well as combinations of these mutations. There are at least two other families in which the use of a cryptic promoter was demonstrated, but no corresponding genomic mutation was defined [5,6]. Furthermore, we came across additional cases/families in which AEXS was clinically suspected, but no causative mutation was identified. Current cutting-edge technologies, especially second-generation (or 'next-generation') DNA sequencing technologies employing paired-end mapping or split-read analysis, could reveal hitherto unrecognized recombination events as they have sufficient power to detect even inversions, which are not detectable by currently available arrays, comparative genome hybridization, single-nucleotide polymorphism genotyping assays or read-depth analysis using a next-generation sequence analyzer [46]. Next-generation technologies may prove useful to identify novel gene mutations, other than those to *CYP19A1*, as causative mutations of AEXS, if any others exist.

Recent progress in high-throughput DNA technology has also shown that genomic rearrangement causing submicroscopic (<5 Mb) copy number variations (CNVs) is far more common in the human genome than previously suspected and can cause hereditary diseases because of a Mendelian or more complex trait as seen in neurogenic disorders and autism. Even if CNVs are identified, it is often difficult to determine a precise genetic mechanism conveying each phenotype because disease phenotypes are complex and the CNV region may harbor multiple genes that function in the progression of disease.

In this context, AEXS provides a unique model to study how these structural variations confer new functions to the human genome. The phenotype highly specific to *CYP19A1* function (estrogen excess) is relatively simple; thus the phenotype–genotype correlation is direct and easy to analyze. This is probably because of the coincidence that estrogen, as a gene product, acts powerfully and specifically, and there exists no neighboring genes that show haplo-insufficiency. Moreover, the alternative promoter structure of *CYP19A1* features novel genetic mechanisms of gain-of-function, namely deletion- and inversion-based adoption of cryptic promoters.

A future 5-year study should be designed to address questions raised by the AEXS study. For example, the inversion-based mechanism of gain-of-function has never been reported except for AEXS; therefore, it would be interesting to determine whether this mechanism is actually exclusive to AEXS, or whether this mechanism is more commonly used for other diseases not previously identified because of technical limitations. Previous studies of mutations in AEXS have revealed that the 15q21 region, especially of upstream of *CYP19A1*, is unstable, suggesting that the number and types of mutations may be more frequent than previously thought. There is considerable diversity in the severity of pubertal gynecomastia, as some cases are phenotypically indistinguishable from mild AEXS. Therefore, it is important to determine whether there exist structural variations or polymorphisms relevant to pubertal gynecomastia. Given that *CYP19A1*recombinations occur as a replication error, somatic cells may also be affected and cause pathologies relevant to excessive estrogen, such as breast cancer and polycystic ovary syndrome; however, such a mutation has not been identified till date [9]. Mammalian *CYP19A1* has evolved through the sequential acquisition of promoters. Thus, it would also be beneficial to determine whether any recombination events found in AEXS are relevant to such evolutionary potential and the history of such mutations in particular cases of familial AEXS [47].

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Key issues

- Aromatase excess syndrome, formerly known as familial gynecomastia, is an autosomal dominant disorder caused by gain-of-function mutations in the aromatase gene (*CYP19A1*).
- Gynecomastia develops during the peri- or pubertal period and continues for life.
- The long bones exhibit accelerated growth at early puberty and then premature epiphyseal closure results in short adult stature.
- Patients appear healthy, except for manifestations of gynecomastia, which may be associated with minor symptoms exclusively related to estrogen excess.
- Serum estradiol levels are elevated in 80% of patients, but are normal in 20%; therefore, a normal serum estradiol level does not exclude a diagnosis of aromatase excess syndrome.
- Use of aromatase inhibitors ameliorates gynecomastia.
- *CYP19A1* mutations serve as fascinating examples to understand how submicroscopic DNA recombination events give rise to gain-of-function mutations.

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RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics PART A

Prenatal Genetic Testing for a Microdeletion at Chromosome 14q32.2 Imprinted Region Leading to UPD(14)pat-like Phenotype

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TO THE EDITOR:

Human chromosome 14q32.2 imprinted region carries several paternally expressed genes (*PEGs*) such as *DLK1* and *RTL1* and maternally expressed genes (*MEGs*) such as *MEG3* (alias, *GTL2*) and *RTL1as* (*RTL1 antisense*), together with the germline-derived primary *DLK1-MEG3* intergenic differentially methylated region (IG-DMR) and the postfertilization-derived secondary *MEG3*-DMR (Fig. 1) [da Rocha et al., 2008; Kagami et al., 2008a]. Consistent with this, paternal uniparental disomy 14 (UPD(14) results in a unique phenotype characterized by facial abnormality, small bell-shaped thorax, abdominal wall defects, placentomegaly, and polyhydramnios [Kagami et al., 2005, 2008a,b]. In this regard, we have recently reported that heterozygous microdeletions and epimutations (hypermethylations) affecting unmethylated DMR (s) of maternal origin also lead to UPD(14)pat-like phenotype [Kagami et al., 2008a, 2010, 2012]. Indeed, after studying 26 patients with UPD(14)pat-like phenotype, we identified UPD (14)pat in 17 patients (65.4%), microdeletions in 5 patients (19.2%), and epimutations in 4 patients (15.4%) [Kagami et al., 2012]. Importantly, although there is no report describing recurrence of UPD(14)pat and epimutation in familial members with a normal karyotype, microdeletions can be transmitted recurrently from mothers with the same heterozygous microdeletions to offsprings [Kagami et al., 2008a]. Here, we report on our experience of a prenatal genetic testing for a microdeletion at the chromosome 14q32.2 imprinted region.

A 33-year-old Japanese woman came to us with her husband seeking for prenatal diagnosis of a fetus at 9 weeks of gestation. The first child and the mother have been reported previously as cases 3 and 11 of Family B in Kagami et al. [2008a]. In brief, the child had upd(14)pat-like phenotype and a maternally derived 411,354 bp microdeletion involving *WDR25*, *BEGAIN*, *DLK1*, *MEG3*, *RTL1/RTL1as*, and *MEG8* (Fig. 1). The mother had UPD(14)mat-like phenotype and the same microdeletion on the paternally derived chromosome 14. The parents hoped to deliver the fetus at a local hospital if there is no microdeletion or at our hospital with a neonatal intensive care unit if a microdeletion is identified.

After thorough consultation, we performed trans-abdominal chorionic villus sampling (CVS) at 12 weeks of gestation. Immediately after the sampling, fluorescence in situ hybridization was carried out with an RP11-566J3 probe detecting a segment within

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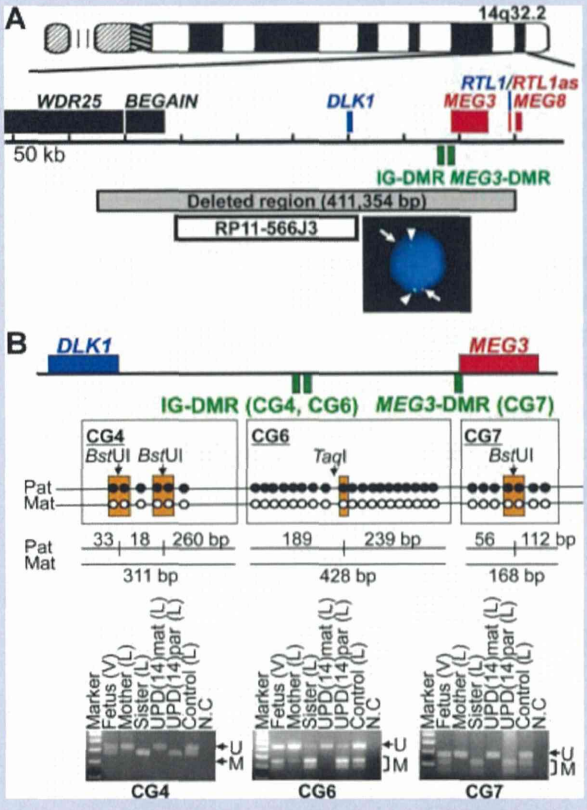

FIG. 1. Summary of the molecular studies. A: The physical map of the 14q32.2 imprinted region and the FISH finding of the fetus. *PEGs* are shown in blue, *MEGs* in red, and the IG-DMR and the *MEG3*-DMR in green. The gray rectangle indicates the 411,354 bp microdeletion identified in the first child and the mother, and the white rectangle denotes the region detected by the RP11-566J3 BAC probe. The FISH analysis reveals two red signals (arrows) identified by the RP11-566J3 BAC probe and two green signals (arrowheads) detected by the RP11-566I2 BAC probe for 14q12 utilized as an internal control. B: The methylation analysis for the IG-DMR (CG4 and CG6) and the *MEG3*-DMR (CG7) by COBRA. The black and white circles indicate methylated and unmethylated cytosines at the CpG dinucleotides, respectively. Pat: paternally derived chromosome; and Mat: maternally derived chromosome. PCR products for CG4 (311 bp) are digested with *Bst*UI into three fragments (33, 18, and 260 bp) when cytosines at the first and the second CpG dinucleotides and the fourth and fifth CpG dinucleotides (indicated with orange rectangles) are methylated. The PCR products for CG6 (428 bp) are digested with *Taq*I into two fragments (189 and 239 bp) when the cytosine at the 9th CpG dinucleotide (indicated with an orange rectangle) is methylated. The PCR products of CG7 (168 bp) are digested with *Bst*UI into two fragments (56 and 112 bp) when the cytosines at the fourth and fifth CpG dinucleotides (indicated with orange rectangle) are methylated. Both methylated (M)- and unmethylated (U)-specific bands are identified in the chorionic villus sample. V, villi; L, leukocytes; and N.C, negative control.

the deleted region of the first child and the mother, delineating two signals on villus cell interphase spreads (Fig. 1). Next combined bisulfite restriction analysis (COBRA) was performed for the IG-DMR and the *MEG3*-DMR using villus cell genomic DNA, identifying both methylated- and unmethylated allele-specific bands (Fig. 1B). These findings clearly excluded the presence of a microdeletion in the fetus by 14 weeks of gestation. Subsequent pregnant course was uneventful, and a phenotypically normal infant was delivered at term by a caesarean section.

To our knowledge, this is the first report describing a prenatal genetic testing for a familial microdeletion affecting the chromosome 14q32.2 imprinted region. Although such a genetic testing is possible only when an accurate genetic diagnosis has been made for the proband, it permitted the precise diagnosis before the second to the third trimester when characteristic UPD(14)pat-like features such as bell-shaped small thorax with coat hanger appearance and polyhydroamnios become detectable by ultrasonographic studies [Suzumori et al., 2010; Yamanaka et al., 2010]. Such an early prenatal diagnosis, though it is associated with a certain risk such as CVS-induced abortion, provides critical information for the clinical management. When a microdeletion is excluded as shown in this case, this releases the parents from the anxiety of having an affected fetus and allows for a standard follow-up during pregnancy. By contrast, when a microdeletion is identified, this will allow for appropriate management during pregnancy (e.g., amnioreduction to mitigate the risk of threatened premature delivery) and pertinent therapeutic interventions for the infant (e.g., respiratory management). Thus, prenatal genetic diagnosis appears to be beneficial for the fetus and the parents, when it is performed at appropriate institutes where a multidisciplinary team including a genetic counselor is available.

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LETTER TO THE EDITOR

Aromatase excess syndrome in a family with upstream deletion of *CYP19A1*

Aromatase excess syndrome (AEXS) is an autosomal dominant disorder caused by overexpression of *CYP19A1* at 15q21.[1] Salient clinical features of AEXS are gynaecomastia and advanced bone age resulting from oestrogen excess.[1–3] To date, six genomic rearrangements at 15q21 have been identified in 23 patients from nine families.[1–3] These rearrangements include duplications involving the promoter region of *CYP19A1*, and deletions and inversions that create chimeric genes consisting of coding exons of *CYP19A1* and promoter-associated exons of neighbouring genes. Given the small number of reported patients, further studies are necessary to clarify molecular basis and phenotypes of AEXS.

Here, we identified a Japanese family with AEXS and hitherto unreported deletion at 15q21·2. This study was approved by the Institutional Review Board Committees at the National Center for Child Health and Development. The proband was a 12-year-old boy ascertained by gynaecomastia. Physical examination revealed age-appropriate sexual development (Fig. 1 and Table S1). His brother and father also exhibited gynaecomastia. The 14-year-old sister experienced early menarche. The mother was clinically normal. Bone age was significantly advanced in the proband and brother. Assessment of growth records revealed that the proband and brother had a peak growth velocity at 6–7 years of age, and the sister showed a high growth velocity at 4–6 years of age (Fig. 1). Blood oestradiol (E_2) values were slightly elevated in the father and remained undetectable in the proband and brother (Table S1).

Array-based comparative genomic hybridization analyses (Agilent Technologies, Palo Alto, CA, USA) identified heterozygous deletions in the proband, brother, sister and father (Fig. 1). The deletion included several exons of the neighbouring genes *DMXL2* and *GLDN*. Long-range PCR of the breakpoint-flanking regions using multiple primers (Table S2) showed that the deletion was 198 662 bp in size and started at a point 154 688 bp upstream of *CYP19A1*. The telomeric and centromeric breakpoints resided in a Line 1 sequence within *DMXL2* intron 1 and in a nonrepeat sequence at the exon–intron boundary of *GLDN* exon 6, respectively (Fig. 1 and S1). The fusion junction was accompanied by 3 bp microhomology. RT-PCR detected a chimeric mRNA clone consisting of *DMXL2* exon 1 and *CYP19A1* exon 2 in the mammary gland and skin of the proband (Fig. 1).

DMXL2 is a widely expressed gene, and exon 1 of *DMXL2* contains promoter-compatible histone marks.[3] Thus, phenotype

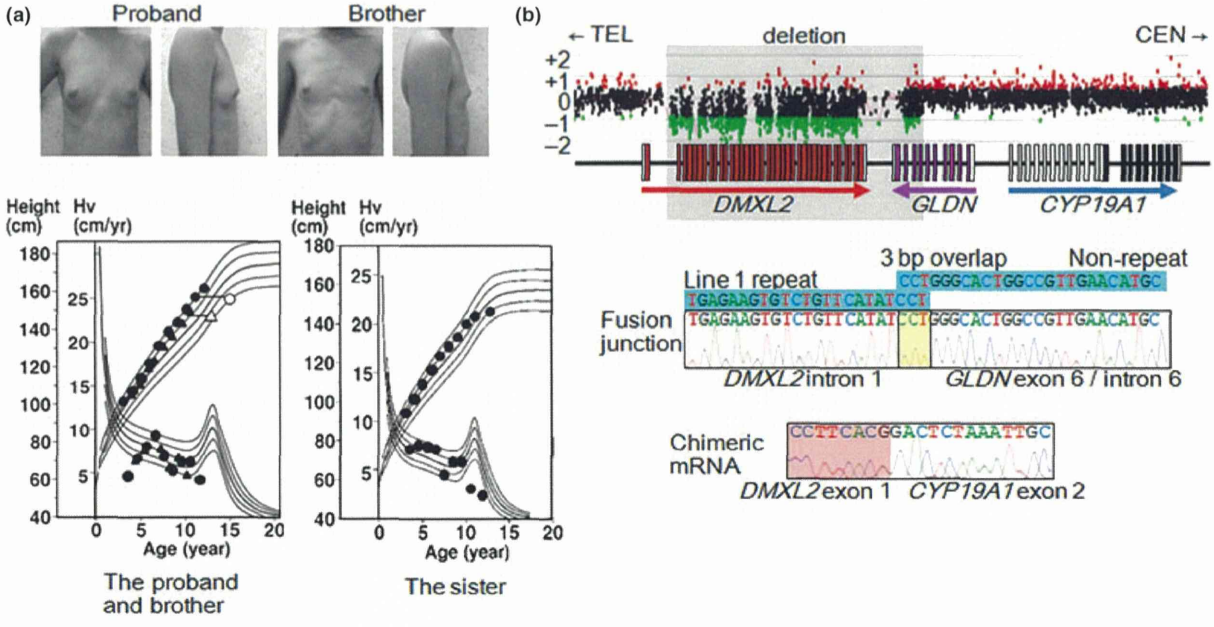

Fig. 1 (a) Clinical features of the family. Upper part: Gynaecomastia in the proband and brother. Lower part: Growth chart. Actual height and growth velocity of the proband (black circles), brother (black triangles) and sister (black circles), together with bone age (a while circle and triangle) are plotted on the growth curves of Japanese children (the mean, ±1·0 SD, and ± 2·0 SD). (b) Molecular findings of the family. Upper part: Comparative genomic hybridization analyses. The arrows indicate positions and transcriptional direction of genes. The coloured and white boxes denote the coding and noncoding exons, respectively. Middle part: DNA sequences at the fusion junction. Lower part: A chimeric mRNA clone consisting of *DMXL2* exon 1 and *CYP19A1* exon 2.

of the present family can be ascribed to *CYP19A1* overexpression due to cryptic use of a widely expressed promoter. To date, two other deletions have been identified in four families with AEXS.[3] This suggests that submicroscopic deletions at 15q21 account a certain part of the aetiology of AEXS. Notably, the breakpoints of the present and previously reported deletions were clustered in small genomic intervals (Fig. S1). These intervals are likely to provide a hotspot for recombination- and replication-mediated errors, because the present deletion is consistent with a replication-mediated error that occurs independently of repetitive sequences and is associated with microhomology at the fusion junctions,[4] while the previously reported deletions are ascribed to recombination-mediated mechanisms.[3]

Two points are noteworthy for the clinical features of this family. First, the phenotypes in the present family are comparable to those in other families with deletions.[3] This supports the previously proposed notion that clinical severity of AEXS is primarily determined by the functional properties of the fused promoters.[3] In addition, the clinical features of the father and sister of our family argue for the assumption that oestrogen excess in AEXS permits normal fertility in males and produces early menarche in females.[2,3] Second, apparent gynaecomastia was observed in the prepubertal brother, and markedly accelerated skeletal growth around 6 years of age was seen in the proband, brother and sister. These data imply that adrenal androgens rather than gonadal androgens serve as the major sources of oestrogens in prepubertal children with AEXS, because in Asian children, adrenarche usually occurs at 6–7 years of age.[5] Indeed, aromatase converts androstenedione, one of the major adrenal androgens, into oestrone (E_1). The undetectable levels of E_2 in the proband and the brother despite apparent gynaecomastia can be explained by assuming that circulating E_1 rather than E_2 mediates the development of gynaecomastia in pre- or peripubertal boys. However, because blood E_1 levels were not examined in this family, this assumption remains speculative.

In summary, this study provides further evidence of allelic heterogeneity and genotype–phenotype correlation in AEXS. Furthermore, our results indicate for the first time that adrenal androgens may be the major source of oestrogens in pre- and peripubertal patients with AEXS.

Conflict of Interest

All authors declare that there is no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical and hormonal findings of the family with aromatase excess syndrome.

Table S2. Primers utilized in the present study.

Figure S1. Schematic representation of the genomic region around the deletions. (a) Deletions in the present family and previously reported families (families C–F described by Fukami *et al.*).[3] The arrows indicate the deleted regions. The colored boxes indicate exons of *DMXL2* and *GLDN*. The genomic positions refer to Human Genome Database (hg19, build 37). (b) Breakpoint-flanking regions of the present family and families C–F. The numbers denote the distance from the 15p telomere.

Aromatase Excess Syndrome: A Rare Autosomal Dominant Disorder Leading to Pre- or Peri-pubertal Onset Gynecomastia

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Abstract

Overexpression of CYP19A1 encoding aromatase results in a rare genetic disorder referred to as aromatase excess syndrome (AEXS). Male patients with AEXS manifest pre- or peri-pubertal onset gynecomastia, gonadotropin deficiency, and advanced bone age, while female patients are mostly asymptomatic. To date, 30 male patients with molecularly confirmed AEXS have been reported. A total of 12 types of submicroscopic rearrangements, i.e., two simple duplications, four simple deletions, two simple inversions, and four complex rearrangements, have been implicated in AEXS. Clinical severity of AEXS primarily depends on the types of the rearrangements. AEXS appears to account for a small number of cases of pre- or peri-pubertal onset gynecomastia, and should be suspected particularly when gynecomastia is associated with an autosomal dominant inheritance pattern, characteristic hormone abnormalities and/or advanced bone age. Treatment with an aromatase inhibitor appears to benefit patients with AEXS, although long-term safety of this class of drugs remains unknown.

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Keywords: aromatase, CYP19A1, estrogen, genomic rearrangement, gynecomastia

Introduction

Aromatase encoded by CYP19A1 is a cytochrome P450 enzyme involved in estrogen biosynthesis. (1) Increased enzymatic activity of aromatase leads to a rare genetic disorder referred to as aromatase excess syndrome (AEXS). (2-4) Male patients with AEXS manifest pre- or peri-pubertal onset gynecomastia, along with gonadotropin deficiency, advanced bone age, and short adult height. (2-8) Female patients are usually asymptomatic, although variable clinical features such as macromastia, precocious puberty, irregular menses, and short stature have been reported in some individuals.(3,4,6,7)