,9,11,36,41]The HOMA index[38] provides useful information on the effects of estrogen treatment on the patients' insulin sensitivity.[36,52]

AREAS OF UNCERTAINTIES

To date, the role of estrogens during childhood in males is still unknown. Serum estradiol in male children is undetectable by commercially available assays. However, very low levels of circulating estradiol can be detected by means of ultrasensistive assays,[31,32,62] as expected on the basis of the aromatase expression observed in children,[63] which accounts for *in situ* estrogen production and both autocrine and paracrine effects. At present the physiological role of this very small amount of estrogens during childhood is unknown.[31,32,62] The long-term consequences of estrogen deprivation during childhood are not foreseeable. The approach advocating the start of estrogen treatment at puberty[4] is empirical and not based on solid evidence. This leaves the issue about the need of estrogen treatment in childhood as well as its relative dosage completely unresolved.

From a pathophysiological viewpoint, there is a major lack of data concerning the possible occurrence and relative phenotype of non-classical partial aromatase deficiency in men, a condition consistent that has been recently described in women.[23,64]

Spermatogenesis is often impaired in adult men with aromatase-deficiency, but while in mice a real cause-effect relationship has been established,[14] this still remains to be shown in humans.[15] To date, however, none of the aromatase-deficient men has generated offspring.

The real impact of estrogen treatment on male sexual behaviour, quality of life and well-being in adult aromatase-deficient men remains still to be determined. All evidence of a positive role in sexuality[15] and subjective feelings can be at least partially attributed to the placebo effect or the positive feeling about finally reaching a diagnosis and starting a specific treatment.[56,57]

The long-term outcome in terms of comorbidities and mortality is not yet available. A tendency towards precocious atherogenesis[9] and susceptibility to cardiovascular diseases is assumed,[1,2,9,10,14,58,59,65] but to date no major adverse cardiovascular event has been reported in aromatase-deficient men.

Furthermore, we do not know whether the peak bone mass reached during adulthood is similar to that which should have obtained at puberty. The delayed achievement of peak bone mass could compromise bone quality and bone strength,[27,51] especially as the skeleton growth continued in adulthood and skeletal malformations (micro- and macro-deformations) are present.[7,50,54]

CONCLUSIONS

Aromatase-deficient men need estradiol replacement treatment lifelong to ensure adequate bone mineralization and good health,[12,16] and to minimize the risk of bone fracture.[27] The androgen-estrogen imbalance can impair the clinical conditions of these patients,[36,65] above all vascular reactivity and endothelial function.[58,59] The best strategy for preventing early- and late-onset manifestations of the disease consists in a early diagnosis. From this viewpoint, gynecologists' acquaintance with the disease should be encouraged so as to enable them to suggest a screening for the disease at birth to all children delivered by mothers with evident signs of virilization during pregnancy. When a early diagnosis is achieved, the treatment should be started at the onset of puberty, although the precise age for starting the treatment has not yet been established.

Additional research are needed to elucidate the full clinical spectrum of this rare disease and strengthen the evidence base regarding the benefits and risks of estrogen replacement treatment in men with aromatase deficiency, especially those above mentioned and not yet very well understood.

Acknowledgements:

We thank Laura Maffei (Consultorios Asociados de Endocrinologia, Buenos Aires, Argentina), Evan R. Simpson (Prince Henry's Institute of Medical Research, Victoria, Clayton, Australia), Marco Faustini-Fustini (Endocrinology, Ospedale Bellaria, Bologna, Italy), Manuela Simoni, Antonio Balestrieri, and Lucia Zirilli (Endocrinology, University of Modena & Reggio Emilia, Modena, Italy), for their longstanding contributions to the field of aromatase deficiency in men.

We are grateful to Giuseppina Rossi (Department of Medicine, Endocrinology and Metabolism, and Geriatrics, Chair of Endocrinology, University of Modena and Reggio Emilia) for technical support in the writing of the manuscript.

We are grateful to Elisa Pignatti (Department of Medicine, Endocrinology and Metabolism, and Geriatrics, Chair of Endocrinology, University of Modena and Reggio Emilia) for her help with the genetic and biomolecular analyses.

We thank Dr. Yael Ukmar for proofreading and editing the manuscript.

Part of this study has been presented at the 32th National Congress of the Italian Society of Endocrinology Verona, Italy, 13-16 June 2007. Some of the underlying concepts and ideas have already appeared, although in germinal form, in the PhD thesis of Dr. Vincenzo Rochira entitled "Fisiopatologia degli estrogeni nel maschio?", supervised by Prof. Cesare Carani and completed in 2004.

Disclosure Statement: The authors have nothing to disclose.

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LEGENDS

Box 1

Estrogen actions in men.

[*Footnote to Box 1*]: for reviews see references 1,2,6,12-15,17,18,41

Box 2

Clinical presentation of men with aromatase deficiency and its relationship with age at onset of the disease.

Box 3

Diagnosis of aromatase deficiency: relevant aspects and diagnostic criteria for suspicion according to the patient's age.

Box 4

Clinical skills available from phisical examination and detailed interview and useful for the diagnosis

Box 5

Procedures for the genetic analysis useful to diagnose aromatase deficiency

[*Footnote to Box 5*] The primer sequences useful for the genetic analysis of the CYP19A1 have been characterized by Mullis et al. (24 Mullis 1997). All the procedures useful for a complete the genetic study have been well characterized and previously described (5 Morishima 1995, 6 Carani C 1997 N Engl J Med, 9 Maffei 2004 JCEM, 10 Maffei 2007 Clin Endocrinol, 21 Mullis 1997, 22 Belgorosky 2003 JCEM, 23 Lin 2007 JCEM).

Box 6

Effectiveness of estrogen treatment in men with aromatase deficiency.

Table 1

Differential diagnosis based on hormonal pattern

[*Footnote to Table 1*] E_2: estradiol; T: testosterone; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone.

Figure 1

Aromatase deficiency: clinical management flow chart.

[footnote for Figure 1] BMD: bone mineral density, T: testosterone, LH: luteinizing hormone, FSH: follicular stimulating hormone, E_2: estradiol.
***** Essential parameters for the follow-up
§ Useful tools for the follow-up
Useful tools for the short-mid term follow-up.

Box 1

Well-established
Epiphyseal fusion
Growth arrest
Achievement of peak bone mass
Maintenance of bone mass throughout adulthood
Harmonic skeletal proportions
Pituitary inhibition of LH and FSH
Hypothalamic inhibition of LH and FSH
Strong evidence
Modulation of insulin sensitivity
Positive effect on lipids
Influence on adiposity and fat distribution
Induction of pubertal growth spurt
Biphasic action on growth plate
Lack of prenatal effects on gender identity and psychosexual orientation
Weak evidence (to be confirmed)
Control of fat accumulation in the liver
Prevention of lipid accumulation in the arterial wall
Maturation of the hypothalamic-pituitary unit during early development
Control of spermatogenesis and sperm maturation
Control of the development of male reproductive structures
Positive influence on male sexual behavior

Box 2

Frequency of the disease
- Rare (8 cases in medical literature)

The onset of the disease
- At birth
- Lack of signs and symptoms during infancy and early adolescence

The onset of signs and symptoms
- Late adolescence (scarce and hardly noticeable signs of the disease: absence of pubertal spurt, initial eunuchoid proportions of the skeleton, delayed bone maturation, lack of growth arrest)

Clinical aspects in adult men at clinical presentation
- *Signs and symptoms likely to be present*
  - Tall stature
  - Widespread bone pain
  - Continuing and progressive linear growth during adulthood
  - Delayed bone maturation
  - Unfused epiphyses
  - Eunuchoid proportions of the skeleton
  - Progressive *genu valgum*
  - Osteopenia or osteoporosis
- *Signs and symptoms almost always present*
  - Abdominal adiposity
  - Acanthosis nigricans
  - Early-onset metabolic syndrome
  - Non-alcoholic fatty liver disease
  - Dyslipidemia
- *Signs and symptoms occasionally present*
  - Oligozoospermia
  - Abnormalities in spermatogenesis
  - Cryptorchidism
  - Increased testis volume

Box 3

Diagnosis-related-aspects

- Difficult to recognize at birth, during childhood and adolescence
- Risk of delayed diagnosis (mean age: 27±2.5 years)
- Risk of overlooking and under-managing the patient's clinical situation

Clinical aspects relevant for suspecting aromatase deficiency

Childhood

- Mother's virilization during the last trimester of pregnancy
- Diagnosis of aromatase deficiency in a patient's relative (sister or brother)

Puberty

- Absence of pubertal spurt
- Delayed bone maturation
- Initial eunuchoid proportions of the skeleton

Adulthood

Relevant criteria

- Continuing linear growth during adulthood
- Delayed bone maturation
- Detection of unfused epiphyses in adult men
- Finding of very low serum estradiol in adult men

All the other clinical features (signs and symptoms) listed in Box 2 becames of relevance only when associated with one or more of the above mentioned 'relevant criteria'

Box 4

Specific issues to address during the clinical interview
Relatives' information ▪Parents' consanguineity ▪Parents' height ▪Maternal virilization during pregnancy ▪History of ambiguous genitalia and/or delayed puberty in sisters
Patient's informations ▪Weight and length at birth ▪Esteem of his target height ▪Patient's early growth ▪Patient's pubertal development ▪History of cryptorchidism ▪Presence of offspring ▪Patient's sexual behavior (sexual identity, sexual orientation, sexual activity)
Specific issues to address during the physical examination
Anthropometric parameters ▪Height ▪Weight ▪BMI ▪Upper and lower skeleton segments lengths ▪Lower segment of the skeleton length ▪Arm span length ▪Waist and hip circumferences
Other clinical examinations ▪Degree of virilization ▪Testicular volume ▪Testes localization ▪Penis size ▪Blood pressure ▪Check for skeletal deformations (e.g. scoliosis or genu valgum) ▪Check for surrogates of insulin resistance (acanthosis nigricans and skin tags)

Box 5

Genetic Analysis of the *CYP19A1* gene

Gene characteristics:

- Cytochrome P450, family 19, subfamily A, polypeptide 1
- Medium size
- 123 kilobases long
- Coding region 30 kilobases long
- Coding region splitted into only IX exons (exons II-to-X)

Steps of Genetic Analysis:

- Patient's peripheral blood collection
- Genomic DNA extraction
- PCR amplification of the coding exons (II-X) and their flanking intronic sequence and of the untraslated exon 1.4
- Purified PCR product is then sequenced by means of a commercially available automated DNA sequencer
- Comparison of the result with the published sequences of the human *CYP19A1*

Study of Gene Function:

- mRNA expression in transfected cells
- Aromatase activity of the mutant protein in transfected cells
- Comparison with mRNA expression and aromatase activity of the wild-type
- Cell lines useful for trensfection: COS-1 or COS-7 cells
- $^{3}H2O$ production from the substrate [1β-^{3}H]androstenedione is the standard for measuring aromatase activity

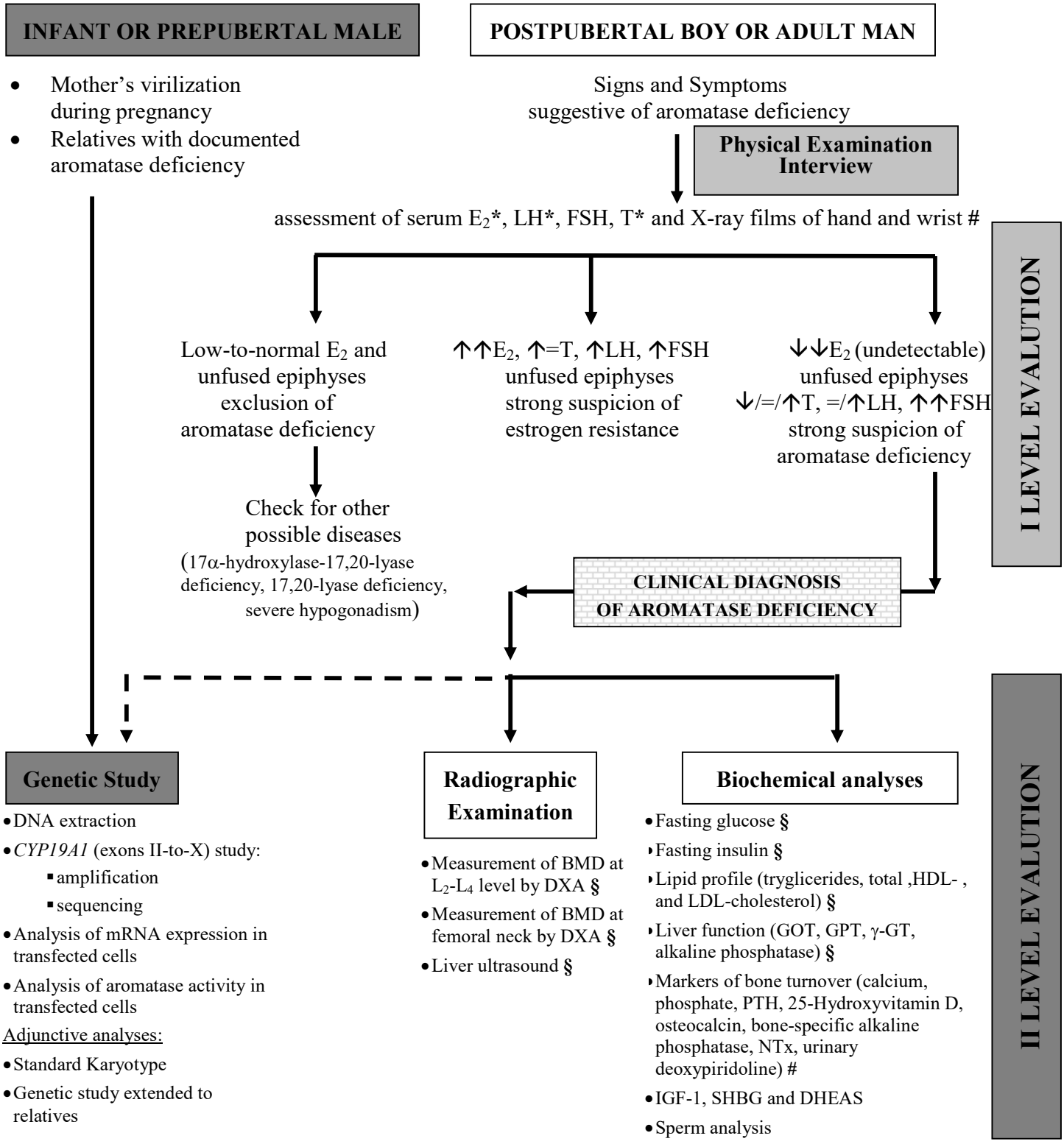
Standard karyotype

Box 6

Effective ▪Bone maturation (epiphyseal closure and growth arrest) ▪Achievement of peak bone mass ▪Maintenance of bone mineral content ▪Improved insulin sensitivity ▪Improved lipid pattern ▪Improvement in NAFLD ▪Restored normal functioning of the hypothalamic-pituitary gonadal axis ▪Skeletal defects (eunuchoid skeleton, *genu valgum*) if not previously present
Ineffective ▪Adiposity (overweight and visceral fat distribution) ▪Male fertility and spermatogenesis ▪Skeletal defects (eunuchoid skeleton, *genu valgum*) when already present

Table 1

	E_2	T	LH	FSH	Adrenal androgens
Aromatase deficiency	↓↓↓↓	↓/=/↑	=/↑	↑↑	=/↑
Estrogen resistance	↑↑	=	↑	↑	?
17α-hydroxylase, 17,20-lyase deficiency	↓	↓↓	↑	↑	↑↑
17,20-lyase deficiency	↓	↓↓	↑	↑	↑↑
Severe hypogonadism	↓	↓↓	↓↓	↓↓	=


INFANT OR PREPUBERTAL MALE
POSTPUBERTAL BOY OR ADULT MAN
• Mother's virilization during pregnancy
• Relatives with documented aromatase deficiency
Signs and Symptoms suggestive of aromatase deficiency
Physical Examination Interview
assessment of serum E2*, LH*, FSH, T* and X-ray films of hand and wrist #
I LEVEL EVALUTION
Low-to-normal E2 and unfused epiphyses exclusion of aromatase deficiency
↑↑E2, ↑=T, ↑LH, ↑FSH unfused epiphyses strong suspicion of estrogen resistance
↓↓E2 (undetectable) unfused epiphyses ↓/=/↑T, =/↑LH, ↑↑FSH strong suspicion of aromatase deficiency
Check for other possible diseases (17α-hydroxylase-17,20-lyase deficiency, 17,20-lyase deficiency, severe hypogonadism)
CLINICAL DIAGNOSIS OF AROMATASE DEFICIENCY
Genetic Study
• DNA extraction
• CYP19A1 (exons II-to-X) study:
▪ amplification
▪ sequencing
• Analysis of mRNA expression in transfected cells
• Analysis of aromatase activity in transfected cells
Adjunctive analyses:
• Standard Karyotype
• Genetic study extended to relatives
Radiographic Examination
• Measurement of BMD at L2-L4 level by DXA §
• Measurement of BMD at femoral neck by DXA §
• Liver ultrasound §
Biochemical analyses
• Fasting glucose §
• Fasting insulin §
• Lipid profile (tryglicerides, total ,HDL- , and LDL-cholesterol) §
• Liver function (GOT, GPT, γ-GT, alkaline phosphatase) §
• Markers of bone turnover (calcium, phosphate, PTH, 25-Hydroxyvitamin D, osteocalcin, bone-specific alkaline phosphatase, NTx, urinary deoxypiridoline) #
• IGF-1, SHBG and DHEAS
• Sperm analysis
II LEVEL EVALUTION