CLINICAL STUDY

Female gonadal development in XX patients with distal 9p monosomy

Tsutomu Ogata, Koji Muroya, Hirofumi Ohashi¹, Hiroshi Mochizuki², Tomoko Hasegawa³ and Masayuki Kaji⁴

Department of Paediatrics, Keio University School of Medicine and Tokyo Electric Power Company Hospital, Tokyo, Japan, Divisions of ¹Medical Genetics and ²Endocrinology and Metabolism, Saitama Children's Medical Centre, Iwatsuki, Japan and Divisions of ³Medical Genetics and ⁴Endocrinology and Metabolism, Shizuoka Children's Hospital, Shizuoka, Japan

(Correspondence should be addressed to Tsutomu Ogata, Department of Paediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan; Email: t-ogata@po.ijnet.or.jp)

Abstract

Objective: A sex determining gene(s) has been mapped to a ~700 kb region distal to the exons of DMRT1 on 9p. The aim of this study was to examine gonadal developmental status in XX patients hemizygous for the 9p sex determining region.

Design: Clinical and molecular studies were performed in an 8-year-old girl with 46,XX,del(9)(p22) (case 1) and in a 2-year-old girl with 46,XX,del(9)(p23) (case 2).

Methods: Ovarian function status was assessed by gonadotrophin-releasing hormone (GnRH) tests. Hemizygosity for the sex determining region was examined by fluorescence *in situ* hybridisation and microsatellite analyses for a total of 17 loci on distal 9p.

Results: GnRH tests indicated mild gonadotrophin hyper responses in both cases (case 1: follicle stimulating hormone 9.2→22.7 IU/l, luteinising hormone 0.7→16.6 IU/l; case 2: follicle stimulating hormone 7.6→38.2 IU/l, luteinising hormone 0.6→9.4 IU/l). Molecular studies showed hemizygosity for the 9p sex determining region in both cases.

Conclusions: The results, in conjunction with previous reports describing sex development in XX and XY patients hemizygous for the 9p sex determining region, imply that haploinsufficiency of the 9p sex determining gene(s) primarily hinders the formation of the indifferent gonad, leading to a wide range of testicular or ovarian development.

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Introduction

A sex determining gene(s) resides on the terminal 9p region (1). This notion is primarily based on clinical findings in XY patients with molecularly defined terminal 9p monosomy. Such XY patients exhibit a wide range of sex development from nearly normal male phenotype to complete sex reversal with defective testis formation (2). The degree of male sex development is unrelated to the size of the monosomic region and independent of the parental origin of the deleted 9p chromosome (2). Thus, it has been suggested that haploinsufficiency of the sex determining gene(s) escaping epigenetic imprinting leads to various degrees of testis formation and resultant male sex development.

The sex determining gene(s) has been located to a ~700 kb DNA sequence at the 9p telomeric region, on the basis of molecular studies in XY sex reversed patients with distal 9p monosomy (3). In this context, it has been reported that, of the candidate

sex determining gene DMRT1 (a human homologue for evolutionary conserved sex determining genes with a DNA binding domain) (4) and its related genes DMRT2 (5) and DMRT3 (6), the promoter region of DMRT1 may be contained in the critical region, but the exons of DMRT1 as well as DMRT2 and DMRT3 map outside the critical region, with the order of 9pter-DMRT1-DMRT3-DMRT2-centromere (3, 6). In addition, mutational analysis in a large series of XY sex reversed patients has failed to identify a definite mutation of DMRT1–3 genes (1). Thus, the sex determining gene(s) is still elusive, although DMRT1 remains the candidate gene at present.

Furthermore, it is uncertain whether ovarian development is preserved or impaired in XX patients missing the sex determining region, because clinical and molecular studies have been poorly performed in such patients. Here, we report on two Japanese XX patients with distal 9p monosomy, and discuss female gonadal development in distal 9p monosomy.

Case reports

Case 1

This girl was born at 39 weeks of gestation after an uncomplicated pregnancy and delivery. At birth, her length was 49.0 cm (± 0 s.d.), her weight was 2.96 kg (-0.4 s.d.), and her head circumference was 31.5 cm (-1.3 s.d.). The parents were non-consanguineous and healthy.

At one month of age, she was referred to Saitama Children's Medical Centre because of dysmorphic features. Physical examination showed up-slanting palpebral fissures, flat nasal ridge, short anteverted nostrils, high arched palate, small and deformed ears, and hypopigmented hair. External genitalia were feminised with hypoplastic labia majora. There were no major anomalies. Thereafter, she had developmental retardation, and was enrolled in a special school for mentally delayed children. At 8 1/12 years of age, her height was 125.2 cm ($+0.1$ s.d.), her weight was 23.7 kg (-0.3 s.d.), and her head circumference was 49.6 cm (-1.0 s.d.). She exhibited no signs of pubertal development. Abdominal ultrasound studies showed a rudimentary uterus and normal urinary system, and failed to detect gonadal structures.

Case 2

This girl was delivered at 37 weeks of gestation by a caesarean section due to breech presentation. At birth, her length was 45.0 cm (-1.3 s.d.), her weight was 2.44 kg (-1.2 s.d.), and her head circumference was 33.0 cm ($+0.2$ s.d.). The unrelated parents and the elder sister were clinically normal.

Shortly after birth, she was referred to Shizuoka Children's Hospital because of cardiac murmur and multiple anomalies. Cardiac evaluation revealed coarctation of the aorta, atrial septal defect, and patent ductus arteriosus, and a surgical operation was carried out for the congenital cardiac anomalies. Her dysmorphic features included trigonocephaly with fusion of the metopic suture, up-slanting palpebral fissures, flat nasal ridge, short anteverted nostrils, and high arched palate. External genitalia were those of normal female infants. Subsequently, she had marked growth and developmental retardation. Abdominal ultrasonography at 2 years of age delineated a small uterus-like structure and normal urinary system, and failed to detect gonadal structures. At 2 4/12 years of age, her height was 69.8 cm (-4.9 s.d.), her weight was 6.37 kg (-4.0 s.d.), and her head circumference was 43.0 cm (-3.1 s.d.). She had no pubertal development.

Methods

Endocrine studies

A gonadotrophin-releasing hormone (GnRH) test (100 $\mu\text{g}/\text{m}^2$ bolus i.v.; blood sampling at 0, 30, 60,

90, and 120 min) was performed in both cases. Serum follicle stimulating hormone (FSH), luteinising hormone (LH) and estradiol (E_2) were measured by the time-resolved fluoroimmunoassay using Delfia kits (Pharmacia-Upjohn, Sweden). The intra-assay coefficients of variation ranged between 2.0 and 5.6% for FSH, between 1.6 and 2.7% for LH, and between 1.9 and 4.6% for E_2 , and the inter-assay coefficients of variation ranged between 3.7 and 4.5% for LH, between 2.5 and 3.0% for FSH, and between 1.2 and 3.0% for E_2 .

Conventional and molecular cytogenetic studies

G-banding chromosome analysis was performed on 50 peripheral lymphocytes of case 1 and her parents and of case 2 and her mother. Fluorescence *in situ* hybridisation (FISH) analysis was carried out for lymphocyte metaphase spreads of cases 1 and 2, with a bacterial artificial chromosome (BAC) probe containing the marker 305J7-T7 (41-L13), a yeast artificial chromosome (YAC) probe containing D9S1779 (757A1), a YAC probe containing D9S1858 (765H2), a BAC probe containing DMRT1 (DMRT-1/BAC), a P1 phage artificial chromosome (PAC) probe containing D9S1136 (34H2), together with a BAC probe for APBA1 at 9q13-21 used as an internal signal control (2, 7-9). The probe for APBA1 was labelled with biotin and detected by avidin conjugated to fluorescein isothiocyanate, and the remaining probes were labelled with digoxigenin and detected by rhodamine anti-digoxigenin.

Microsatellite analysis

Leukocyte genomic DNA of case 1 and her parents and of case 2 and her mother was amplified by polymerase chain reaction (PCR) with fluorescently labelled forward primers and unlabelled reverse primers defining 14 loci on 9p: D9S1779, D9S1858, D9S129, D9S143, D9S54, D9S1871, D9S288, D9S286, D9S168, D9S285, IFNA, D9S169, D9S165, and D9S1874. The primer sequences and the PCR conditions were as described in Genome Database (<http://www.gdb.org>). The PCR products were determined for fragment size on an autosequencer using GeneScan software 2.1 (ABI PRISM 310; Applied Biosystems).

Results

Endocrine studies

The results are summarised in Table 1. GnRH tests showed mild hyper responses of FSH and LH in case 1, and FSH dominant mild gonadotrophin hyper responses in case 2. Serum E_2 was undetected in both cases.

Table 1 The results of GnRH test (100 µg/m² bolus i.v.) in case 1 and case 2.

	Time (min)				
	0	30	60	90	120
Case 1 (8 1/12 years)					
FSH (IU/l)	9.2	11.9	22.1	22.7	20.5
LH (IU/l)	0.7	12.4	16.6	13.2	8.8
Estradiol (pmol/l)	<30	N.D.	N.D.	N.D.	N.D.
Case 2 (2 4/12 years)					
FSH (IU/l)	7.6	25.4	35.9	38.2	34.7
LH (IU/l)	0.6	7.5	9.4	8.7	6.0
Estradiol (pmol/l)	<30	N.D.	N.D.	N.D.	N.D.

Reference values in prepubertal girls: FSH, baseline <5.3 and peak 2.2–16.4 IU/l; LH, baseline <1.0 and peak 2.6–4.7 IU/l; and estradiol, baseline <30 pmol/L (10, 11).
N.D.: not determined.

Conventional and molecular cytogenetic studies

The karyotype was 46,XX,del(9)(p22) in case 1 (loss of a 9p region distal to the breakpoint at 9p22) and 46,XX,del(9)(p23) in case 2 (loss of a 9p region distal to the breakpoint at 9p23). The parents of case 1 and the mother of case 2 had normal karyotypes. The five FISH probes defining different distal 9p regions (41-L13/BAC, 757A1/YAC, 765H2/YAC, DMRT-1/BAC, and 34H2/PAC) failed to detect positive signals on the abnormal chromosome 9 of cases 1 and 2 (Fig. 1 and Table 2).

Microsatellite analysis

The results are summarised in Table 2. In case 1, genotyping analysis confirmed distal 9p monosomy with the breakpoint between D9S285 and D9S168, and showed the paternal origin of the deleted chromosome 9. In case 2, single peaks only were detected for all the 10 loci distal to IFNA, providing further support for the distal 9p monosomy; comparison of the fragment sizes between the patient and the mother was consistent with the single peaks being derived from the mother.

Discussion

Cases 1 and 2 had molecularly confirmed distal 9p monosomy. In particular, the results of FISH analysis with 41-L13/BAC containing 305J7-T7, 757A1/YAC containing D9S1779, 765H2/YAC containing D9S1858, and DMRT-1/BAC, as well as those of microsatellite analysis for D9S1779 and D9S1858, are important (Table 2), because 305J7-T7, D9S1779, D9S1858, and DMRT-1 reside at positions ~190 kb, ~250 kb, ~400 kb, and ~700 kb respectively from the 9p telomere (1, 9). The results imply that cases 1 and 2 had hemizygosity for the ~700 kb sex determining region distal to the exons of DMRT1 (3), although it might be possible that the abnormal chromosome 9 had an undetected cryptic complex rearrangement involving the sex determining region such as interstitial deletion preserving the telomeric 9p segment distal to 305J7-T7.

Figure 1 FISH analysis with BAC probes for DMRT1 and APBA1 in case 1. Only a single signal is detected for DMRT1 (arrows), whereas two signals are found for APBA1 used as an internal signal control (arrowheads).

Table 2 The results of FISH and microsatellite analyses in case 1 and case 2.

Locus/region	Chromosomal location	Methods	Case 1			Case 2	
			Father	Patient	Mother	Patient	Mother
41-L13/BAC	9p24.3	FISH	N.E.	—*	N.E.	—*	N.E.
D9S1779	9p24.3	MS	137, 139	141*	123, 141	141	121, 141
757A1/YAC	9p24.3	FISH	N.E.	—*	N.E.	—*	N.E.
D9S1858	9p24.3	MS	244	232*	232, 244	244	244
765H2/YAC	9p24.3	FISH	N.E.	—*	N.E.	—*	N.E.
DMRT1/BAC	9p24.3	FISH	N.E.	—*	N.E.	—*	N.E.
D9S129	9p24.3	MS	130	130	130	134	130, 134
D9S143	9p24.3	MS	115	105*	105, 113	114	114
D9S54	9p24.3–p24.1	MS	113	117*	115, 117	113	107, 113
34H2/PAC	9p24.3–p24.1	FISH	N.E.	—*	N.E.	—*	N.E.
D9S1871	9p24.3–p24.1	MS	140, 142	142	142, 174	140	140
D9S288	9p24.3–p24.1	MS	124	128*	128, 130	122	122, 124
D9S286	9p23	MS	147	151*	145, 151	143	143, 147
D9S168	9p23	MS	226, 232	230*	230, 232	232	232
D9S285	9p22	MS	107, 111	111, 115	113, 115	109	109
IFNA	9p22	MS	149	149, 151	141, 151	141, 149	141
D9S169	9p21.1	MS	257	257, 267	267, 269	261, 267	261
D9S165	9p21.1	MS	217, 221	215, 217	215	211, 215	207, 211
D9S1874	9p12	MS	192, 196	190, 196	190	188, 200	188, 198

MS: microsatellite analysis; N.E.: not examined.

The (—) symbol for FISH analysis represent the absence of positive signals on the abnormal chromosome 9.

The locus order and the chromosomal location are based on the previous reports (3, 19, 20).

*Confirmed hemizygous loci.

Endocrine studies showed mild gonadotrophin hyper responses in both cases, as compared with the reference values (10, 11). Interestingly, case 1 showed hyper responses of both FSH and LH whereas case 2 showed FSH dominant gonadotrophin hyper responses. In this regard, it has been reported that in Turner's syndrome a GnRH test usually results in FSH dominant gonadotrophin hyper responses in prepubertal age and hyper responses of both LH and FSH in pubertal and adult age (12, 13). Thus, the variation in the gonadotrophin hyper response between cases 1 and 2 may be due to the age difference between the two cases. However, further clinical observation would be necessary to clarify whether the mild gonadotrophin hyper responses in the prepubertal age indeed represent mild primary hypogonadism (hypergonadotrophic hypogonadism). In addition, although ultrasound studies failed to delineate gonadal structures in both cases, this would not indicate defective ovarian development because of the difficulty in ovarian detection in prepubertal girls.

Nevertheless, the present study provides some information on the gonadal development in XX females hemizygous for the 9p sex determining region. To date, gonadal assessment is possible only in two XX patients with distal 9p monosomy, and a striking contrast is suggested for gonadal developmental status in the two females. Muroya *et al.* (2) described a 17-month-old girl with molecularly defined distal 9p monosomy missing the sex determining region who had a markedly elevated basal serum FSH level of 41.0 IU/l. Calvari *et al.* (3) reported a fertile female with an apparently

46,XX karyotype missing the 700 kb sex determining region who gave birth to two sex reversed 46,XY siblings with the same submicroscopic 9p deletion. The mild gonadotrophin hyper responses in cases 1 and 2 may exhibit an intermediate gonadal phenotype, thereby connecting the contrasting gonadal features of the two previously reported XX females. Collectively, it is inferred that hemizyosity of the sex determining region in XX females results in a wide range of gonadal development, from normally functioning ovary to severely dysgenetic ovary.

It is likely, therefore, that the 9p sex determining gene(s) is involved in both testicular and ovarian development. This provides further support for the notion that the 9p sex determining gene(s) is operating in the formation of the indifferent gonad common to both sexes, rather than in the sex-specific testicular or ovarian development from the indifferent gonad (2). It appears that haploinsufficiency of the 9p sex determining gene(s) primarily hinders the formation of the indifferent gonad, leading to a wide range of testicular or ovarian development. In this context, it should be pointed out that such phenotypic variability in haploinsufficiency is not specific to the 9p sex determining gene(s) but common to many genes involved in human development (14). For example, haploinsufficiency of SOX9 results in a wide spectrum of sex development in XY patients, ranging from nearly normal male phenotype to nearly complete female phenotype (15). Probably, haploinsufficiency of developmental genes leads to a wide phenotypic spectrum, depending on other genetic and/or environmental factors.

Case 1 had hypoplastic labia majora, and both cases had hypoplastic or rudimentary uterus on ultrasound examinations. Such abnormal external genitalia, as well as anal and renal anomalies, have also been described in several XX patients with distal 9p monosomy (16), although uterine structures have been poorly examined. This suggests that the 9p sex determining gene(s), or other gene(s) on distal 9p, may be involved in the development of urogenital regions.

Cases 1 and 2 also had a constellation of minor anomalies and mental retardation consistent with 9p monosomy syndrome (16, 17). In this context, Christ *et al.* (18) have performed genotype–phenotype correlations in a total of 24 patients with partial 9p deletions and clinical features compatible with 9p monosomy syndrome including trigonocephaly, up-slanting palpebral fissures, midface hypoplasia (flat nasal ridge), and mental retardation, localizing the critical region for 9p monosomy syndrome to a 4–6 Mb segment between D9S286 and D9S285. Although it is unknown how many genes are involved in the development of 9p monosomy syndrome, this location of the critical region would primarily explain the presence of clinical features consistent with 9p monosomy syndrome in both cases reported here (Table 2). In this regard, the absence of trigonocephaly in case 1 may be noteworthy, because trigonocephaly constitutes one of the salient clinical features in 9p monosomy syndrome (16–18). There are two possible explanations for the lack of trigonocephaly in case 1: (i) the deleted 9p chromosome of case 1 retains the gene for trigonocephaly and is missing the gene(s) for other features; and (ii) the gene for trigonocephaly has also been deleted from the abnormal chromosome 9 of case 1, but the expressivity has remained at a subclinical level. This matter will be clarified when the gene(s) for 9p monosomy syndrome has been cloned.

In summary, it appears that haploinsufficiency of the 9p sex determining gene(s) results in diverse sex development in XX as well as XY patients. Further studies will permit a better clarification of the phenotypic spectrum in haploinsufficiency of the 9p sex determining gene(s).

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References

- Ottolenghi C & McElreavey K. Deletion of 9p and the quest for a conserved mechanism of sex determination. *Molecular Genetics and Metabolism* 2000 **71** 397–404.

- Muroya K, Okuyama T, Goishi K, Ogiso Y, Fukuda S, Kameyama J *et al.* Sex determining gene(s) on distal 9p: clinical and molecular studies in six cases. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 3094–3100.
- Calvari V, Bertini V, De Grandi A, Peverali G, Zuffardi O, Ferguson-Smith M *et al.* A new submicroscopic deletion that refines the 9p region for sex reversal. *Genomics* 2000 **65** 203–212.
- Raymond CS, Shamu CE, Shen MM, Seifert KL, Hirsch B, Hodgkin J *et al.* Evidence for evolutionary conservation of sex-determining genes. *Nature* 1998 **391** 691–695.
- Raymond CS, Parker ED, Kettlewell JR, Brown LG, Page DC, Kusz K *et al.* A region of human chromosome 9p required for testis development contains two genes related to known sexual regulators. *Human Molecular Genetics* 1999 **8** 989–996.
- Ottolenghi C, Veitia R, Quintana-Murci L, Torchard D, Scapoli L, Souleyreau-Therville N *et al.* The region on 9p associated with 46,XY sex reversal contains several transcripts expressed in the urogenital system and a novel doublesex-related domain. *Genomics* 2000 **64** 170–178.
- Fleijter WL, Fergestad J, Gorski J, Varvilli T & Chandrasekharappa S. A gene involved in XY sex reversal is located on chromosome 9, distal to marker D9S1779. *American Journal of Human Genetics* 1998 **63** 794–802.
- Ning Y, Roschke A, Smith ACM, Macha M, Precht K, Riethman H *et al.* A complete set of human telomeric probes and their clinical application. *Nature Genetics* 1996 **14** 86–89.
- Knight SJL, Lese CM, Precht KS, Kuc J, Ning Y, Lucas S *et al.* An optimized set of human telomere clones for studying telomere integrity and architecture. *American Journal of Human Genetics* 2000 **67** 320–332.
- Ito J, Tanaka T, Horikawa R, Okada Y, Morita S, Koitaji M *et al.* Serum LH and FSH levels during GnRH tests and sleep in children (in Japanese). *Journal of Japan Pediatric Society* 1993 **97** 1789–1796.
- Japan Public Health Association Normal biochemical values in Japanese children (in Japanese). Tokyo: Sanko Press, 1996.
- Illig R, Tolksdorf M, Mürset G & Prader A. LH and FSH response to synthetic LH-RH in children and adolescents with Turner's and Klinefelter's syndrome. *Helvetica Paediatrica Acta* 1975 **30** 221–231.
- Ross JL, Loriaux DL & Cutler GB Jr. Developmental changes in neuroendocrine regulation of gonadotropin secretion in gonadal dysgenesis. *Journal of Clinical Endocrinology and Metabolism* 1983 **57** 288–293.
- Fisher E & Scambler P. Human haploinsufficiency – one for sorrow, two for joy. *Nature Genetics* 1994 **7** 5–7.
- Cameron FJ, Hageman RM, Cooke-Yarborough C, Kwok C, Goodwin LL, Silience DO *et al.* A novel germ line mutation in SOX9 causes familial campomelic dysplasia and sex reversal. *Human Molecular Genetics* 1996 **5** 1625–1630.
- Huret JL, Leonard C, Forestier B, Rethoré MO & Lejeune J. Eleven new cases of del(9p) and features from 80 cases. *Journal of Medical Genetics* 1988 **25** 741–749.
- Jones KL. *Smith's Recognizable Patterns of Human Malformations*. Philadelphia: WB Saunders, 1997.
- Christ LA, Crowe CA, Micale MA, Conroy JM & Schwartz S. Chromosome breakage hotspots and delineation of the critical region for the 9p-deletion syndrome. *American Journal of Human Genetics* 1999 **65** 1387–1395.
- Bouzyk M, Bryant SP, Evans C, Guioli S, Ford S, Schmidt K *et al.* Integrated radiation hybrid and yeast artificial chromosome map of chromosome 9p. *European Journal of Human Genetics* 1997 **5** 299–307.
- Povey S, Attwood J, Chadwick B, Frezal J, Hains JL, Knowles M *et al.* Report of the fifth international workshop on chromosome 9. *Annals of Human Genetics* 1997 **61** 183–206.

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