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Reversibility in male idiopathic osteoporosis possible

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Summary

A 44-year-old athletic man presented in 2009 with severe low back pain. Dual-energy x-ray absorptiometry revealed severe osteoporosis; serum testosterone was 189 ng/dL while serum estradiol (E_2) measured by liquid chromatography/mass spectrometry was 8 pg/mL. DNA was extracted and sequenced from a blood sample from the patient since his maternal first cousin also had low bone mass and both patients were screened for aromatase dysfunction by PCR analysis for the *CYP19A1* gene, which encodes aromatase. No known pathologic mutations were observed in the coding exons, but novel single nucleotide polymorphisms were detected both in the proband and in his cousin. Treatment with topical testosterone started in August 2010. Over the next 8 years, testosterone dosage was varied and switched from topical gel to injections and maintained on depo-injections of testosterone at about 60 mg once per week. Re-examination in March 2012 included a brain MRI to exclude pituitary lesions; hyperparathyroidism was ruled out (normal serum parathyroid hormone, calcium, and calcium to phosphorous ratio) and celiac disease was excluded (negative transglutaminase antibodies). Follow-up in October 2018 showed improved bone mineral density of the lumbar spine by 29% and of the left femoral hip by 15% compared to baseline measurements. This reveals the importance of measuring serum E_2 for making the correct diagnosis, as well as for monitoring a therapeutic effect. Herein, we propose treatment of male osteoporosis where serum E_2 levels are below about 20 pg/mL with testosterone to reverse osteoporosis.

Learning points

- Estrogen deficiency in the diagnosis of male idiopathic osteoporosis.
- Importance of serum estradiol in male osteoporosis.
- Role of polymorphisms in aromatase gene on bone health.
- Reversal of osteoporosis.
- Tailored testosterone treatment for bone health.

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Background

Primary and secondary hypogonadism with serum total testosterone concentrations <200 ng/dL is a well-recognized cause of osteoporosis in men of all ages (1, 2, 3, 4). In men with low or low-normal serum testosterone levels, however, such as patients with Klinefelter syndrome, bone mineral density (BMD) may be preserved without testosterone replacement (5). In contrast, loss-of-function mutations in the CYP19A1 gene, which encodes for aromatase, the enzyme responsible for converting testosterone to estradiol (E_2), is a rare syndrome characterized by the presence of unfused epiphyses, osteopenia or osteoporosis, eunuchoid skeletal proportions, and, importantly, undetectable serum E_2 levels which requires lifelong estrogen replacement therapy (6, 7).

In clinical settings different from congenital estrogen deficiency, BMD decrease is inversely correlated with low serum E_2, but not with serum T, as noted in several population studies (8, 9), suggesting that in cases of low T, BMD is impaired only when serum E_2 is low as in hypogonadal men (3, 9). Similarly, low serum E_2 due to mild estrogen deficiency may theoretically cause osteopenia or osteoporosis in eugonadal men (10, 11).

Case presentation

The patient, a 44-year-old 6-foot 2-inch (1.87 m), 178 pounds (81 kg), BMI of 22.9, non-smoker, with minimal alcohol consumption complaining of chronic low back pain was first seen by Dr Michael C. Ullery in January 2009. A complete physical examination showed a healthy athletic male who exercised aerobically and with weights for approximately 60 min a day, ruling out RED-S. No blue discoloration of his sclera was observed ruling out osteogenesis imperfecta. Multiple myeloma and marfanoid syndrome were also excluded. Initial x-rays and CT scans suggested degenerative disc disease and the patient was conservatively treated with chiropractic and physiotherapy but with no lasting relief. The first dual-energy x-ray absorptiometric (DEXA) scan done in January 2009 showed a BMD of the lumbar spine of 0.784 g/cm^2 with a T score of −2.8 and a Z score of −2.6 and left femoral neck value of 0.718 g/cm^2 with a T score of −2.1 and a Z score of −1.8, respectively, indicative of severe osteoporosis of the lumbar spine and osteopenia of the left hip. A blood test revealed low serum testosterone (189 ng/dL) and a low E_2 of 8 pg/mL. Repeated blood tests were performed in order to obtain a diagnosis between January 2009 and August 2010 (pre-treatment) and showed low to normal testosterone levels with consistently low serum E_2 levels of 8–17 pg/mL (Table 1). Serum E_2 was measured by an ultrasensitive E_2 test based on liquid chromatography/mass spectrometry (LC/MS) (Quest Diagnostics Nichols Institute San Juan Capistrano, CA, USA) with a sensitivity of 4 pg/mL. In February 2009, the patient underwent a thorough examination at the Mayo Clinic (Scottsdale, Arizona, USA) and a repeat DEXA scan there confirmed osteoporosis/osteopenia. The patient was put on calcium (1200 mg/daily) and Vitamin D3 (800 IU/daily) supplementation with bisphosphonate therapy. The latter consisted of alendronate (Fosamax®) 10 mg/daily p.o. from 16 to 23 February 2009 (7 days) when the drug was stopped as the patient developed diarrhea and abdominal cramps. He was next treated with risedronate (Actonel®) at 5 mg/daily p.o. from 2 to 19 March 2009 (18 days). The patient developed jaw pain and this drug was also discontinued.

Investigation

In an effort to uncover the cause of the osteoporosis, the patient was next seen in August 2009 by an endocrinologist at the University of California San Francisco Medical Center (San Francisco, CA, USA). Differential diagnoses included marfanoid syndrome and hypogonadism but were each excluded by physical examination and laboratory tests.

In October 2009, the patient was seen by an endocrinologist, Dr Massimiliano Rochietti March at the Ospedale S. Andrea in Rome, Italy.

Back in the USA, laboratory tests in August 2010 showed serum testosterone of 275 ng/dL in the low-normal range, and serum E_2 of 8 pg/mL, the latter well below the reference range for adult males (10–35 pg/mL) and below the threshold of 20 pg/mL below which bone health is not protected.

In October 2010, the patient (patient 1) reported that his then-48 years old maternal first cousin (patient 2), born of the twin of the patient's mother, had documented osteopenia, normal serum testosterone (300 ng/dL), and still low serum E_2 (17 pg/mL).

DNA was extracted and sequenced from a blood sample from patient 1 and from his maternal first cousin (patient 2) and analyzed by PCR for the *CYP19A1* gene, which encodes aromatase, in Modena, Italy. No known pathologic mutations were observed in the coding exons, but novel single nucleotide polymorphisms (SNPs) were detected in both the proband (patient 1) and his cousin (patient 2) which might explain the consistently low serum E_2.

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Table 1 Serum testosterone and estradiol in patient 1.

	Pre-treatment				Post-treatment									
	January 2009	February 2009	August 2009	August 2010	December 2010	August 2011	April 2012	February 2013	December 2013	August 2015	July 2016	November 2017	September 2018	
Testosterone														
Total (ng/dL)	189	353	336	275	296	422	435	309	869	557	472	1006	626	
Free (pg/mL)	7.5	66.8	67.9	50.8	62.5	112	NA	NA	NA	118.7	NA	NA	148.5	
Estradiol-E_2 (pg/mL)	8	17	15	8	21	43	37	22	36	43	40	41	31	

Treatment

Patient 1 was first treated with topical testosterone (AndroGel® 1.62%, 5 g/day) beginning in August 2010 in an attempt to force testosterone conversion to E_2 by increasing the concentrations of the androgenic substrate thereby increasing the patient's serum E_2. By December 2010, serum testosterone was 296 ng/dL, but serum E_2 had increased to 21 pg/mL and remained stable above 20 pg/mL over time during testosterone treatment (Table 1). Over the next decade, testosterone dosing was varied and switched from topical gel to injections and maintained on depo-injections at about 60 mg testosterone once per week.

Pituitary lesions (brain MRI), hyperparathyroidism (laboratory investigations), and celiac disease (negative antibodies) were excluded.

Table 1 shows the changes in testosterone and E_2 over time in patient 1, and Table 2 shows the changes in BMD of the lumbar spine and left femoral neck in patient 1. Figure 1 is a plot of serum E_2 vs lumbar spine BMD indicating that serum E_2 above about 20 pg/mL is associated with consistent increases in BMD in patient 1.

The patient (patient 1), 57 in 2018, is doing well and his BMD at the lumbar spine appears to have peaked as of October 2018 (Table 2). Overall, his lumbar spine BMD improved by 29% from the lowest measured value baseline in 2010 while BMD in the left femoral hip improved by 15% over this period.

His maternal first cousin (patient 2), now 60 years of age, opted not to undergo any treatment and showed normal testosterone (337 ng/dL) with low E_2 (17 pg/mL) in October 2009. A summary of his blood work is presented in Table 3. As of January 2016, he continued to have osteopenia of his lumbar spine. BMD changes are given in Table 4. The genetic analyses of the aromatase gene for both the proband (patient 1) and his first cousin (patient 2) are given in Table 5.

Outcome and follow-up

The patient was doing well at follow-up in 2018 and to date (2023).

Discussion

The increase of serum E_2 from under 20 pg/mL due to testosterone treatment improved BMD markedly over a period of about 10 years. This is in line with experimental data coming from rare cases of men with aromatase deficiency (6) showing that the increase of serum E_2 leads

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Table 2 Changes in bone mineral density (g/cm²) over time in patient 1.

	January 2009	January 2011	October 2011	January 2013	August 2014	April 2017	October 2018
Lumbar spine	0.784	0.754	0.796	0.830	0.862	0.802	0.975
T score	−2.8	−3.1	−2.7	−2.4	−2.1	−2.6	−2.1
Z score	−2.6	−2.8	−2.4	−2.1	−1.7	−2.2	−1.9
Left femoral neck	0.718	0.740	0.731	0.749	0.791	0.778	0.825
T score	−2.1	−1.9	−2.0	−1.9	−1.6	−1.7	−1.9
Z score	−1.8	−1.7	−1.7	−1.6	−1.3	−1.3	−1.2

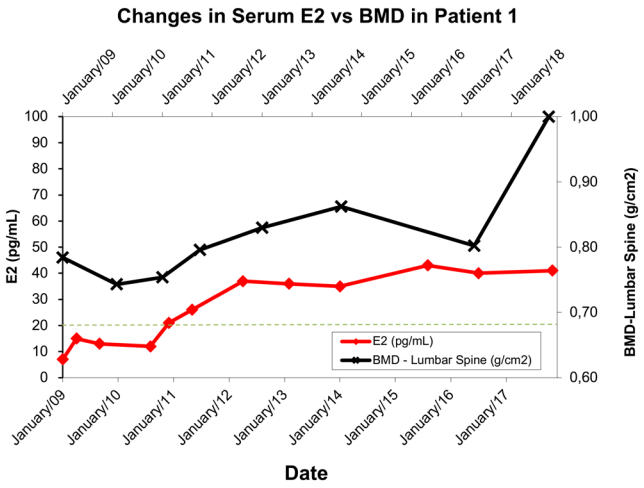

Figure 1
Changes in serum E_2 vs BMD in patient 1.

Table 3 Serum testosterone and estradiol in patient 2.

	October 2009	May 2016
Testosterone		
Total (ng/dL)	300	353
Free (pg/mL)	66.1	66.8
Estradiol (pg/mL)	17	16.7

Table 4 Changes in bone mineral density (g/cm²) over time in patient 2.

	January 2009	October 2014	October 2016
Lumbar spine	0.966	0.958	0.958
T score	−1.1	−1.2	−1.2
Z score	−0.9	−0.8	−0.7
Left femoral neck	0.794	0.774	0.661
T score	−1.6	−1.7	−2
Z score	−1.3	−1.4	−1.1

to a parallel increase in BMD in a dose-dependent fashion (7). Furthermore, the data reported here confirm that BMD in men requires serum E_2 levels to be consistently above 20 pg/mL (9, 11), as already demonstrated in different settings such as aromatase-deficient men (12) and in population studies (13, 14). As a matter of fact, the same concept was introduced by Nethander *et al.* who demonstrated that a 1 S.D. (9.6 pg/mL) decrease in serum E_2 is associated with a 0.38 S.D. decrease of BMD and to an increase in fracture risk (15). Caution about the assay used for serum E_2 measurement (LC/MS method used in this study) is mandatory since many commercially available assays have poor accuracy for the measurement of E_2 in the low range in males (10, 16). For this reason, the routine measurement of serum E_2 is not recommended in men with or without impaired BMD (10, 17), except in cases where an endocrinologist is aware of an accurate and sensitive method for E_2 assay used by the referral laboratory (10). Accordingly, the rapid diffusion of the LC-tandem mass spectrometry in clinical laboratories together with the availability of immunoassays for E_2 that are the most accurate and sensitive for serum E_2 in the range of 10 to 30 pg/mL (18, 19) may be of value in clinical practice in order to ruling in/ruling out relative estrogen deficiency in men with hypogonadism or with low to normal serum testosterone (10, 11), such as in the case here described. Accordingly, when ultrasensitive assays are used, serum E_2 changes may be monitored to check if testosterone treatment is able to ensure values above 20 pg/mL and to optimize the dose of testosterone treatment looking also at bone health (10, 11).

Patient 1 described here showed a peculiar pattern of various genetic polymorphisms of the aromatase gene and this pattern was shared with his first cousin (patient 2) (their mothers were maternal twins) who both presented with low serum E_2 and osteopenia/osteoporosis. The result of the genetic analysis raises the question of the possible causal effect of these polymorphisms in lowering aromatase function and, consequently, serum E_2. Recently, several genetic studies have demonstrated an association between some aromatase polymorphisms and lower levels of serum E_2 in men (20, 21, 22). Even though none of the SNPs and/or polymorphisms found in our patient and his cousin are known to be related to aromatase dysfunction, it is possible that one or more of them may be at the basis of their observed low serum E_2.

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Table 5 Results of the genetic analysis of the *CYP19A1* gene (encoding for the enzyme aromatase) of patient 1 and of his first cousin patient 2. The two cousins showed the same identical pattern of polymorphisms of the aromatase gene. Underlined, the intron 4 tetranucleotide repeat polymorphism associated with estrogen levels (23).

Patient 1	Patient 2
Wildtype	Wildtype
g.106531A>G in homozygosity (SNP, rs3759811)	g.106531A>G in homozygosity (SNP, rs3759811)
g.106684A>G (Val80Val) in homozygosity (SNP, rs700518)	g.106684A>G (Val80Val) in homozygosity (SNP, rs700518)
IVS4 TTTA repeat 7/7 (STR polymorphism, rs60271534)	IVS4 TTTA repeat 10/10 (STR polymorphism, rs60271534)
g.124928T>G in homozygosity (SNP, rs4324076)	g.124928T>G in homozygosity (SNP, rs4324076)
g.125094A>T in homozygosity (SNP, rs1143704)	g.125094A>T in homozygosity (SNP, rs1143704)
Wildtype	Wildtype
Wildtype	Wildtype
Wildtype	Wildtype
g.132810C>T in homozygosity (SNP, rs10046)	g.132810C>T in homozygosity (SNP, rs10046)
g.132952T>G in homozygosity (SNP, rs4646)	g.132952T>G in homozygosity (SNP, rs4646)

In conclusion, this case demonstrates the importance of measuring serum E_2 in male patients with idiopathic osteoporosis and low or low to normal serum T. In this case, the pivotal role of serum E_2 in improving BMD over 8 years in a male patient with consistently low serum E_2 (<20 pg/mL) and low to normal serum testosterone and a peculiar SNP pattern in his aromatase gene similar to that of his first cousin with osteopenia/osteoporosis. At present, serum E_2 is rarely checked in men in clinical practice but should be considered if an ultrasensitive method for E_2 assay is available (10, 16).

It is indisputable that the physiological concept that only serum E_2 above a certain value can ensure optimal bone health. With this in view, these two cases of male first cousins add new insights to this issue. We propose that testosterone treatment of male osteoporosis patients where serum E_2 levels are consistently below about 20 pg/mL and low or low to normal testosterone may help reverse osteoporosis. In order to exert beneficial effects on BMD, testosterone replacement treatment of hypogonadal men must ensure serum E_2 levels above 20 pg/mL.

Declaration of interest
The authors declare there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent
Written informed consent for publication of their clinical details was obtained from the patient.

Author contribution statement
All authors contributed to the diagnosis and treatment of the patients in this study, in the evaluation of the data presented and the drafting of this manuscript.

References

1 Stahl F, Dörner G, Knappe G & Schnorr D. Total and free testosterone in plasma of hypo- and agonadal men. *Endokrinologie* 1975 **66** 152–157.
2 Fink HA, Ewing SK, Ensrud KE, Barrett-Connor E, Taylor BC, Cauley JA & Orwoll ES. Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 3908–3915. (https://doi.org/10.1210/jc.2006-0173)
3 Rochira V, Balestrieri A, Madeo B, Zirilli L, Granata AR & Carani C. Osteoporosis and male age-related hypogonadism: role of sex steroids on bone (patho)physiology. *European Journal of Endocrinology* 2006 **154** 175–185. (https://doi.org/10.1530/eje.1.02088)
4 Finkelstein JS, Lee H, Leder BZ, Burnett-Bowie SA, Goldstein DW, Hahn CW, Hirsch SC, Linker A, Perros N, Servais AB, *et al.* Gonadal steroid-dependent effects on bone turnover and bone mineral density in men. *Journal of Clinical Investigation* 2016 **126** 1114–1125. (https://doi.org/10.1172/JCI84137)
5 Bonomi M, Rochira V, Pasquali D, Balercia G, Jannini EA & Ferlin A. Klinefelter syndrome (KS): genetics, clinical phenotype and hypogonadism. *Journal of Endocrinological Investigation* 2016 **40** 123–134. (https://doi.org/10.1007/s40618-016-0541-6)
6 Rochira V & Carani C. Aromatase deficiency in men: a clinical perspective. *Nature Reviews. Endocrinology* 2009 **5** 559–568. (https://doi.org/10.1038/nrendo.2009.176)
7 Rochira V, Faustini-Fustini M, Balestrieri A & Carani C. Estrogen replacement therapy in a man with congenital aromatase deficiency: effects of different doses of transdermal estradiol on bone mineral density and hormonal parameters. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 1841–1845. (https://doi.org/10.1210/jcem.85.5.6583)
8 Vandenput L & Ohlsson C. Estrogens as regulators of bone health in men. *Nature Reviews. Endocrinology* 2009 **5** 437–443. (https://doi.org/10.1038/nrendo.2009.112)
9 Rochira V, Kara E & Carani C. The endocrine role of estrogens on human male skeleton. *International Journal of Endocrinology* 2015 **2015** 165215. (https://doi.org/10.1155/2015/165215)
10 Rochira V & Carani C. Estrogen deficiency in men. In *Endocrinology of the Testis and Male Reproduction, Endocrinology*. M Simoni & I

Huhtaniemi Eds. Cham: Springer 2017. (https://doi.org/10.1007/978-3-319-29456-8_27-1)
11 Rochira V. Late-onset hypogonadism: bone health. *Andrology* 2020 **8** 1539–1550. (https://doi.org/10.1111/andr.12827)
12 Lanfranco F, Zirilli L, Bald M, Pignatti E, Cornelia G, Gheg E, Aimaretti G, Carani C, Rochira V. A novel mutation in the human aromatase gene: insights on the relationship among serum estradiol, longitudinal growth and bone mineral density in an adult man under estrogen replacement treatment. *Bone* 2008 **43** 628–635. (https://doi.org/10.1016/j.bone.2008.05.011)
13 Mellström D, Vandenput L, Malian H, Holmberg AH, Lorentzon M, Oden A, Johansson H, Orwoll ES, Laborie F, Carlson MK, *et al.* Older men with low serum estradiol and high serum SHBG have an increased risk of fractures. *Journal of Bone and Mineral Research* 2008 **23** 1552–1560. (https://doi.org/10.1359/jbmr.080518)
14 Khosla S, Bellido TM, Drezner MK, Gordon CM, Harris TB, Kiel DP, Kream BE, LeBoff MS, Lian JB, Peterson CA, *et al.* Forum on aging and skeletal health: summary of the proceedings of an ASBMR workshop. *Journal of Bone and Mineral Research* 2011 **26** 2565–2578. (https://doi.org/10.1002/jbmr.488)
15 Nethander M, Vandenput L, Eriksson AL, Windahl S, Funck-Brentano T & Ohlsson C. Evidence of a causal effect of estradiol on fracture risk in men. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 433–442. (https://doi.org/10.1210/jc.2018-00934)
16 Rosner W, Hankinson SE, Sluss PM, Vesper HW & Wierman ME. Challenges to the measurement of estradiol: an endocrine society position statement. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 1376–1387. (https://doi.org/10.1210/jc.2012-3780)
17 Rochira V, Antonio L & Vanderschueren D. EAA clinical guideline on management of bone health in the andrological outpatient clinic. *Andrology* 2018 **6** 272–285. (https://doi.org/10.1111/andr.12470)
18 Sluss PM, Hayes FJ, Adams JM, Barnes W, Williams G, Frost S, Ramp J, Pacenti D, Lehotay DC, George S, *et al.* Mass spectrometric and physiological validation of a sensitive, automated, direct immunoassay for serum estradiol using the Architect. *Clinica Chimica Acta; International Journal of Clinical Chemistry* 2008 **388** 99–105. (https://doi.org/10.1016/j.cca.2007.10.020)
19 Ketha H, Girtman A & Singh RJ. Estradiol assays--the path ahead. *Steroids* 2015 **99** 39–44. (https://doi.org/10.1016/j.steroids.2014.08.009)
20 Eriksson AL, Perry JRB, Coviello AD, Delgado GE, Ferrucci L, Hoffman AR, Huhtaniemi IT, Ikram MA, Karlsson MK, Kleber ME, *et al.* Genetic determinants of circulating estrogen levels and evidence of a causal effect of estradiol on bone density in men. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 991–1004. (https://doi.org/10.1210/jc.2017-02060)
21 Yeap BB, Knuiman MW, Handelsman DJ, Ho KKY, Hui J, Divitini ML, Arscott GM, McQuillan B, Hung J & Beilby JP. A 5α-reductase (SRD5A2) polymorphism is associated with serum testosterone and sex hormone-binding globulin in men, while aromatase (*CYP19A1*) polymorphisms are associated with oestradiol and luteinizing hormone reciprocally. *Clinical Endocrinology* 2019 **90** 301–311. (https://doi.org/10.1111/cen.13885)
22 Gennari L, Nuti R & Bilezikian JP. Aromatase activity and bone homeostasis in men. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 5898–5907. (https://doi.org/10.1210/jc.2004-1717)
23 Mao JJ, Su HI, Feng R, Doelso ML, Aplenc R, Rebbeck TR, Stanczyk F & DeMichele A. Association of functional polymorphism in *CYP19A1* with aromatase inhibitor associated arthralgia in breast cancer survivors. *Breast Cancer Research* 2011 **13** R8. (https://doi.org/10.1186/bcr2813)

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