A case of a parthenogenetic 46,XX/46,XY chimera presenting ambiguous genitalia

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Received: 17 December 2019 / Revised: 12 March 2020 / Accepted: 12 March 2020 / Published online: 10 April 2020
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Abstract

Sex-chromosome discordant chimerism (XX/XY chimerism) is a rare chromosomal disorder in humans. We report a boy with ambiguous genitalia and hypospadias, showing 46,XY[26]/46,XX[4] in peripheral blood cells. To clarify the mechanism of how this chimerism took place, we carried out whole-genome genotyping using a SNP array and microsatellite analysis. The B-allele frequency of the SNP array showed a mixture of three and five allele combinations, which excluded mosaicism but not chimerism, and suggested the fusion of two embryos or a shared parental haplotype between the two parental cells. All microsatellite markers showed a single maternal allele. From these results, we concluded that this XX/XY chimera is composed of two different paternal alleles and a single duplicated maternal genome. This XX/XY chimera likely arose from a diploid maternal cell that was formed via endoduplication of the maternal genome just before fertilization, being fertilized with both X and Y sperm.

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Supplementary information The online version of this article (<https://doi.org/10.1038/s10038-020-0748-4>) contains supplementary material, which is available to authorized users.

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A chimera is defined as the fusion product of two different zygotes in a single embryo, whereas a mosaic results from a mitotic error in a single zygote. Sex-chromosome discordant chimerism in humans (XX/XY chimerism) is a rare chromosomal abnormality. Although the first case was described in 1962 [1], its incidence is still unknown. The XX/XY chimera manifests variable genital phenotypes, ranging from normal male or female genitalia to different degrees of ambiguous genitalia. Chimeras are thought to result from a defect in the processes near the time of fertilization. The XX/XY chimera is classified into some subtypes; tetragametic chimeras [2–5], parthenogenetic chimeras [5–9], androgenetic chimeras [5, 10, 11] and sesquizygotic twinning chimera [12]. Whereas tetragametic chimeras are known as the most common subtype of XX/XY chimeras, which are derived from the simple fusion of two different zygotes, parthenogenetic chimeras and androgenetic chimeras [8, 11] undergo endoreplication of one of the gametic genomes. Therefore, genotyping of XX/XY chimeras is important not only to clarify its developmental mechanism but also for diagnosis and treatment, as such patients occasionally present with fertility problems. In this report, we present a case of a parthenogenic chimera with a karyotype of XX/XY, together with a literature review.

Fig. 1 Clinical findings of the patient. **a** On examination, the patient showed cryptorchidism (left), hypospadias (right), and anterior scrotum (right). **b** On operation, epididymis were identified macroscopically, but the tunica albuginea of the testis was absent bilaterally (left). Laparoscopy demonstrated that the vas deferens and gonadal veins flowed normally into the bilateral inguinal rings, and ovaries and a uterus were not detected (right). The precise position of the external urethral opening was normal. **c** The ultrasound echoic level of both

gonads was homogeneous. No other tissue-like structures with margins, suggestive of ovarian tissue, were detected. **d** Testis biopsy of the patient (left). Compared to control tissue (right, age-matched normal testis), interstitial tissue of the patient was edematous and more prominent than the seminiferous tubules, which were tortuous and diverging. The seminiferous tubules appeared dysplastic, but detailed analysis demonstrated that they were not dysplastic. Bar, 100 μ m

The patient was born from healthy parents at an affiliated hospital as a boy with ambiguous genitalia, hypospadias cryptorchidism, and an anterior scrotum (Fig. 1a). The pregnancy was not a result of in vitro fertilization treatment. His father was 43 years old and mother was 31 years old. Abdominal magnetic resonance imaging displayed no uterus or ovaries. His growth milestone at 1 year was normal. The patient's toy preference was wheeled vehicles and superheroes. Macro- and microscopically, the patient had ambiguous external genitalia, male-type internal genitalia (epididymis), and bilateral testes (Fig. 1b–d). The family chose a male sex to assign. Hypospadias was repaired. The parents were recommended to undergo genetic counseling regarding detailed genetic analysis as well as recurrence risk. G-banding from peripheral blood cells of the patient showed 46,XY[26]/46,XX[4]. To analyze the XX/XY ratio further, we performed fluorescence in situ hybridization (FISH) analysis on interphase nuclei from the patient's buccal mucosal cells with specific probes for chromosomes X and Y by AneuVysion Assay Kit (Abbott, Tokyo, Japan). FISH showed nuc ish XX[83]/XY[17]. The XY/XX ratio in buccal cells (17%) was lower than that in peripheral blood cells (87%). After receiving approval from the Ethics Review Board for Human Genome Studies at Fujita

Health University and written informed consent from the parents to participate in our study, genetic diagnosis was performed.

To confirm chimerism, we carried out SNP microarray analysis using a CytoScan 750 K Array (Affymetrix, Santa Clara, CA). We used genomic DNA, which was isolated from nail as a template for microarray analysis. Scan data were visualized using ChAS 3.2 software (Affymetrix). The copy number state of chromosome X was 1.5, which means that the ratio of XX to XY was about 50% (Fig. 2a). Next, to confirm chimerism, we analyzed the B-allele frequency of autosomes and the X chromosome [13, 14]. We found both a region showing five allele combinations, where possible SNP genotypes were BB–BB, BB–BA, BB–AA, BA–AA, and AA–AA, which means that there were three or four chromosome sets, and a region showing three allele combinations, where the possible SNP genotypes were BB–BB, AB–AB, and AA–AA, which means that there were two identical chromosome sets in the autosomes (Fig. 2b). The region showing four allele combinations, where the possible SNP genotypes were BB–B, BB–A, AA–B, and AA–A, were on chromosome X (Fig. 2b). These allele combinations were detected throughout the whole genome (Supplementary Fig. 1). This indicates the

Fig. 2 SNP array and STR analysis. **a** Smooth signal track of the patient's X chromosome. Copy number (Y axis) showed 1.5, suggesting that the patient has more than two sets of X chromosomes. **b** A B-allele frequency (BAF) plot and possible allele patterns of the patient's nail DNA, showing a XX to XY ratio of 50% in SNP array

analysis. The blue and red colored chromosomes in the circles are both of paternal origin. The yellow and pink colored chromosomes are of maternal origin. **c** STR maker analysis. Red and blue numbers indicate paternal origin, and yellow and pink numbers indicate maternal origin

presence of two different genomes, demonstrating that the patient does not show mosaicism but is an XX/XY chimera. Haplotype sharing region encompasses 1.5 Gbps, which is approximately half of the entire genome (Supplementary Fig. 1), suggesting that the chimera is possibly tetragenic, parthenogenetic or androgenetic. However, SNP array is not able to classify chimerism.

To investigate the parental origin of the genome in the patient, we performed microsatellite marker analysis, as known as short tandem repeat (STR) analysis. This time, genomic DNA was extracted from the patient's nails and parental peripheral blood cells. We chose STR markers located in the region showing the five allele combinations in autosomes or four allele combinations on chromosome X on SNP array analysis. We first determined the patient's genotype as well as the genotype of the parents. Next, the proband's genotype was determined by using completely different markers in autosomes. All markers showed the contribution of two paternal alleles and one maternal allele in the chimera (Fig. 2c). Therefore, we determined the dispermic origin of the chimera. We concluded that the

patient is a parthenogenetic chimera, which is composed of two paternal alleles and one duplicated maternal allele (Fig. 3a).

Although there are various mechanisms that lead to the development of chimeras, our results suggested that this patient was a parthenogenetic chimera [9]. Oocyte possibly started first mitosis before the disappearance of the pronuclear.

From the point of view of genetic counseling, the recurrence risk of this condition in this family is low because of the lack of a family history. On the other hand, careful follow-up of testicular function is important in considering future family planning and gender identity for the patient. However, as the chimeric ratio in this patient is different among various tissues in the body [15], androgen or estrogen exposure to the entire body cannot be assessed and it is difficult to predict the risk of problems, such as gonadoblastoma (Fig. 3b). In addition, genetic counseling should be recommended so that the patient's gender identity can be determined autonomously when the pathological condition is explained to the patient.

Fig. 3 Schematic drawing of how a parthenogenetic chimera is produced. **a** Schematic drawing of how a parthenogenetic chimera is produced. Endoduplication of the maternal genome occurs just before fertilization with two spermatozoa. The colors of the cells are consistent with Fig. 2b, c. **b** Top, diagram of XX and XY chimeric ratios of three tissues from the patient. Orange cells indicate 46,XX, and

green cells indicate 46,XY. Bottom, whereas G-banding from the peripheral blood cells of the patient showed 46,XY[26]/46,XX[4] (XX:XY ratio, 1:9), FISH showed nuc ish XX[83]/XY[17] (XX:XY ratio, 8:2) in buccal cells, and the SNP array suggested that both XX and XY are present at about 50% (XX:XY ratio, 5:5)

In the present case, genotyping determined that the patient had a rare condition (XX/XY chimerism). Furthermore, it was suggested that the patient was not the common tetragametic chimera, but a parthenogenetic chimera. Genotyping of XX/XY chimeras is important not only for clarifying the pathogenesis of chimeras but also for understanding of the disease. Therefore, we expect this method to become the standard for patients who are chimeric for the XX/XY karyotype.

Acknowledgements We would like to thank the patient and the family for participating in this study. We would also like to thank Dr Akiko Helena Popiel for valuable comments, Tatsuro Ikeda for drawing of the Figures. This study was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (grant number 17K00481 to RK, to 17K11259 to TK).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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