Short reports

Impaired male sex development in an infant with molecularly defined partial 9p monosomy: implication for a testis forming gene(s) on 9p

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Abstract

This paper describes a genetically male infant with impaired male sex development and partial 9p monosomy. The external genitalia were ambiguous with microphallus (penile length at birth 10 mm, mean age matched normal length 29 mm (SD 5)), hypospadias, and hypoplastic scrotum. The testes were undescended and severely hypoplastic (testis size at 12 months of age, right 8×5×4 mm and left 4×3×2 mm; mean age matched normal size, length 18 mm (SD 2), width 11 mm (SD 1)). Cytogenetic studies showed a 46,XY,del(9)(p23) karyotype in all the 30 peripheral lymphocytes and 20 skin fibroblasts examined. Microsatellite analysis for a total of 13 loci assigned to the 9p22-24 region showed that the deleted chromosome 9 was of paternal origin and was missing a region distal to D9S168. Southern blot analysis for D9S47 also confirmed the 9p deletion. The sequence of SRY was normal. The results provide further support for the previously proposed hypothesis that a gene(s) for testis formation is present on the distal part of 9p and indicate in molecular terms that the putative testis forming gene(s) resides in the region distal to D9S168.

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A gene(s) for testis formation has been suggested to be on the distal part of 9p, on the basis of karyotype-phenotype correlations in six genetically male patients with impaired male sex development and monosomy of distal 9p.[1–6] However, there are only a small number of patients and gonadal structure has been examined in only three patients.[1 3 6] Furthermore, since molecular studies for the 9p deletion have not been carried out to date, the precise chromosomal location of the putative testis forming gene(s) remains to be determined. In this report, we describe clinical, cytogenetic, and molecular findings in a genetically male infant with impaired male sex development and partial 9p monosomy.

This infant was referred to Toyohashi Municipal Hospital shortly after birth because of minor anomalies and ambiguous genitalia. Dysmorphic features included prominent forehead, anteverted nostrils, low set ears, high arched palate, micrognathia, puffy hands and feet, and funnel chest. The phallus was 10 mm in length (mean age matched penile length of normal Japanese boys, 29 mm (SD 5))[7] and associated with hypospadias. The scrotum was severely hypoplastic and there was no vaginal formation. Small testis-like masses were felt in the bilateral inguinal regions. There were no major anomalies except for oesophageal hiatus hernia, and there were no urinary tract abnormalities other than the hypospadias. On the basis of the above findings and the cytogenetic results described later, female sex was assigned to the infant.

At 12 months of age, gonadectomy was carried out by a local inguinal incision to prevent gonadal malignancy and further masculinisation. Macroscopic examination of the internal genitalia at the time of the operation showed that both testes were severely hypoplastic and accompanied by vasa deferentia and epididymides. The size was 8×5×4 mm for the right testis and 4×3×2 mm for the left testis (mean age matched testis size of normal Japanese boys, length 18 mm (SD 2), width 11 mm (SD 1)).[8] Spermatic cords were associated with hydroceles. Microscopic examination of the testes showed that seminiferous tubules were well preserved and consisted of Sertoli cells and spermatogonia (fig 1). There was no thickening of peritubular connective tissue. The interstitium was oedematous and contained immature Leydig cells that were associated with vacuoles suggestive of fatty degeneration. Ovarian or dysgenetic tissue was not detected in any gonadal sections examined. Vasa deferentia and epididymides showed normal histological findings.

Endocrine studies showed no obvious abnormalities. At 2 months of age, serum testosterone was 4.4 nmol/l at a baseline level (mean age

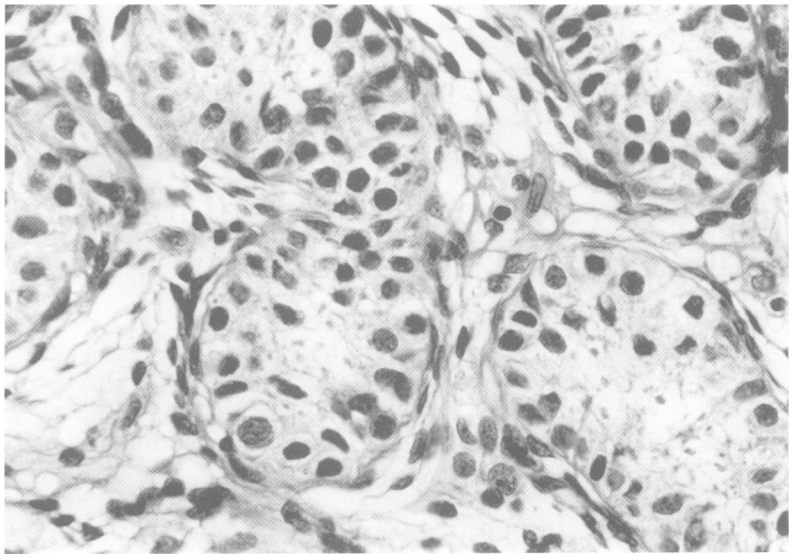

Figure 1 Histological finding of the right testis (haematoxylin-eosin stain).

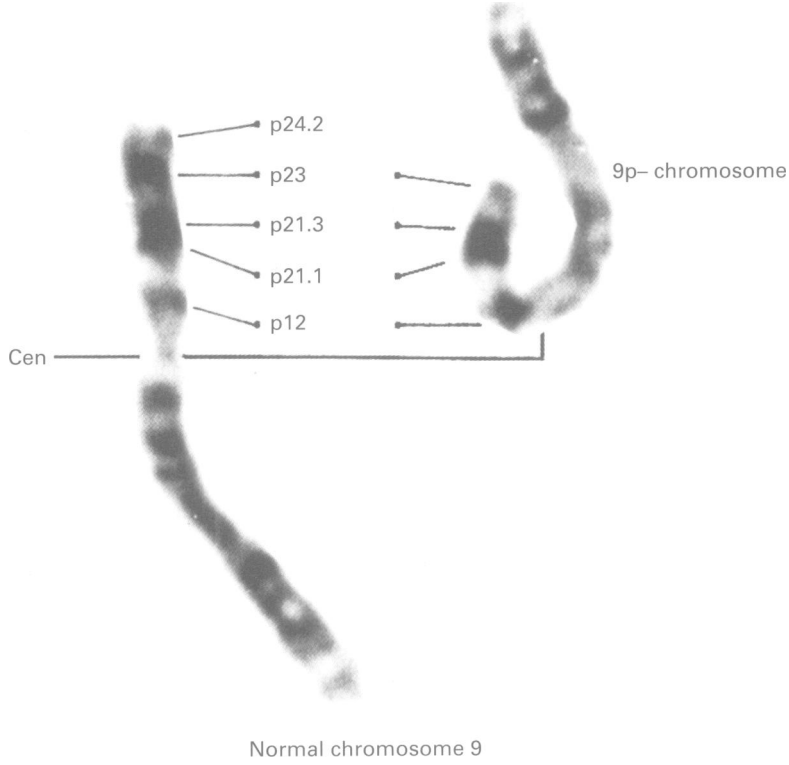

Figure 2 The normal chromosome 9 (left) and the 9p- chromosome (right) of the patient.

matched normal level 6.8 nmol/l (SD 4.1))[9] and increased to 9.4 nmol/l after a human chorionic gonadotrophin (hCG) stimulation (4000 IU/m^2/dose intramuscularly for three consecutive days; blood sampling on the fourth day), and serum dihydrotestosterone was 0.6 nmol/l at a baseline level and increased to 1.3 nmol/l after hCG stimulation. Urinary steroid hormone metabolites determined by gas liquid chromatograph mass spectrometry were normal, indicating intact enzymatic activity for steroidogenesis. At 15 months of age, a gonadotrophin releasing hormone test (100 μg/m^2 bolus intravenously, blood sampling at 0, 30, 60, 90, and 120 minutes) showed increased responses of luteinising hormone (1→14 IU/l) and follicle stimulating hormone (20→56 IU/l), being consistent with a postgonadectomised status with normal hypothalamic-pituitary function.

Cytogenetic studies showed that the karyotype of the patient was 46,XY,del(9)(p23) in all the 30 peripheral lymphocytes and 20 skin fibroblasts analysed (fig 2). The paternal karyotype was 46,XY and the maternal karyotype 46,XX.

To confirm the 9p deletion in molecular terms, microsatellite analysis was carried out for a total of 13 different loci assigned to the 9p22-24 region (table 1). Genomic DNA of the patient and the parents was amplified by polymerase chain reaction (PCR) with Cy-5 (Pharmacia) labelled forward primer and unlabelled reverse primer, and the size of the PCR products was determined on ALF*red* sequencer by Fragment Manager V1.1 (Pharmacia). The primer sequences and the PCR conditions were as described in the references shown in table 1. For D9S178, D9S288, D9S132, D9S281, and D9S286, paternal markers were not inherited by the patient and maternal markers alone were transmitted to the patient, indicating that these five loci were present in a single copy. For D9S168, D9S285, and D9S274, both parental markers were inherited by the patient, confirming that these three loci were present in two copies. The results of the remaining five loci, D9S143, D9S129, D9S269, D9S267, and D9S162, were not informative for the copy number in the patient. Thus, it was shown that the deleted chromosome 9 was derived from the father and was missing a region distal to D9S168 (according to the genetic map reported in the Fourth International Workshop on Chromosome 9,[13] D9S168 is the most distal locus among the three loci shown to be present in two copies in the patient).

The 9p deletion was also examined by Southern blotting for D9S47 assigned to the 9p23-24 region.[13] Genomic DNA of the patient and the parents was digested with *Eco*RI, and was hybridised with the probe ovc 2.2 for D9S47.[14] For an internal band intensity control, the same filter was also hybridised with the probe for the TK gene on 17q.[15] Comparison of the band intensity between D9S47 and TK indicated that D9S47 was present in a single copy in the patient (fig 3).

In addition, mutational analysis of SRY was carried out by previously described methods.[16] The SRY sequence was normal in the patient (data not shown).

The infant had ambiguous external genitalia and severely hypoplastic testes, in addition to dysmorphic features compatible with 9p- syndrome,[17] under partial 9p monosomy distal to D9S168. The ambiguous external genitalia would be the result of defective testis formation, because normal testis formation is the pivotal event for male sex development.[18] Thus, although the hormonal studies postnatally were grossly normal and the histological findings were similar to those of age matched patients

Table 1 The results of microsatellite analysis

Locus	Chromosomal location*	Product size (bp)			Copy number of the patient	Reference
		Father	Mother	Patient		
D9S143	9p24.3 (distal)	117	117	117	NI	10
D9S129	9p24.3 (distal-middle)	133	133	133	NI	11
D9S178	9p24.3 (middle) –9p24.1 (middle)	98	96	96	1	12
D9S288	9p24.2 (proximal) –9p24.1 (distal)	132, 136	126, 138	126	1	12
D9S132	9p24.2 (proximal) –9p24.1 (middle)	158, 162	158, 170	170	1	11
D9S281	9p23 (distal)	188, 190	204, 216	216	1	12
D9S286	9p23 (distal)	140, 152	154, 156	154	1	12
D9S168	9p23 (distal-middle)	231, 233	237, 239	233, 237	2	12
D9S269	9p23 (middle)	173	173, 183	173	NI	12
D9S267	9p23 (middle-proximal)	163, 171	163	163	NI	12
D9S285	9p23 (proximal)	126, 128	128	126, 128	2	12
D9S274	9p23 (proximal)	162, 164	162	162,164	2	12
D9S162	9p22	172	172	172	NI	12

*According to the report on the Fourth International Workshop on Chromosome 9.[13] NI=not informative.

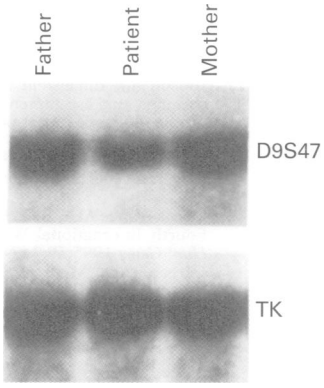

Figure 3 Southern blot analysis. Shown are EcoRI digests hybridised with ovc 2.2 for D9S47 and the probe for the TK gene on 17q (same filter).

with idiopathic cryptorchidism,[19] it is inferred that the severely hypoplastic testes were incapable of producing a sufficient amount of androgens to masculinise the external genitalia in the fetus, especially in the critical period for sex development. In support of this, it has been suggested that, in genetic males, defective androgen production involving the critical period usually results in female external genitalia or ambiguous external genitalia with microphallus, whereas impaired androgen production after the critical period usually leads to male external genitalia with micropenis.[18 20] Although Wolffian ducts were well developed compared with the external genitalia, this is consistent with the notion that a relatively small amount of testosterone allows Wolffian duct development while a fairly large amount of testosterone, which is converted into dihydrotestosterone by 5α-reductase activity in the target tissue, is required to masculinise the external genitalia.[21] Therefore, the results of our patient indicate a link between defective testis formation and partial 9p monosomy distal to D9S168.

Our patient is similar to six previously reported genetic male patients with partial 9p monosomy and impaired male sex development (table 2). The data of the total seven patients, including our patient, can be summarised as follows: (1) monosomy of the 9p24 region is shared in common by all the seven patients; (2) the extent of developmental defects of the external genitalia and sexual ducts is variable among the patients, ranging from an ambiguous intersex phenotype to a nearly complete female phenotype; (3) all four patients examined for gonadal structure have defective testes or dysgenetic gonads; and (4) there is no correlation between the size of the monosomic region and the degree of impaired male sex development. These findings suggest that the monosomic region common to the seven patients is responsible for defective testis formation and resultant impaired male sex development of various degrees.

The relationship between monosomy of distal 9p and defective testis formation is consistent with a testis forming gene(s) residing in the

Table 2 Cytogenetic and clinical findings in patients with partial 9p monosomy and impaired male sex development

Case	Karyotype	Monosomic region	External genitalia	Wolffian structure	Müllerian structure	Gonadal structure	Reference
1	46,XY,der(9) t(9;13)(p21;q21)mat	9p21-pter	Ambiguous (vagina+)	—	Bifid uterus Oviducts	Immature testes*	1
2	46,XY,der(9) t(3;9)(p21;p22.1)mat	9p22.1-pter	Female	—	—	—	2
3	46,XY,der(9) t(7;9)(q31.1;p23)pat	9p23-pter	Female	Remnants	Uterus Oviducts	Hypoplastic testes†	3
4	46,XY,der(9) t(2:9)(p11;p24)de novo	9p24-pter	Female	—	Uterus	—	4
5	46,XY,der(9) t(4;9)(q?;p24?)pat	9p24?-pter	Female	—	—	—	5
6	46,XY,del(9)(p2305) de novo	9p2305-pter	Female	Remnants	Uterus Oviducts	Streak gonads ‡	6
7	46,XY,del(9)(p23) de novo	9p23-pter	Ambiguous (vagina−)	Epididymides Vasa deferentia	—	Hypoplastic testes§	Present case

*Examined at 4 months of age; size unknown; Sertoli cells present; Leydig cells absent; germ cells, no information.
†Examined at 5 months of age; size 7×3×3 mm bilaterally; Sertoli cells present; Leydig cells, no information; germ cells absent.
‡Examined in infancy; size unknown; Sertoli cells absent; interstitial cells present; germ cells absent.
§ Examined at 12 months of age; size, right 8×5×4 mm, left 4×3×2 mm; Sertoli cells present; Leydig cells present; germ cells, present.

distal 9p region.[3 4 6] However, a simple explanation in terms of the gene dosage effect or epigenetic imprinting is difficult for the defective testis formation: (1) most patients with partial 9p monosomy apparently missing the distal 9p region have developed as males in the presence of the Y chromosome[17]; and (2) the origin of the deleted 9p can be both maternal and paternal in patients with impaired male sex development (table 2). Nevertheless, there are three possible explanations as to why the 9p deletion involving such an apparently recessive and non-imprinted gene(s) could lead to impaired testis formation of various degrees. First, haploinsufficiency of the 9p gene(s) may disturb testis formation in several patients who are highly liable to have defective testis development because of genetic and environmental factors other than the 9p gene. Second, the testis forming gene(s) on the cytogenetically normal 9p may be prezygotically mutated.[4 6] Third, the originally intact testis forming gene(s) on the normal 9p may undergo a postzygotic somatic mutation in developing testicular cells, under the loss of heterozygosity condition caused by the 9p deletion.

In summary, the results of our patient provide further support for the previously proposed hypothesis that a gene(s) for testis formation is present on the distal part of 9p and indicate in molecular terms that the putative testis forming gene(s) resides in the region distal to D9S168.

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