ORIGINAL ARTICLE

Estrogen Excess Associated with Novel Gain-of-Function Mutations Affecting the Aromatase Gene

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ABSTRACT

BACKGROUND

Gynecomastia of prepubertal onset may result from increased estrogen owing to excessive aromatase activity in extraglandular tissues. A gene in chromosome 15q21.2 encodes aromatase, the key enzyme for estrogen biosynthesis. Several physiologic tissue-specific promoters regulate the expression of aromatase, giving rise to messenger RNA (mRNA) species with an identical coding region but tissue-specific 5′-untranslated regions in placenta, gonads, brain, fat, and skin.

METHODS

We studied skin, fat, and blood samples from a 36-year-old man, his 7-year-old son, and an unrelated 17-year-old boy with severe gynecomastia of prepubertal onset and hypogonadotropic hypogonadism caused by elevated estrogen levels.

RESULTS

Aromatase activity and mRNA levels in fat and skin and whole-body aromatization of androstenedione were severely elevated. Treatment with an aromatase inhibitor decreased serum estrogen levels and normalized gonadotropin and testosterone levels. The 5′-untranslated regions of aromatase mRNA contained the same sequence (FLJ) in the father and son and another sequence (TMOD3) in the unrelated boy; neither sequence was found in control subjects. These 5′-untranslated regions normally make up the first exons of two ubiquitously expressed genes clustered in chromosome 15q21.2–3 in the following order (from telomere to centromere): *FLJ*, *TMOD3*, and aromatase. The aromatase gene is normally transcribed in the direction opposite to that of *TMOD3* and *FLJ*. Two distinct heterozygous inversions reversed the direction of the *TMOD3* or *FLJ* promoter in the patients.

CONCLUSIONS

Heterozygous inversions in chromosome 15q21.2–3, which caused the coding region of the aromatase gene to lie adjacent to constitutively active cryptic promoters that normally transcribe other genes, resulted in severe estrogen excess owing to the overexpression of aromatase in many tissues.

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Aromatase is the key enzyme for estrogen biosynthesis. The aromatase gene (also referred to as *CYP19*) on chromosome 15q21.2 encodes aromatase messenger RNA (mRNA), which produces aromatase, an enzyme that converts C19 steroids to estrogens.[1,2] The human aromatase gene is transcribed under the control of tissue-specific promoters.[2] These promoters are dispersed in a large, 90-kb regulatory region.[2] Each promoter is activated by a different set of hormones and regulates the expression of aromatase, giving rise to mRNA species with an identical coding region but variable tissue-specific 5′-untranslated regions in placenta, gonads, brain, fat, and skin.[2] Thus, the 5′-untranslated regions of aromatase mRNA may be viewed as a signature of the promoter used in a particular tissue.[2]

In ovulatory women, estrogen is produced primarily by aromatase in ovarian granulosa cells by means of the proximally located promoter II, activated by follicle-stimulating hormone.[2] In men and postmenopausal women, however, estrogen is produced primarily in extraglandular tissues such as fat and skin, which expresses low levels of aromatase by means of distal promoters I.3 and I.4 located 0.2 and 73 kb, respectively, upstream of the coding region.[2,3]

Excess estrogen in boys causes gynecomastia, a premature growth spurt, early fusion of epiphyses, and decreased adult height. One cause of gynecomastia of prepubertal onset involves the secretion of estrogen by testicular Sertoli-cell tumors associated with the Peutz–Jeghers syndrome.[4-7] Increased conversion of steroid precursors to estrogens in extraglandular tissues represents another cause of estrogen excess. Hemsell et al. described a feminized, prepubertal, adopted boy in whom large amounts of estrone and estradiol were produced by extraglandular aromatization of plasma androstenedione.[8] Subsequently, three families were described in which several members had estrogen excess (manifested as gynecomastia in boys and men and premature thelarche in girls) as a result of increased extraglandular aromatization inherited in an autosomal dominant fashion.[9-11]

We evaluated for genetic abnormalities three male patients who had severe gynecomastia of prepubertal onset and strikingly elevated circulating estrone and estradiol levels owing to severely increased extraglandular aromatase activity.

CASE REPORTS

PATIENT 1

Patient 1 was a 36-year-old man who had progressive gynecomastia and a linear growth spurt at the age of 5 years, which was quickly followed by the development of pubic hair and penile enlargement. He stopped growing at the age of 14 years when his height was below the 1st percentile. He underwent bilateral mastectomy at the age of 16 years. He had a son (Patient 2) when he was 30 years old. Physical examination revealed a high-pitched voice, lack of facial hair, mastectomy scars, and unremarkable external genitalia. His 30-year-old wife was healthy.

PATIENT 2

Patient 2 was the seven-year-old son of Patient 1. Gynecomastia and accelerated linear growth first occurred at the age of five years: his height and weight were above the 99th percentile, breast development was Tanner stage 3, and he had normal prepubertal external genitalia. His bone age was 13 years at a chronologic age of 5½ years.

PATIENT 3

Patient 3 was a 17-year-old boy who was unrelated to Patient 1 or 2. Progressive gynecomastia first occurred at the age of seven years and was soon followed by premature puberty. Linear growth stopped at the age of 15 years, when he underwent bilateral mastectomy. His height was below the 1st percentile. He had scarce facial hair and normal penile size and testicular volume. Neither his parents nor his five siblings had a history of estrogen excess.

METHODS

BIOCHEMICAL AND GENETIC STUDIES

Karyotypes of all three patients were 46,XY with no gross rearrangements. Adrenal and testicular tumors had previously been ruled out by computed tomography, ultrasonography, and testicular biopsies. The patients' hormone levels are provided in Table 1.

The rate at which plasma androstenedione is converted to estrone in the entire body (transfer constant) was determined as previously described.[8] The radioactive tracers [6,7-^{3}H]estrone and [4-^{14}C]androstenedione were injected intravenously and were followed by a 72-hour urine collection. The trans-

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Table 1. Effects of Anastrozole, an Aromatase Inhibitor, and Dexamethasone on Hormone Levels in a Father, His Son, and an Unrelated Boy with Estrogen Excess.*

Patient No.	Age	Estrone	Estradiol	Androstenedione	Testosterone	LH	FSH
	yr	*pmol/liter*		*nmol/liter*		*IU/liter*	
1, Father	36						
At base line		3341	844	2.76	11.08	1.7	1.5
During treatment†		103	253	ND	37.39	2.9	5.6
Normal range‡		37–183	29–128	2.62–7.16	15.64–46.01	1.5–9.0	2.0–9.2
2, Son	7						
At base line		441	55.8	1.21	<0.13	0.06	0.26
During treatment§		<18	<18	<0.2	<0.13	ND	ND
Normal range‡		<18–62	<18–33	0.28–1.75	<0.13–0.45	0.02–0.3	0.26–3.0
3, Unrelated boy	17						
At base line		2056	1439	3.67	8.09	4.3	2.7
During treatment¶		330	217	ND	18.54	8.9	5.6
Normal range‡		74–165	37–132	2.27–7.33	15.64–43.33	0.4–7.0	2.6–11.0

* To convert values for estrone to picograms per milliliter, divide by 3.699. To convert values for estradiol to picograms per milliliter, divide by 3.671. To convert values for androstenedione to micrograms per liter, divide by 3.492. To convert values for testosterone to nanograms per milliliter, divide by 3.467. LH denotes luteinizing hormone, FSH follicle-stimulating hormone, and ND not done.

† The patient received 4 mg of anastrozole per day orally.

‡ The age-adjusted normal range is shown.

§ The patient received 0.5 mg of dexamethasone per day orally.

¶ The patient received 2 mg of anastrozole per day orally.

fer constant was computed from the ^{3}H:^{14}C ratio of isolated, purified urinary estrone and that of the injected tracers.[8]

Biopsy specimens of buttock and forearm skin (fibroblast cultures), subcutaneous fat from the buttocks, thighs, and abdomen, and lymphocytes were obtained from the three patients, from five unrelated normal male subjects (ages, 17, 26, 32, 37, and 63 years), from the unaffected mother of Patient 2, and from the unaffected brother (age, 31 years) of Patient 3. Fat and skin biopsies and measurement of aromatase activity of skin fibroblasts were performed as described previously.[12-14] Aromatase mRNA levels were measured with use of a quantitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) method and an internal standard.[15]

Fat and skin RNA samples were subjected to rapid amplification of 5′–complementary DNA (cDNA) ends to identify novel 5′-untranslated regions of aromatase mRNA in the patients,[16-18] and 11 to 20 clones were sequenced per sample. We then used rapid amplification of 3′-cDNA ends to identify the full complement of mRNAs with these novel 5′-untranslated regions. We used the data from the Human Genome Project and screened a bacterial-artificial-chromosome plasmid library to map *FLJ14957* (an uncharacterized gene cloned from normal fetal brain), the tropomodulin 3 (*TMOD3*) gene, and the aromatase gene to the human genome.[2,19] We used transformed lymphocytes for fluorescence in situ hybridization with labeled bacterial-artificial-chromosome clones.[20,21] Genomic Southern hybridization was performed with use of a full-length aromatase cDNA. The known promoter sequences and coding region of the gene were sequenced directly.

AstraZeneca provided anastrozole, an oral aromatase inhibitor.[22] Anastrozole was used to block the activity of the aromatase enzyme and thus stop estrogen production. Protocols and consent forms were approved by the institutional review board of the University of Texas Southwestern Medical Center at Dallas. Written informed consent was obtained for all biopsies. Patient 1 and Patient 3 gave consent for radiolabeled steroid injections and anastrozole treatment.

RESULTS

CONVERSION OF PLASMA ANDROSTENEDIONE TO ESTRONE

In Patients 1 and 3, the rates at which plasma androstenedione was converted to estrone were 58.8

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percent and 54.9 percent, respectively, as compared with 1.7 percent and 1.3 percent in age-matched controls. In Patients 1 and 3, treatment with the aromatase inhibitor anastrozole reduced serum estradiol and estrone levels to nearly normal (Table 1). The initial dose of anastrozole was 1 mg per day; the dose was increased monthly until estradiol was suppressed, and the dose was then adjusted in order to maintain testosterone levels above 15.64 nmol per liter (4.5 ng per milliliter). Suppression of estradiol restored luteinizing hormone, follicle-stimulating hormone, and testosterone levels. In Patient 2, suppression of androstenedione with the use of oral dexamethasone (0.5 mg per day, used as part of routine care) was sufficient to suppress estrogen levels, since androstenedione was the only circulating substrate in the absence of detectable testosterone.

AROMATASE ACTIVITY AND mRNA LEVELS IN SKIN FIBROBLASTS AND ADIPOSE TISSUE

As compared with the values in the control subjects, aromatase activity was increased by a factor of 11 to 24 in cultured fibroblasts from buttock and forearm skin from the three patients (Fig. 1A and 1B). We also measured aromatase mRNA levels in subcutaneous fat samples from the buttocks and thighs to determine whether overproduction of the enzyme was generalized. In all three patients, levels of aromatase mRNA in the buttocks and thighs were 14 to 24 times as high as those in the control subjects (Fig. 1C).

ANALYSIS OF AROMATASE, *FLJ*, AND *TMOD3* mRNA

The 5′-untranslated regions of aromatase mRNA can be used to identify the promoter that induced transcription. We used rapid amplification of 5′-cDNA ends to identify the 5′-untranslated regions of aromatase mRNA in fat and skin samples from the three patients to characterize the promoters responsible for the overexpression of aromatase. Sequencing of the resultant clones revealed two novel 5′-untranslated regions (Fig. 2A and 2B). In Patient 1 and his son (Patient 2), a single novel 5′-untranslated region made up 86 to 100 percent of aromatase mRNA in fat tissue from the buttocks and thighs and in skin fibroblasts (Fig. 2A). This 45-bp sequence — I.FLJ — was not detected in aromatase mRNA from skin or fat samples from the unaffected mother of Patient 2 or from four unrelated male controls (ages, 17, 26, 32, and 37 years) either by rapid amplification of 5′-cDNA ends (Fig. 2A) or by exon-specific RT-PCR (Fig. 2B). Another novel 5′-untranslated region of 170 bp (I.TMOD3) was discovered in 80 to 82 percent of aromatase mRNA in samples of buttock and thigh fat and skin from Patient 3 but not in tissue samples from his unaffected brother or four unrelated male controls (Fig. 2A and 2B). All products of rapid amplification of 5′-cDNA ends and RT-PCR were confirmed by sequencing (Fig. 2A and 2B).

We performed rapid amplification of 3′-cDNA ends using fat and skin RNA samples to determine whether these two abnormal 5′-untranslated regions of aromatase mRNA were present in other mRNA species under physiologic circumstances. In all three patients and all control subjects, the 45-bp sequence cloned from tissues of Patients 1 and 2 was identical to the 5′-untranslated region of an mRNA encoded by the *FLJ14957* gene (GenBank accession number AK027863), which has not to our knowledge been characterized previously.[23] The product of the *FLJ14957* gene has been found to be weakly similar to the myosin heavy-chain smooth-muscle isoform. The 170-bp sequence isolated from Patient 3 was identical to the 5′-untranslated region of another mRNA normally encoded by the *TMOD3* gene (Fig. 2B).[24] *TMOD3* is the only ubiquitously expressed member of the tropomodulin gene family that encodes actin-capping proteins.[24] The function of the protein that is encoded by the *TMOD3* gene is unknown.

ANALYSIS OF THE AROMATASE, *FLJ*, AND *TMOD3* GENES

We isolated the genomic clones that contained the *FLJ14957* and *TMOD3* genes. We mapped these genes in chromosome 15q21.2–3 from telomere to centromere in the following order: *FLJ14957*, *TMOD3*, and aromatase (Fig. 3 and 4). The genome data base indicated that *FLJ14957* and *TMOD3* are transcribed from the same DNA strand toward the telomere, whereas the aromatase gene is transcribed from the opposite strand toward the centromere. In all three genes, activation of a distal promoter separated by an intron causes splicing of the first exon (the 5′-untranslated region) to the coding region (Fig. 3 and 4).

The regulatory regions upstream of both *FLJ14957* and *TMOD3* promoters were similar. Each had typical characteristics of a TATAless promoter, which included a transcription initiator–cap sequence and multiple DNA motifs that bind the tran-

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Figure 1. Mean (±SD) Base-Line Aromatase Activity after a Two-Hour Incubation in Monolayers of Cultured Skin Fibroblasts from Patient 1 and His Son (Patient 2) (Panel A), Patient 3 (Panel B), and Related and Unrelated Controls and Aromatase Messenger RNA (mRNA) Levels in Adipose Tissue from Buttock and Thigh Specimens from Patient 3 and a 17-Year-Old Control (Panel C).

Panel A shows the aromatase activity in buttock-skin fibroblasts from Patients 1 and 2 and two controls — the 30-year-old mother of Patient 2 and an unrelated 37-year-old man. Values were expressed as picomoles of converted substrate per milligram of protein during a two-hour incubation. In a separate experiment (Panel B), aromatase activity was compared in forearm-skin fibroblasts from the 17-year-old boy (Patient 3), a 63-year-old unrelated male control, and a 17-year-old unrelated male control. In Panel C, aromatase mRNA levels in adipose tissue from the buttocks and thighs are 14 to 21 times as high in Patient 3 as in a control boy of the same age. A 272-bp segment flanking three coding exons of aromatase mRNA was amplified. As an internal standard, a homologous rat aromatase complementary RNA (cRNA) sequence was coamplified in each sample. This enabled us to control and correct for the differences in the efficiency of primer extension and amplification between samples. Amplified products for each sample were divided in half and transferred by slot–blot to separate membranes. Each membrane was hybridized with species-specific oligonucleotide probes labeled with phosphorus-32. The radioactivity of the bands was quantified with use of a computerized image analyzer. The aromatase mRNA level of each sample was expressed as a ratio of the human product to the rat product. A standard curve for this reaction has been constructed, which demonstrated that, as total RNA quantities in human fat were serially doubled, the ratios increased in a linear fashion (data not shown).[12,15]

scription factors, promoter-selective transcription factor-1, and activator protein-2. Messenger RNAs for both genes were present in multiple tissues, indicating their ubiquity as opposed to the more tightly regulated and tissue-selective expression of the aromatase gene in control subjects (data not shown).

ANALYSIS OF CHROMOSOME 15 FOR GAIN-OF-FUNCTION MUTATIONS

Samples of genomic DNA from all three patients were digested with the restriction enzymes *Hi*, *Pv*, and *Xb* and subjected to Southern hybridization with use of a full-length aromatase cDNA probe. This ruled out any multiplication or gross defects in the

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coding region of the gene (data not shown). Furthermore, in all three patients direct sequencing of the entire coding region and previously described promoter regions failed to reveal any mutations. Thus, we sought to demonstrate other rearrangements that may cause the formation of cryptic promoters for the aromatase gene. We analyzed the specific order of the three genes and the direction of transcription. The *FLJ* and *TMOD3* genes are both located telomeric to the aromatase gene and are transcribed in the direction that is opposite that of the aromatase gene (Fig. 3 and 4).

PATIENTS 1 AND 2

To explain the genetic alteration responsible for abnormal aromatase mRNA species containing I.FLJ as the 5′-untranslated region in Patients 1 and 2, we hypothesized that an inversion involving a segment of approximately 6.1 Mb caused the *FLJ* promoter to lie adjacent to the aromatase coding region (Fig. 3). We used fluorescence in situ hybridization with two pairs of fluorescent-labeled bacterial-artificial-chromosome clones as probes. The first pair of probes was designed to be centromeric to the estimated break points (Fig. 3). The mutation was predicted to cause the fusion of the red and green signals in a single chromosome 15. Fluorescence in situ hybridization showed that this rearrangement was in the affected father and his son but not in the child's mother (Fig. 3) — a finding consistent with an autosomal dominant mode of transmission. Next, we designed probes immediately telomeric to the predicted break points and again predicted that the two signals were fused in one chromosome of the affected father and son. This second set of probes also confirmed the presence of the heterozygous rearrangement involving the inversion of this segment of approximately 6.1 Mb (data not shown). Each abnormal result was observed in 100 lymphocytes in interphase.

PATIENT 3

Since the *TMOD3* promoter also lies telomeric to the aromatase gene and the two genes are transcribed from opposite strands of DNA, we hypothesized that an inversion of a segment flanking the *TMOD3* promoter caused this cryptic promoter to lie adjacent to the aromatase coding region (Fig. 4). Using a red probe complementary to the aromatase coding region and a green probe complementary to the *TMOD3* promoter region, we demonstrated in 100 lymphocytes that a portion of the green signal was split and merged with the red signal in only one chromosome, whereas the signals in the sister chromosome were not altered (Fig. 4). This heterozygous small inversion was consistent with formation of another cryptic aromatase promoter. Control lymphocytes from the unaffected brother of Patient 3 had no rearrangements (Fig. 4).

Figure 2 (facing page). Genomic Location and Tissue Distribution (Panel A) and Sequences (Panel B) of Normal and Abnormal Promoter-Specific Aromatase Messenger RNA (mRNA) Species.

In Panel A, tissue-specific promoters direct the expression of aromatase by causing splicing of untranslated first exons or 5'-untranslated regions (UTRs) onto a common splice junction upstream of the aromatase coding region. Each 5'-untranslated region in aromatase mRNA may be viewed as a signature of the promoter used in a particular tissue. Primarily promoter I.4 is used in fat and skin of control subjects. Using rapid amplification of 5'-cDNA ends, we identified two abnormal 5'-untranslated regions in the aromatase mRNA of the three patients with estrogen excess. Patient 1 and his son (Patient 2) had the same 5'-untranslated region (I.FLJ), which is normally found in the mRNA encoded by the *FLJ14957* gene. Patient 3 had a different 5'-untranslated region (I.TMOD3), one that is normally found in the mRNA encoded by the tropomodulin 3 gene. Both genes were mapped to the chromosome 15q21.2–3 region. Aromatase mRNA in fat and skin samples from four control subjects contained primarily the first exon I.4, as expected.[16,18] Panel B shows normal and chimeric (abnormal) sequences of the aromatase mRNA species from the patients. The common splice junction upstream of the ATG translation start site and the encoded protein are identical in both instances. Sense and antisense oligonucleotides used for reverse-transcriptase–polymerase-chain-reaction amplification of promoter-specific mRNA species are indicated. We could amplify the I.FLJ-specific aromatase mRNA (upper blot) only from samples of RNA from buttock (Bu) fat from Patient 1 (lane 3) and Patient 2 (lane 2), not from a buttock-fat sample from the mother of Patient 2 (lane 4) or a male control (lane 5), a sample of abdominal fat (Ab) from another male control (lane 6), a sample of breast-cancer (BC) tissue from a 49-year-old woman (lane 7), or a control sample (lane 8) with no reverse transcriptase (nRT). Likewise, we could amplify the I.TMOD3-specific aromatase mRNA (lower blot) only from skin fibroblasts (SF) and a buttock-fat sample from Patient 3 (lanes 5 and 4, respectively), not from cells or tissues of his unaffected brother (lanes 3 and 2, respectively) or from a control sample (lane 6). Amplification products of the normally used promoter I.4–specific aromatase mRNA species from these samples were used as controls. The transcription start site in the normal I.FLJ–*FLJ* coding region mRNA is 18 bp farther upstream than the cryptic start site for aromatase found in Patients 1 and 2.

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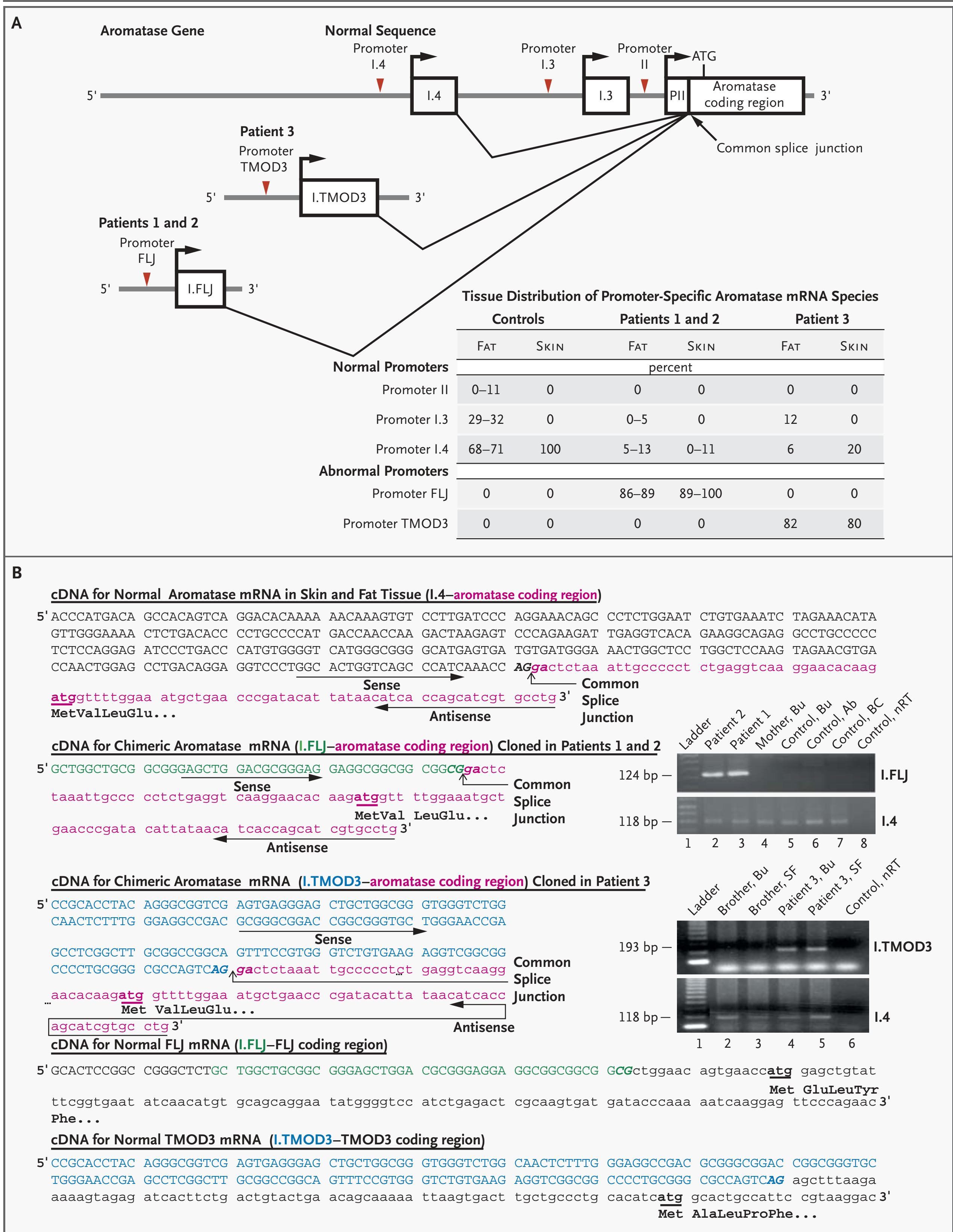

Tissue Distribution of Promoter-Specific Aromatase mRNA Species

	Controls		Patients 1 and 2		Patient 3	
	Fat	Skin	Fat	Skin	Fat	Skin
	percent					
Normal Promoters						
Promoter II	0–11	0	0	0	0	0
Promoter I.3	29–32	0	0–5	0	12	0
Promoter I.4	68–71	100	5–13	0–11	6	20
Abnormal Promoters						
Promoter FLJ	0	0	86–89	89–100	0	0
Promoter TMOD3	0	0	0	0	82	80

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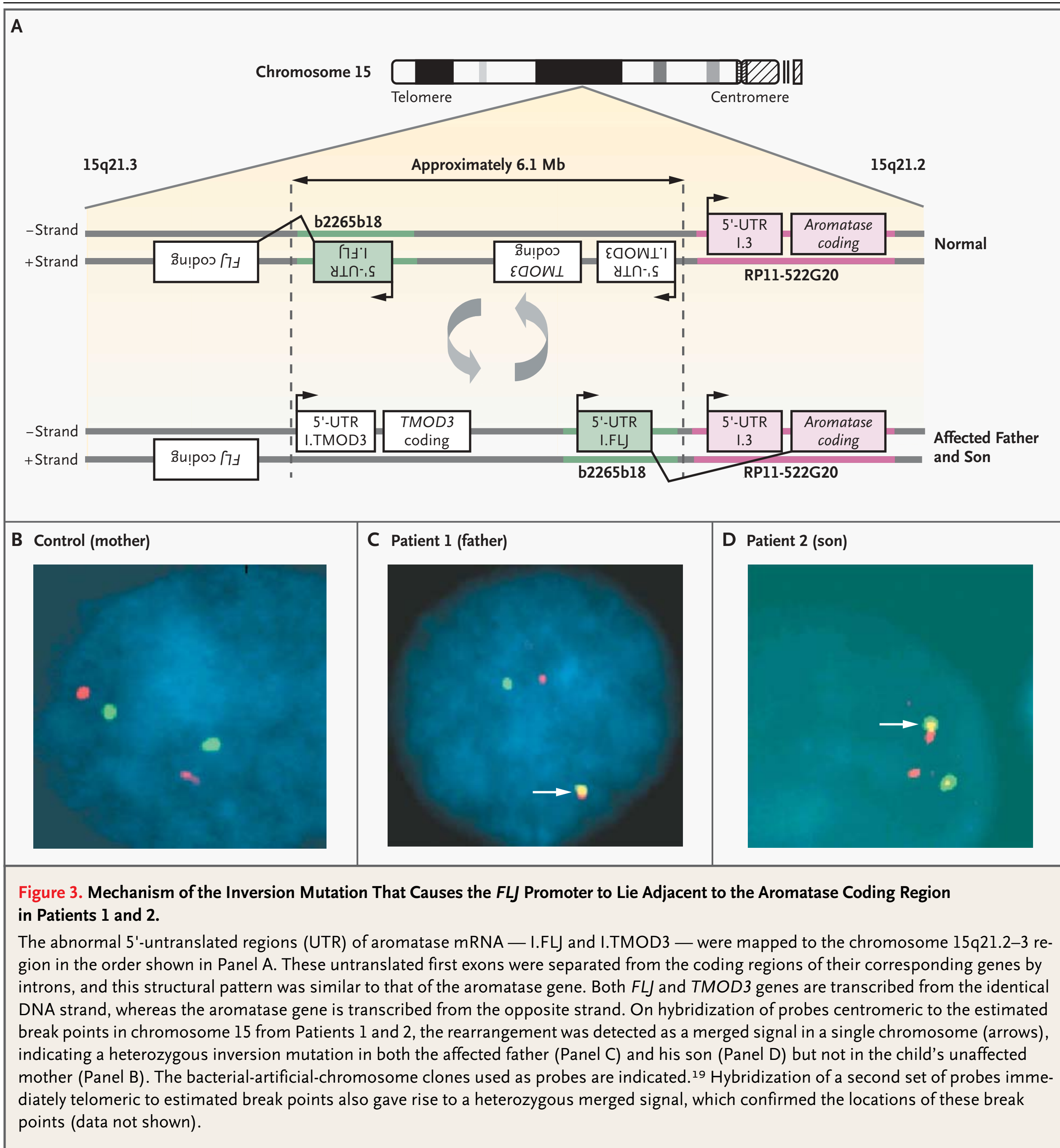

Figure 3. Mechanism of the Inversion Mutation That Causes the *FLJ* Promoter to Lie Adjacent to the Aromatase Coding Region in Patients 1 and 2.

The abnormal 5'-untranslated regions (UTR) of aromatase mRNA — I.FLJ and I.TMOD3 — were mapped to the chromosome 15q21.2–3 region in the order shown in Panel A. These untranslated first exons were separated from the coding regions of their corresponding genes by introns, and this structural pattern was similar to that of the aromatase gene. Both *FLJ* and *TMOD3* genes are transcribed from the identical DNA strand, whereas the aromatase gene is transcribed from the opposite strand. On hybridization of probes centromeric to the estimated break points in chromosome 15 from Patients 1 and 2, the rearrangement was detected as a merged signal in a single chromosome (arrows), indicating a heterozygous inversion mutation in both the affected father (Panel C) and his son (Panel D) but not in the child's unaffected mother (Panel B). The bacterial-artificial-chromosome clones used as probes are indicated.[19] Hybridization of a second set of probes immediately telomeric to estimated break points also gave rise to a heterozygous merged signal, which confirmed the locations of these break points (data not shown).

DISCUSSION

Patients 1 and 3 had hypogonadotropic hypogonadism. Both had scarce facial hair but normal penile and testicular size, indicating that hypogonadism was not severe. During treatment with an aromatase inhibitor, their estrogen levels declined and testosterone, luteinizing hormone, and follicle-stimulating hormone levels rose to normal. This response suggests a crucial role of estrogen in the suppression of both gonadotropins in men. Despite low testosterone levels in these patients, luteinizing hormone remained suppressed, possibly owing to the high levels of circulating estrogen. These mutations

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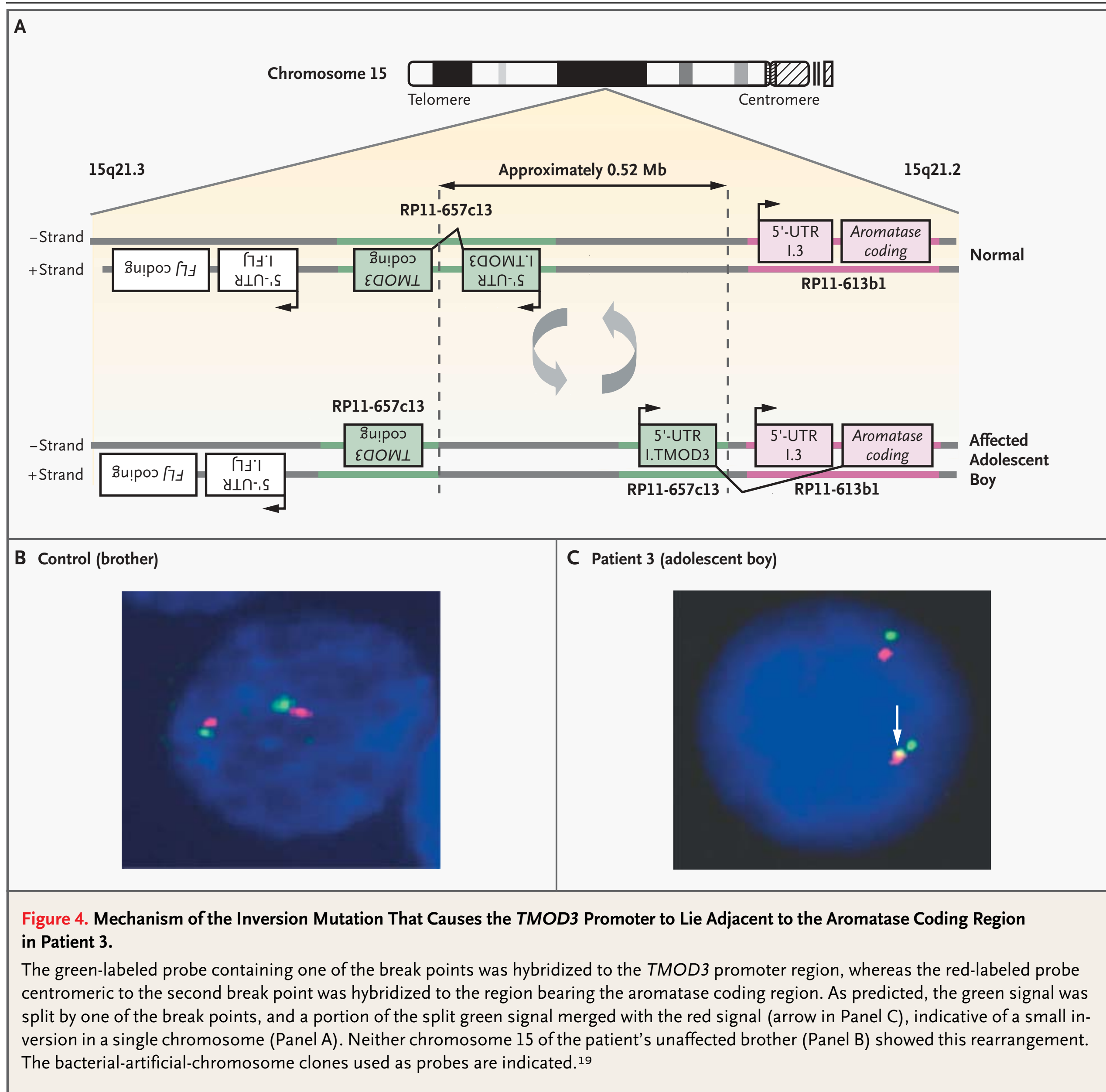

Figure 4. Mechanism of the Inversion Mutation That Causes the *TMOD3* Promoter to Lie Adjacent to the Aromatase Coding Region in Patient 3.

The green-labeled probe containing one of the break points was hybridized to the *TMOD3* promoter region, whereas the red-labeled probe centromeric to the second break point was hybridized to the region bearing the aromatase coding region. As predicted, the green signal was split by one of the break points, and a portion of the split green signal merged with the red signal (arrow in Panel C), indicative of a small inversion in a single chromosome (Panel A). Neither chromosome 15 of the patient's unaffected brother (Panel B) showed this rearrangement. The bacterial-artificial-chromosome clones used as probes are indicated.[19]

may have given rise to the overexpression of aromatase in the brain and thus to increased local estrogen production, which might also have contributed to the suppression of gonadotropins.

The potential objective of long-term treatment with an aromatase inhibitor is to restore gonadal function, but this approach is not clearly justified, for several reasons. First, men with this condition are not thought to be at risk for osteoporosis. Second, although men with estrogen excess may be subfertile, infertility is not a uniform feature of the syndrome, as indicated by the fact that Patient 1 fathered a son with the same genetic trait. Third, the consequences of long-term exposure to high levels of estrogen in men are not known. We suggest that these men should periodically be evaluated for breast and prostate disease, given the potentially deleterious effects of estrogen on these tissues.

We found that overproduction of estrogen arose from novel gain-of-function mutations in chromo-

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some 15, giving rise to the formation of cryptic promoters that regulate the aromatase gene. These constitutively active promoters normally serve to transcribe two ubiquitously expressed genes — *FLJ* and *TMOD3* — that encode products homologous to muscle proteins in many human tissues. The functions of *FLJ* and *TMOD3* are not known.[23,24] In Patients 1 and 2, the same mutation was transmitted in an autosomal dominant manner. The cryptic promoter in Patient 3 differed from that in the first two patients. The relatives of Patient 3 were not affected. Therefore, this appeared to be a new mutation.

It is not clear whether transcription from the normal chromosome compensated for heterozygous disruption of the *FLJ* or *TMOD3* gene in the affected chromosome. Since the functions of these genes are unknown, a mild phenotype may not have been readily detected. It is also likely that these rearrangements were not extensive or genome-wide, since there were no apparent phenotypic abnormalities other than the overexpression of aromatase.

In these patients, 80 to 100 percent of aromatase mRNA arose from a gain-of-function mutation in a single allele, whereas the normal promoters I.4 and I.3 in skin and fat contributed a much smaller portion of total aromatase mRNA. This is explicable by the much higher level of activity of the cryptic promoter, as compared with the normal promoters.

Our findings are similar to those described by Wilson and McPhaul and their colleagues in henny-feathered Sebright roosters.[25-27] In birds, aromatase is normally expressed in the ovaries and brain but not in skin fibroblasts.[1,27] In Sebright chickens with the (autosomal dominant) henny-feathered trait, aromatase is overexpressed in skin fibroblasts, leading to a female pattern of feather development in roosters.[25,26] Furthermore, the 5′-untranslated region of aromatase mRNA in skin fibroblasts suggested that a unique promoter regulated the expression of aromatase.[26]

The upstream region of the aromatase gene may represent a "hot spot" for mutations. Occasional mutations, such as those we describe, cause extremely high aromatase activity and striking clinical consequences. More common rearrangements may go clinically unrecognized and cause subtle degrees of estrogen excess, which may increase the risks of estrogen-dependent disease, such as breast and endometrial cancer and endometriosis.[28,29]

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