Deletion of 9p associated with gonadal dysfunction in 46,XY but not in 46,XX human fetuses


A locus on distal 9p has been reported to be deleted in patients with mental retardation, craniofacial dysmorphic traits, notably trigonocephaly, and a high frequency of genital and/or gonadal anomalies (Alfi’s syndrome). More recently, some cases of 46,XY male to female sex reversal without dysmorphic traits have been described in association with distal deletions of 9p. 46,XY patients described so far have had gonadal anomalies ranging from streak gonads, to partially masculinised dysgenetic gonads, to normal testes. A family of genes containing a unique zinc finger DNA binding domain, which has been called DM, as it is contained in two proteins involved in invertebrate sex determination, doublesex and mab-3, has recently been recognised. Three genes belonging to this family have been identified on 9p24.3, immediately proximal to the centromeric boundary of the minimal region, which is required in two copies for testis development. The first gene identified was termed DMRT1 and the two additional DM gene family members are placed in tandem more distally to the breakpoint of the minimal deleted region. DMRT1 is transcribed only in the embryonic gonads of both sexes and in fetal and adult testis, and is required for postnatal differentiation of both somatic and germ cells in the mouse testis. Therefore, this gene is a good candidate to explain the 46,XY sex reversal associated with 9p deletions. Recently, a 1 year old 46,XX female patient with premature ovarian failure and sex reversal associated with 9p deletions was studied with two BACs containing two markers placed at the two ends of the minimal interval, corresponding to the sex reversal critical region, were not informative (data not shown). No mutations were detected in the coding regions of DMRT1.

Here, we report two cases of human fetuses with deletions of 9p, one with a dysgenetic testis and ambiguous genitalia with a 46,XY karyotype, and the other had a 46,XX karyotype and no gonadal or genital anomalies.

RESULTS

Fetus 1

The second echograph in the pregnancy at 24 weeks showed ambiguous genitalia with clitoral hypertrophy in a fetus with normal growth, morphology, and vitality. The karyotype from amniotic fluid cells showed a 46,XY karyotype and a large deletion in the short arm of chromosome 9 (p22-piger) with R-banding in 37 mitotic cells (fig 1B). Therapeutic abortion was proposed because of the poor prognosis associated with deletions of 9p. In this fetus the deletion of 9p spanned all the four FISH probes (fig 1A, C-E). In addition, we performed conventional microsatellite analysis with markers positioned on 9p. Hemizygosity was ascertained at VLDLR and D9S1813, whereas more distal markers (D9S1858 and D9S1779), corresponding to the sex reversal critical region, were not informative (data not shown). No mutations were detected in the coding regions of DMRT1.

The fetus showed mild craniofacial dysmorphism (fig 2A) and ambiguous genitalia (fig 2B). The internal genitalia showed normal regression of Müllerian ducts and macroscopically normal development of Wolffian derivatives. Two pelvic testes were found with no macroscopic abnormality. Microscopically, one of the testes had a poorly developed albuginea. Two-thirds of the gonad contained well differentiated tubules with Sertoli cells and rare spermatogonia. Leydig cells were present in the interstitium. The remaining one-third of the gonad at one pole was maldeveloped with ill defined, large trabecula that were rich in germ cells with non-centred nuclei and vacuolated cytoplasm. The cells appeared cohesive, thus differing from primitive cords. There was no follicle differentiation in the deeper part. There was no apparent myoid cell layer at the periphery of the cords. WT1 protein was positive in the nuclei of the well developed Sertoli cells as well as in the poorly differentiated part of the testis. PLAP was positive in most spermatoogonia in the well

Immunohistochemistry

Immunohistochemistry was performed on 4 µm sections of formalin fixed gonadal tissue embedded in paraffin. The sections were rehydrated and submitted to antigen retrieval by microwave oven exposure in citrate buffer pH 6 for 2 × 5 minutes at 95°C. Immunostaining was done in a Dako system TM. The primary antibody was SRY monoclonal antibody 1/1000 (a gift from L Salas-Cortes), WT1 rabbit polyclonal antibody 1/100 (Santacruz), and AMH/MIS monoclonal antibody 1/500 (a gift from R Rey). A secondary antibody combined with streptavidin peroxidase amplification was shown by the H2O2-DAB (diaminobenzidine) reaction. Nuclei were counterstained with methyl green followed by dehydration and mounting.

DNA sequencing

The five exons of the DMRT1 gene were amplified from DNA and sequenced with primers as described elsewhere.

METHODS

FISH analysis

To study the subtelomeric region, we used two probes, one PAC (P1 derived artificial chromosome) (RPCI-8J4) containing the DMRT1-3 genes and placed in a cluster located about 700-900 kb from the telomere. An additional BAC probe (bacterial artificial chromosome) (CEPH-B275C7) contains a more telomeric part of band 9p24.3 (at about 250-350 kb), including marker D9S1779, at 0.1 cM. The Alfi syndrome critical region was studied with two BACs containing two markers placed at the two ends of the minimal interval.

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differentiated part, while only a few germ cells were positive in the poorly differentiated one (data not shown). Haematoxylin-eosin staining (HES) is shown for normal and poorly differentiated portions of the testis (fig 3A and C, respectively). AMH/MIS was strongly positive in the well differentiated Sertoli cells (fig 3B), while the staining was less intense, though clearly present, in the poorly differentiated part (fig 3D) with a gradient of positive signal from the normal part to the periphery of the maldeveloped gonad. SRY was positive in the nuclei of some germ cells, mainly in the maldeveloped part. Only a faint positive signal was found in the nuclei of the well developed Sertoli cells (data not shown). The contralateral testis was normal.

**Fetus 2**

The second echograph of pregnancy at 24 weeks in a 47 year old woman was suggestive of hypoplasia of the left heart cavities in addition to a single umbilical artery in a fetus with normal growth. A karyotype with R banding showed a deletion of the short arm of chromosome 9 (p22-pter) in a 46,XX female fetus (fig 4C), leading to therapeutic abortion of the pregnancy. The fetus had normal development of the female external (fig 4B) and internal genitalia. Facial dysmorphism was observed, including hypertelorism, bulging ocular globes, flat nose, low set ears with abnormal auricles, a large mouth, bilateral choanal atresia, and a short neck (fig 4A). Fetal dissection showed heart anomalies involving the two cava vessels, a large pulmonary artery, hypoplasia of the arterial

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**Figure 1** Molecular data on a fetus with genital ambiguities associated with a 46,XY,del(9)(p22-pter) karyotype. (A) Schematic representation of the chromosome 9 short arm; cytogenetic bands are depicted over the bar and microsatellites are indicated underneath. Deleted regions detected by microsatellite and FISH analysis are shown by Δ. (B) Deletion of the chromosome 9 distal short arm detected by karyotype analysis (R banding). (C) Staining of 9p and 9q telomeres (green and red, respectively). (D) FISH results for CEPH-B275C7 BAC probe (0.1 cM). (E) FISH results for RP11-87N24 PAC probe (20.4 cM).
and MIS were detected in the dysgenetic testis, this suggests that the locus on 9p associated with 46,XY sex reversal is required for the maintenance of testis differentiation rather than as a factor in primary sex determination. In addition, analysis of the 46,XX female fetus with a 46,XX,del(9)(p22-pter) karyotype showed apparently normal ovarian development, suggesting that deletion of 9p22-24 does not contain a locus required for early ovarian differentiation. Although we cannot exclude incomplete penetrance for this locus, our findings are in agreement with the phenotype of Dmrt1-/- mice, where XX females are fertile and have normal ovaries.

**DISCUSSION**

We provide the first report of a fetus with sexually ambiguous genitalia associated with a deletion of 9p. The karyotype of the fetus was 46,XY,del(9)(p22-pter) and the gonadal appearance was similar to that obtained in the postnatal testis by targeted inactivation of mouse Dmrt1. Since expression of WT1, SRY,
Figure 3  Immunohistochemistry of fetus 1. (A, B) Normal testicular tissue. (A) HES staining. (B) Anti-AMH antibody staining. (C, D) Polar region of the dysgenetic testis, showing the primitive seminiferous cords. (C) HES staining. (D) Anti-AMH antibody staining.

**Key points**

- Haploinsufficiency of DMRT1 and, possibly, of additional doublesex related genes, termed DMRT2 and DMRT3, is thought to be responsible for 46,XY gonadal dysgenesis associated with deletions of the distal short arm of chromosome 9.
- Recently, a case of deletion of 9p was reported to be associated with premature ovarian failure in 46,XX subjects.
- Here, we report on one fetus with a 46,XY,del(9)(p22-pter) karyotype and ambiguous external genitalia. One testis had poorly differentiated seminiferous tubules at one pole. Immunohistochemical data suggest that the gene(s) on 9p is required for maintenance of testis differentiation rather than for sex determination.
- A second, female fetus with 46,XX,del(9)(p22-pter) showed apparently normal ovarian development, suggesting that deletion of 9p22-24 does not contain a locus required for early ovarian differentiation.
Figure 4  Clinical and molecular data on a fetus with 46,XX,del(9)(p22-pter) karyotype. (A) Lateral view of the head. (B) External genitalia 46,XX. (C) Deletion of the chromosome 9 distal short arm detected by karyotype analysis (R banding). (D) FISH results of RPCI-8J4 PAC probe (0.1 cM). (E, F) Immunohistochemistry of gonadal tissue. (E) HES staining. (F) Anti-AMH antibody (background) staining.
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