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Case Report

Autosomal chromosome microdeletions in three adolescent girls with premature ovarian insufficiency: a case report

Ke Yuan, Minfei He, Yanlan Fang, Jianfang Zhu, Li Liang, Chunlin Wang

Department of Pediatrics, The First Affiliated Hospital, College of Medience, Zhejiang University, China.

ABSTRACT

Background. Premature ovarian insufficiency (POI) in the pediatric age group is most commonly related to X chromosome abnormalities such as Turner syndrome. Autosomal chromosome microdeletions in ovarian failure are relatively rare. The present study identified new autosomal deletions in three girls with POI.

Case. We present three adolescent girls aged 14–15 years who had not attained menarche. Upon physical examination, there was a lack of breast tissue and no prominent secondary sexual characteristics. Clinical evaluation, hormonal tests, abdominal ultrasonography, and chromosome karyotyping were performed. Chromosome microarray analysis (CMA) was also performed to detect DNA copy number changes. Luteinizing hormone level was significantly increased, while follicular stimulating hormone level was >25 IU/L with low estradiol levels. Autosomal deletions were detected in all three cases by CMA. The first patient had 0.454 Mb deletion on 15q25.2, the second patient had 1.337 Mb deletion on 19p13.3, and the third patient had 0.163 Mb deletion on 16p11.2.

Conclusions. POI is rare in children and is most commonly associated with X chromosome abnormalities. However, normal karyotype does not exclude the presence of chromosomal abnormality. CMA should be considered in cases with POI to detect microdeletions in autosomal chromosomes.

Key words: premature ovarian insufficiency, chromosome microarray analysis, chromosome karyotype, chromosomal abnormalities, autosomal.

Premature ovarian insufficiency (POI) refers to the development of ovarian dysfunction prior to the age of 40 years. The main manifestations of POI are abnormal menstruation (amenorrhea, oligomenorrhea, or polymenorrhea), elevated gonadotropin levels (FSH >25 IU/L), and a general decrease in the levels of female hormones. POI is further classified into primary POI and secondary POI, according to whether there had been spontaneous menstruation or not.[1,2] In children, POI is usually associated with short stature and delayed puberty. Genetic, iatrogenic, immune, and environmental factors have been implicated in the causation of POI. Nonetheless, the underlying cause remains unclear in more than half of all patients with POI (idiopathic POI). Approximately 20–30% of cases of POI are due to genetic aberrations, including chromosomal abnormalities and genetic variations. Among these, chromosomal abnormalities account for approximately 10-13% of cases, and 94% of aberrations involve the X chromosome. Other genes and autosomal abnormalities account for only 2% of patients with POI.[3-9] Besides, in the majority of POI cases with autosomal abnormalities, the autosomal abnormalities are considered coincidental. The onset of POI is commonly seen amongst older adults; however, cases of POI presenting in adolescence are rare.

✉ Chunlin Wang
hzwangcl@zju.edu.cn

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Current guidelines recommend the use of chromosome analysis to rule out potential chromosomal abnormalities in patients with POI.[10] In our previous work, we found that microdeletion may lead to POI, and we further discussed the genes affected by microdeletion. Our research indicated that POI may be related to genes, such as *BNC1*.[11] In the subsequent research, we found that new microdeletions may still lead to POI, indicating the need for further research on the correlation between chromosome microdeletions and POI. The present paper is a corollary to our previous work. In this study, we describe three adolescent girls with POI who had normal karyotype. Chromosomal microarray analysis (CMA) revealed three different microdeletions in autosomal chromosomes.

Case Report

Case 1 was a 14-year-old girl admitted to our clinic with growth retardation for 12 years. Her body weight was 33 kg (-2.2 standard deviation (SD)) and her height was 147 cm. She was admitted to our clinic due to a lag in sexual development and a lack of secondary sexual characteristics. She was diagnosed with intellectual disability shortly after birth. During her early kindergarten education, she was only able to say a few sentences and had a poor academic performance. According to the Wechsler Intelligence Scale for Children, her language IQ was 50, active IQ was 57, and total IQ was 44. Physical examination revealed no peripheral lymphadenopathy, tonsil enlargement, or hepatosplenomegaly. She had not achieved menarche. She was the first child of Han Chinese non-consanguineous parents. Her birth bodyweight (BBW) was 3.3 kg. Her father's height was 170 cm, and her mother's height was 157 cm. She showed poor development of the mammary glands, with widely spaced nipples.

On laboratory investigations, her sex hormone profile was: luteinizing hormone (LH), 35.05 IU/L (normal reference range: 50–9.30 IU/L); follicle stimulating hormone (FSH), 105.6 IU/L (1.4–18.1 IU/L); estradiol, 17.3 pg/mL (0–44.5 pg/mL); testosterone, 19.6 ng/dL (10.83–56.94 ng/dL); prolactin, 14.1 ng/mL (2.1–17.7 ng/mL); progesterone, 0.55 ng/mL (0.28–1.22 ng/mL); dehydroepiandrosterone sulfate (DHEAS), 144.6 μg/dL (34.5–568.9 μg/dL); and androstenedione 1.1 ng/mL (0.6–3.1 ng/mL). Her chromosome karyotype was 46, XX. The *FMR1* gene showed no variation, which excluded fragile X syndrome. On abdominal ultrasound, the uterine size was 1.9×0.7×1.2 cm^3 with no signs of thickening. The cervix was approximately 1.5 cm long, and the cervical intima was thin.

Case 2 was a 15-year-old girl admitted to our clinic for growth delay for 1 year. She also had a lag in prominent secondary sexual characteristics. She was yet to achieve menarche. There was no previous history of surgery or major disease.

She was the third child of Han Chinese non-consanguineous parents. She was born from a full-term spontaneous normal delivery with no complications; her BBW was 2.4 kg. Her father's height was 168 cm, and her mother's height was 155 cm. Her breast tissue was poorly developed. Further examination of the mammary gland showed no abnormal or web neck phenomenon.

Her sex hormone profile was: LH, 24.29 IU/L; FSH, 87.1 IU/L; estradiol, 18.2 pg/mL; testosterone, 34.7 ng/dL; prolactin, 17.9 ng/mL; progesterone, < 0.21 ng/mL; DHEAS, 97.1 μg/L; and androstenedione 0.6 ng/mL. The chromosome karyotype was 46, XX. On abdominal ultrasound, the uterus was 1.4×0.5×1.3 cm^3 in size, and the uterine wall was not thickened. The cervix was approximately 1.7 cm long and the intima was thin.

Case 3 was a 14-year-old girl brought to our outpatient clinic by her parents with complaints of growth retardation for 11 years. She had not yet achieved menarche. Her past history was unremarkable. She was borne of a full-term normal delivery with no complications;

her BBW was 2.4 kg. There was no family history of immunodeficiency or recurrent infections. Physical examination showed good mental development with optimal speech communication for her age. However, she had a short stature (height 136.4 cm, -4.0 SD) for her age, short neck, and underdeveloped breast tissue.

Her sex hormone profile was: LH, 17.02 IU/L; FSH, 61.3 IU/L; estradiol, 16.3 pg/mL; testosterone, 11.9 ng/dL; prolactin, 11.3 ng/mL; and progesterone < 0.21 ng/mL. The chromosome karyotype was 46, XX. Her uterus size was 1.4×1.4×0.6 cm^3, and the uterine wall was not thickened. The cervix was approximately 1.9 cm long and had a thin intima.

Prior to genetic testing, written informed consent was obtained from the probands and their parents (reference number of the research ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University: 2018-727). Genomic DNA of the three children was collected, and copy number variance was analyzed using the Phalanx Biotech's Cyto-One-Array Chromosome Chip. The chip contains a total of 33,255 57-63mer oligonucleotide probes covering a total of 331 specific diseases and all the sub telomeric regions designed according to the UCSC hg19 human gene bank (NCBI build 37). The probe resolution was 10–30 kb in disease-specific regions and 1.5–2 Mb resolution in non-disease-specific backbone areas. The three patients were sporadic cases. The family history of the patients revealed no similar case.

All three cases presented in this study are typical cases of ovarian dysfunction developing before the age of 40 years. None of the patients showed signs of secondary sexual development. Notably, the FSH levels of the patients far exceeded the POI standards. Moreover, their estrogen levels were significantly lower. Thus, a diagnosis of POI was established based on the clinical manifestations and sex hormone profile. CMA analysis revealed deletion aberrations in all three cases.

Case 1 had a DNA loss change on 15q25.2 with deletion size of about 0.454 Mb starting from position 83,581,573 to position 84,035,357 (Fig.1). Case 2 had 1.337 Mb (1,043,392-2,380,865) deletion on 19p13.3 (Fig.2). Of note, there are a total of 67 reported genes in this area. However, there is no previously reported association of

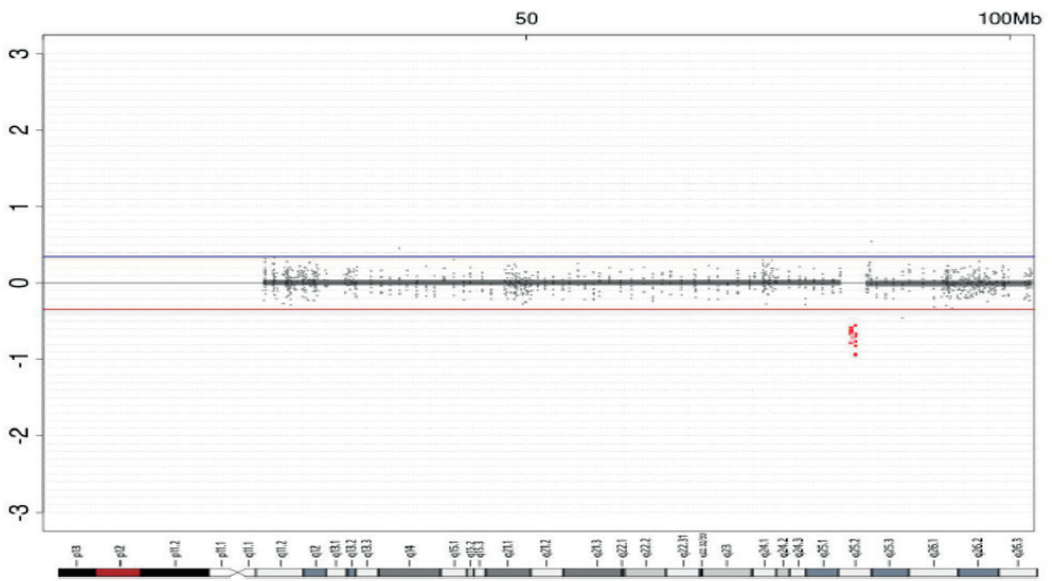

Fig. 1. Case 1 had a DNA loss change on 15q25.2 with deletion size of about 0.454 Mb starting from position 83,581,573 to position 84,035,357.

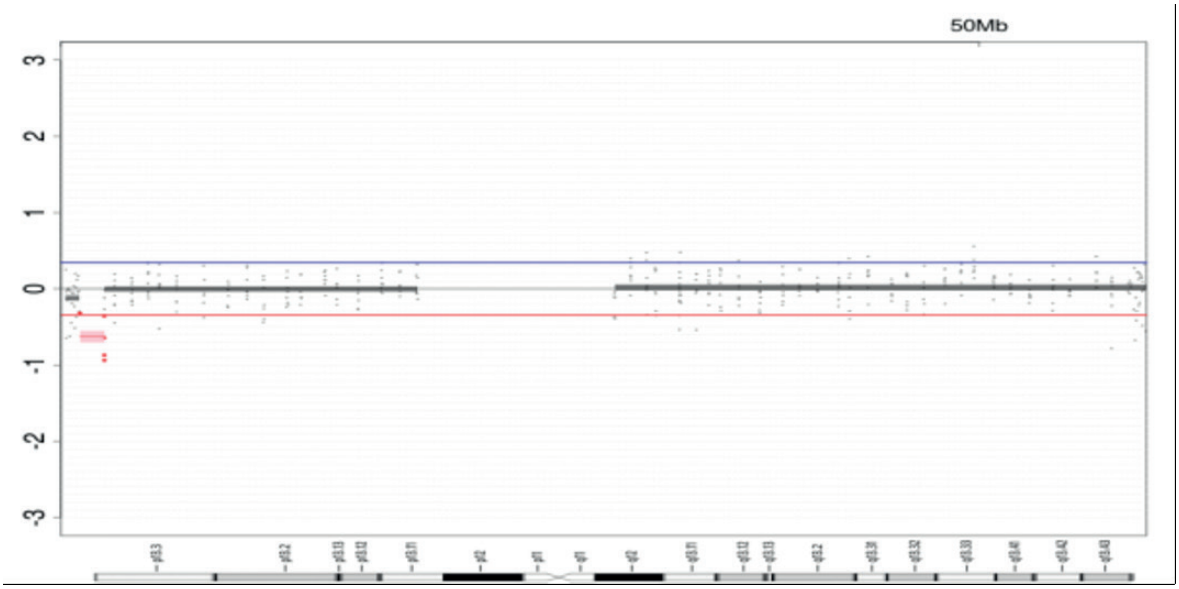

Fig. 2. Case 2 had 1.337 Mb (1,043,392 - 2,380,865) deletion on 19p13.3.

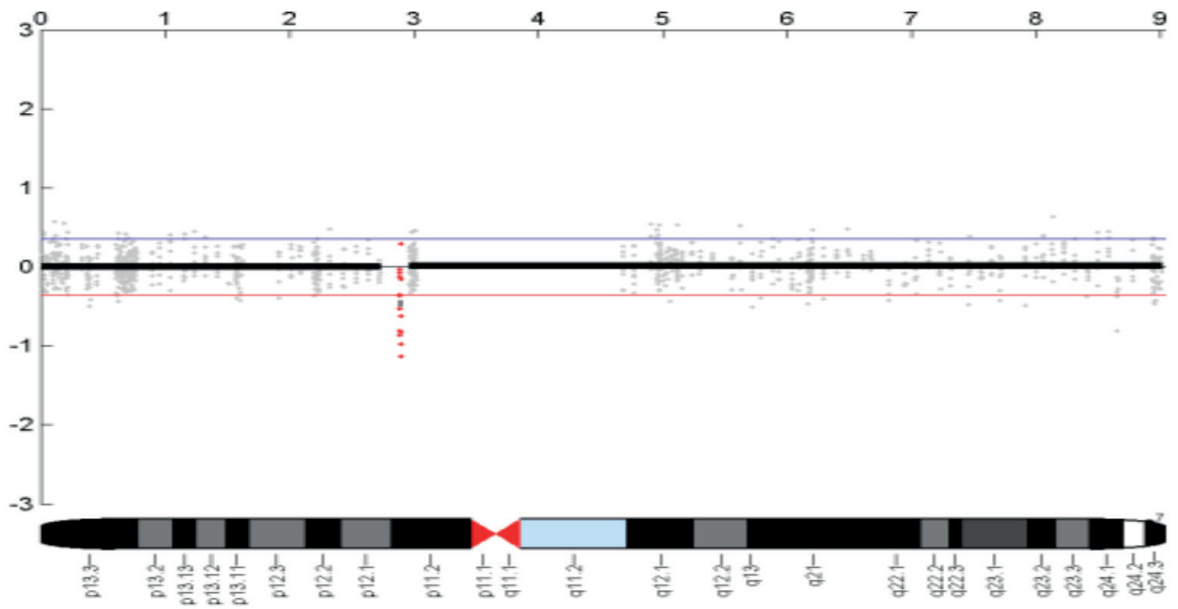

Fig. 3. Case 3 had 16p11.2 deletion with size of about 0.163 Mb (28,824,793 -28,987,798).

these genes with early ovarian insufficiency. However, case 3 had 16p11.2 deletion with size of about 0.163 Mb (28,824,793-28,987,798) (Fig.3). There are a total of 12 reported genes in this area; nonetheless, none of the genes have been reported to be associated with early ovarian insufficiency (Table I).

All 3 patients were treated with estrogen and progesterone for the establishment of artificial menstruation. All achieved menarche and established regular menstruation after treatment. The clinical course has been uneventful to date (as of 3-year follow-up).

Table I. Features of patients with premature ovarian insufficiency.

	Case 1	Case 2	Case 3
Age	14 y/o	15 y/o	14 y/o
Genotype	Del 15q25.2	Del 19p13.3	Del 16p11.2
Position	83,581,573-84,035,357	1,043,392-2,380,865	28,824,793-28,987,798
Size (Mb)	0.454	1.337	0.163
Secondary sexual characteristics	none	none	none
LH (IU/L)	35.05	24.29	17.02
FSH (IU/L)	105.6	87.1	61.3
E2 (pg/mL)	17.3	18.2	16.3
Ovary volume (mL)	0.798	0.455	0.588

LH: luteinizing hormone, FSH: follicle stimulating hormone, E2: estradiol

Discussion

As previously reported, POIs are usually associated with X chromosome abnormalities, and only a few POIs are associated with autosomal abnormalities. The pathophysiology of POIs falls into two categories: follicular dysfunction and accelerated depletion of the primordial follicles. There are several X chromosomal genes related to ovarian follicular dysfunction and apoptosis, such as *USP9X*, *ZFX*, *BMP15*, *SHOX*, *XIST*, *POF1B*, *DIAPH2*, *XPNPEP2*, and *FMR1*. However, there are other genes controlling the apoptosis and functions of autosomal follicles such as *BAX*, *BCL2*, *CDKN1B*, *CYP19A1*, *ESR1*, *FOXL2*, *CASP2*, and *CASP3*.[7,12,13]

The clinical manifestations of Case 1 in this case-series were very similar to those of fragile X syndrome. Fragile X syndrome is the most common cause of hereditary dementia and is the most prevalent genetic cause of autism and intellectual disability. The clinical manifestations may range from minor learning and mental disorders, and autism, to severe intellectual disability. Based on these characteristic features, the first case was suspected to be that of fragile X syndrome. However, after detecting the *FMR1* gene, fragile X syndrome was ruled out.[14] Previous studies suggested that the deletion of the *CPEB1* gene on 15q25.2 may cause POI or stunting. However, 15q25.2 (0.454 Mb) microdeletion in our case did not include the *CPEB1* gene, which, therefore, shows that POIs may not be directly affected by the *CPEB1* gene deletion.[15-18]

To the best of our knowledge, our case series is the first to show that the deletion on 15q25.2 may result in the simultaneous occurrence of POI and stunting.[15,16] Nonetheless, further studies should investigate the pathogenetic mechanism by which 15q25.2 deletions cause POI.

Microdeletion of 16p11.2 was detected in the third case. Aboura et al.[19] had reported a case of a female Caucasian with POI, a microdeletion of 16p11.2, and having a total of 21 genes. *CD19* gene may be associated with the development of POI. Nonetheless, the deletion in the third case was smaller when compared to that reported by Aboura et al.[19], but, however, included the *CD19* gene. Thus, the *CD19* gene deletion might be involved in the pathogenesis of POI.

Microdeletion on 19p13.3 detected in the second case might be the first reported to be associated with POI. Previous reports usually focused on signs such as developmental delays, deformity of the eye, extraocular distance, low set of ear lobes, retinopathy, and erythrocytes abnormalities with the deletion of the 19p13.3 region.[20-22] Our clinical findings suggest that there is a new microdeletion-19p13.3 chromosome phenotype.

Karyotype analysis of adolescents with POI was often used to exclude sex chromosomes or autosome related diseases. Karyotype analysis can quickly find a large deletion of chromosome fragments; however, it has a much lower sensitivity for the detection of chromosome microdeletions compared with CMA. Thus, CMA is the optimal method for genetic testing in order to identify chromosomal aberrations in clinically diagnosed POI patients.

CMA is widely used for prenatal diagnosis and genetic analysis of children with congenital heart disease and growth retardation.[23,24] Our series of studies have demonstrated the feasibility of CMA for the diagnosis of POI in children and adolescents, and reaffirmed the importance of CMA in the genetic diagnosis of childhood diseases.

In conclusion, although autosomal microdeletions are known to be a rare cause of POI, the application of CMA may unravel the involvement of more autosomal microvariations in the causation of POI. CMA may help identify the various causes of POIs and play a vital role in genetic counseling.

Ethical statement

Part of the manuscript was reported and discussed at the 2018 European Society for Pediatric Endocrinology Annual Meeting. The relevant information of case 1 was reported by the members of our research group in September 2020 with a 15q25.2 microdeletion phenotype for premature failure in a Chinese girl: a case report and review of literature.[11] The manuscript mainly discusses the possible effects of the microdeletion site on BNC1 gene, which is different from the research direction of this manuscript.

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Ethical approval

The Research Ethics Committee of First Affiliated Hospital, College of Medicine, Zhejiang University approved the study. Written informed consent was obtained from the parents. Reference number of the research ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University: 2018-727.

Author contribution

The authors confirm contribution to the paper as follows: designed the research and drafted the manuscript: KY, LL, CW; interpreted the data: KY, MH, YF; performed the literature search and scientific overview of our case: KY, JZ, CW. All authors critically read and reviewed the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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