Androgen receptor gene mutations in 46,XY females with germ cell tumours

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We present clinical findings and molecular characterization in two patients previously diagnosed as 46,XY female gonadal dysgenesis with germ cell tumour. Both patients showed a female general phenotype with unambiguously female external genitalia and primary amenorrhoea compatible with complete androgen insensitivity syndrome. The first patient, at the age of 31 years, developed a dysgerminoma measuring $8 \times 13 \times 10$ cm in one abdominal testis. Genetic analysis revealed a single nucleotide substitution on exon 4 in the hormone-binding domain of the androgen receptor (AR) gene, resulting in a change of codon 681 GAG (glutamic acid) to AAG (lysine). The second patient, at the age of 17 years, developed a dysgerminoma measuring $12 \times 10 \times 7$ cm in one abdominal testis and gonadoblastoma in the other testis. Genetic analysis showed a point mutation on exon 3 in the DNA-binding domain of the AR gene resulting in a change of codon 607 CGA (arginine) to CAA (glutamine). Arg607-Gln and Arg608-Lys point mutations in the DNA-binding domain of the AR gene have been associated with male breast cancer in partial androgen insensitivity syndrome. A codon 607 mutation in the DNA-binding domain of the AR gene in our patient 2 is associated with early development of germ cell tumour. We suggest regular molecular genetic analysis of the AR gene in 46,XY females with germ cell tumour and androgen insensitivity syndrome to detect differences in the specific regions of AR gene involved in early progression toward oncogenesis of the dysgenetic gonads.

Key words: androgen receptor gene/complete androgen insensitivity syndrome/germ cell tumour/molecular genetics

Introduction

The incidence of androgen insensitivity syndrome (AIS) or testicular feminization syndrome has been estimated at 1:20 400 (Bangsbøll et al., 1992) to 1:62 400 males (Jagiello and Atwell, 1962). Individuals with complete androgen insensitivity syndrome (CAIS) have a 46,XY complement, bilateral testes, female external genitalia, a short, blind-ended vagina, absent or rudimentary Mullerian derivatives, normal breast development, pubertal feminization and slightly increased height over that of normal women. The spectrum of partial androgen insensitivity syndrome (PAIS) can range from individuals with ambiguous external genitalia characterized by phallic enlargement and partial labioscrotal fusion to infertile normal males (Simpson, 1997). The human androgen receptor (AR) gene has been mapped to chromosome Xq11-12 (Brown et al., 1989). The pathogenesis of CAIS involves a defective AR gene and end-organ insensitivity to androgen, and that of PAIS involves a decreased number or qualitative defect of AR gene (Simpson, 1997).

AIS is inherited as a single X-linked recessive disorder. However, the same mutation of the AR gene in different affected 46,XY males can cause variable clinical phenotypes (McPhaul et al., 1992; Tincello et al., 1997). About 5% of dysgerminomas are associated with 46,XY gonadal dysgenesis, 45,X/46,XY mixed gonadal dysgenesis and AIS (Berek et al., 1996). Dysgerminomas or gonadoblastomas develop in about 20 to 30% of 46,XY gonadal dysgenesis patients and about 15 to 20% of 45,X/46,XY mixed gonadal dysgenesis patients, in whom the tumours often arise in the first or second decade (Simpson, 1997). However, the risk of germ cell tumour in CAIS is considerably lower (probably no greater than 5%) and occurs at a later age (after 25 to 30 years of age) than with other male pseudohermaphrodites (Simpson, 1997). The rarity of this condition and the interest in its genetic origin and malignant transformation prompted this presentation.

Materials and methods

Clinical subjects

Case 1, a Chinese female, presented with primary amenorrhoea and a left abdominal mass at 31 years of age. The patient was 158 cm in height and 49 kg in weight. Physical examination showed moderately developed breasts, sparse pubic hair, absence of axillary hair and normal external genitalia (Figure 1). Pelvic examination revealed the absence of the cervix and uterus. The vagina ended in a blind pouch and measured 6 cm in length. The left gonad revealed a firm $8 \times 10$ cm mass with slight tenderness. An abdominal ultrasound (Figure 2A) showed a left solid adnexal mass measuring $11.8 \times 8.4$ cm. A uterus was not visualized. A computer tomography scan study (Figure 2B) revealed a huge, well-encapsulated, pelvic mass with areas of central necrosis. A karyotype was performed, and it revealed a 46,XY complement. Laboratory studies revealed: follicle stimulating hormone (FSH), 18.49 mIU/ml; luteinizing hormone (LH), 86.49 mIU/ml; oestradiol, 30.09 pg/ml; testosterone, 3.16 ng/ml (normal male: 3–10 ng/ml); lactate dehydrogenase (LDH), 784 U/L (normal 90–220 U/L); α-fetoprotein (AFP), 6 ng/ml; β-human chorionic gonadotrophin (β-HCG), 8.26 mIU/ml and CA125, 10.89 U/ml.
Surgical exploration revealed the absence of a uterus, a grossly normal right gonad and a nodal enlarged left gonad measuring 15 × 12 × 10 cm in size (Figure 3A, B). Bilateral gonadectomy, partial omentectomy, bilateral pelvic lymph node dissection and para-aortic lymph node sampling were performed. The frozen section revealed the presence of dysgerminoma (Figure 4A). Histology of the right gonad showed testicular tissue composed of seminiferous tubules without spermatogenesis (Figure 4B). Placental alkaline phosphatase (PALP) positivity was seen in the tumour cell membrane and cytoplasm (Figure 4C). Fluorescent in-situ hybridization on nuclei from tumour cells of dysgerminoma showed an XY complement (Figure 4D). The omentum, pelvic lymph nodes and para-aortic lymph nodes were free of tumour cells. The surgical staging was Ia. The patient was doing well 2.5 years after operation. The LDH level was normal and there was no evidence of metastasis during postoperative follow-up.

Case 2, a Chinese female, presented with primary amenorrhoea and a left abdominal mass at 17 years of age. The patient was 160 cm in height and 46.5 kg in weight. Physical examination showed moderately developed breasts and pubic hair, scant axillary hair, and normal external genitalia. Pelvic examination revealed the presence of a small cervix. The vagina was 7 cm in length. The lower abdomen had a firm tender fetal-head sized mass. An abdominal ultrasound showed a 10 × 14 cm solid intra-abdominal mass. Intravenous pyelography revealed left hydroureter and hydronephrosis. The patient was found to have a karyotype of 46,XY, t(3;10)(p21;q24). Laboratory studies revealed: FSH, 48.1 mIU/ml; LH, 42 mIU/ml; oestriol, 0.132 ng/ml; oestradiol, 0 pg/ml; testosterone, 0.625 ng/ml (normal male: 3–10 ng/ml); prolactin, 7.01 ng/ml; β-HCG, 4.6 mIU/ml; CEA, 0.66 ng/ml; and AFP, 0 ng/ml.

Surgical exploration revealed the presence of a small uterus measuring 5 × 3 × 1.8 cm, bilateral Fallopian tubes, an ovoid shaped right gonad measuring 2 × 1 × 1 cm and a large greyish solid left adnexal mass measuring 12 × 10 × 7 cm (Figure 5A, B). Bilateral gonadectomy was performed. Histology showed dysgerminoma in the right gonad (Figure 6A) and gonadoblastoma in the left gonad (Figure 6B). PALP positivity was seen in the tumour cells (Figure 6C and Figure 6D). Surgical staging revealed no extension of the disease. The patient was doing well 12 years after operation. There was no evidence of metastasis of the disease and the breasts were checked normal during postoperative follow-up.

**DNA analysis**

Genomic DNA was extracted from the peripheral blood of the patients using a standard method (Maniatis et al., 1982). For polymerase chain reaction (PCR), 30 ng of DNA was amplified with primers flanking the coding region of the human AR gene. Ten pairs of specific oligonucleotide primers were used to amplify the exons and their intronic junctions (Lubahn et al., 1989). The amplification was carried on in a thermocycler (Perkin Elmer-Cetus, Foster City, CA, USA) under the following conditions: at 94 °C for 50 s; at 55–58 °C for 55 s; and at 72 °C for 35 cycles. PCR amplicons were analysed by agarose gel electrophoresis. The amplified DNA was then purified by ethanol precipitation and used as templates in cycle sequencing. Sequencing reactions were performed using the Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS (Applied Biosystems Inc., Foster City, CA, USA). Fluorescent dye labelled DNA fragments were then analysed on a semiautomated DNA sequencer (ABI PRISM 377 DNA Sequencer; Applied Biosystem Inc., Foster City, CA, USA). Each DNA fragment was sequenced repetitively in both sense and antisense directions.

**Results**

After the entire AR gene had been sequenced, in the CAG polyglutamine tract, case 1 had 21 repeats and case 2 had 25 repeats which were within normal limits. Additionally, in case 1, there was a single nucleotide substitution on exon 4 in the hormone-binding domain of the AR gene, resulting in a change of codon 681 GAG (glutamic acid) to AAG (lysine) (Figure 7A, B). In case 2, there was a point mutation on exon 3 in the DNA-binding domain of the AR gene resulting in a change of codon 607 CGA (arginine) to CAA (glutamine) (Figure 8A, B).
Figure 3. (A) Surgical exploration of case 1 revealing the absence of a uterus, a grossly normal right gonad (arrow) and a nodular dysgerminoma of the left gonad. (B) Bisected dysgerminoma of the left gonad. Scale bar = 3 cm.

Figure 4. (A) Histology of dysgerminoma of the left gonad in case 1 showing aggregates of large cells separated by connective tissue stroma infiltrated by lymphocytes. The cells are polyhedral and have vesicular nuclei, slightly eosinophilic cytoplasm, and distinguishable borders. (H&E). Scale bar = 200 µm. (B) Histology of the right gonad in case 1 showing testicular tissue composed of seminiferous tubules and numerous Leydig cells in the interstitium. Spermatogenesis is absent. (H&E). Scale bar = 200 µm. (C) The tumour cells of dysgerminoma exhibit positive reactivity with placental alkaline phosphatase in cell membranes and cytoplasm. (PALP). Scale bar = 200 µm. (D) Fluorescent in-situ hybridization with direct-labelled probe specific for chromosome X (orange fluorescence) and chromosome Y (green fluorescence) on nuclei from tumour cells of dysgerminoma show an XY complement. Scale bar = 200 µm.

Discussion

The androgen receptor, a steroid receptor, is a ligand-activated nuclear transcription factor that binds androgens and mediates the biological effects of androgens on the target cells by activating transcription of different androgen-regulated genes (Brinkmann et al., 1992). The receptor has three functional domains, i.e. transcription-regulation, DNA-binding, and hormone-binding domains. The AR gene was cloned in 1988 (Chang et al., 1988; Lubahn et al., 1988; Trapman et al., 1988; Lubahn et al., 1989; Tilley et al., 1989). The gene is composed of eight exons, of which exon 1 encodes the N-terminal transcriptional activation domain, exons 2 and 3 encode the DNA-binding domain comprising two zinc binding motifs, and exons 4–8 encode the hormone-binding domain. In a normal population, a series of CAG repeat sequences, ranging from 11 to 31 repeats, are located at a polymorphic region in exon 1 of the AR gene (Patterson et al., 1994a). Enlargement...
of the CAG repeat numbers, ranging from 40 to 52 or more than six standard deviations above the normal mean CAG repeat numbers, has been linked to spinal and bulbar muscular atrophy or Kennedy’s disease (La Spada and Fischbeck, 1991; La Spada et al., 1991). The size of the CAG repeat in our cases is within normal limits. Our case 1 had a mutation in the hormone-binding domain, the most common site of AR mutations. Mutations in this region can change the androgen binding affinity and/or specificity of AR. Our case 2 had a mutation in the DNA-binding domain. Mutations in this region can result in the inability of AR to bind DNA and activate gene transcription.

The development of the gonadoblastoma has been associated with a Y chromosome gene, GBY, in dysgenetic gonads (Page, 1987), loss of heterozygosity for a tumour suppressor gene, RB1 (Antonini et al., 1997), and 9p24 monosomy (McDonald et al., 1997). Jørgensen et al. (1997) suggested that germ cell tumours associated with gonadoblastoma arise from carcinoma in-situ cells inside the gonadoblastoma nests. The present report concerns the risk of developing germ cell neoplasia among patients with different mutations in the AR gene. The risk of neoplasia is low below 25 to 30 years of age in patients with CAIS, however, our case 2 developed germ cell tumour at the age of 17. This case raises the question of whether a codon 607 mutation in the DNA-binding domain of the AR gene in CAIS is associated with an increased risk of early development of germ cell tumour. Zinc finger motifs in the DNA-binding domain have been demonstrated to be important for positive control of transcription (Schena et al., 1989). Arg607 and Arg608 have been proved to be partially surface-exposed and located in adjacent areas in the AR DNA-binding domain (Poujol et al., 1997). These two arginines are involved in protein–protein interactions and are important for the correct functionality of the AR gene (Poujol et al., 1997). Arg607–Gln and Arg608–Lys point mutations in the DNA-binding domain of the AR gene have been associated with male breast cancer (Wooster et al., 1992; Lobaccaro et al., 1993; Poujol et al., 1997). Several point mutations in the hormone-binding domain have also been reported in prostate cancer tissues (McKusick, 1998). Whether codon 607 mutation in the DNA-binding domain has a potentially worsening effect of early development of germ cell tumour is unknown and deserves further attention. In particular, more work is required to determine if different mutations in the AR gene correlate with the development of germ cell tumour in CAIS.

Our case 2 had a t(3;10) balanced translocation with two breakpoints 3p21 and 10q24 harbouring tumour suppressor genes. Loss of heterozygosity on chromosome 3p has been reported in various cancers, including lung, renal, breast, ovarian, testicular, cervical, and head and neck cancer (Latif et al., 1997). Lothe et al. (1989) suggested that loss of 3p or 11p alleles is associated with testicular cancer tumours. Several genes assigned to 3p21 have been analysed as potential candidates for tumour suppressor genes, i.e. ACY1 (Kok et al., 1997), CNAI2 (Magovcevic et al., 1992), CTNNB1 (Kok et al., 1997), GPX-1 (Moscow et al., 1994), hMLH1 (Papadopoulos et al., 1994; Benachenhou et al., 1998), ITGA4L (Hibi et al., 1994), NY-LU-12 (Güre et al., 1998), SEMA III/F (Xiang et al., 1996), SEMA IV (Roche et al., 1996), SEMA V (Sekido et al., 1996) and UBE1L (Kok et al., 1995). Deletions of chromosome 10q have also been described in gliomas, malignant meningiomas, endometrial carcinomas, and melanoma (Petersen et al., 1997). Various genes on 10q24 have been associated with neoplastic evolution, i.e. FGF-8 (Payson et al., 1996), HOX11 (Lichty et al., 1995) and MXII (Eagle et al., 1995). The chromosomal translocation on the segments 2p21 and 10q24 might involve certain tumour suppressor genes and possibly contributed to early tumour formation of our case 2.

To date, at least 200 point mutations of the AR gene have been described and identified in all eight exons of the AR gene, most commonly in the hormone-binding domain and the DNA-binding domain (Quigley et al., 1995; Hiort et al., 1996). Glu681–Lys has been reported in patients with CAIS and Arg607–Gln in patients with partial AIS (Hiort et al., 1996). It has been shown that phenotypic diversity exists in subjects within an affected family with AIS despite the same AR gene mutation (Batch et al., 1993; Evans et al., 1997). Therefore, molecular alteration of the AR gene cannot reliably predict the phenotypic presentation of affected patients (Wiener et al., 1997). In this study, unlike previous reports, our patient 2 with Arg607–Gln presented with CAIS and Müllerian duct remnants. The timing for gonadal extirpation in patients with CAIS remains controversial. Traditionally, the testes are left in place after pubertal feminization because the risk of early development of germ cell tumour in CAIS is rare (Simpson, 1997). But some investigators recommend orchidectomy as
Figure 6. (A) Histology of dysgerminoma of the left gonad in case 2 showing islands of uniform tumour cells surrounded by connective tissue stroma containing lymphocytes. (H&E). Scale bar = 200 µm. (B) Histology of gonadoblastoma of the right gonad in case 2 showing cellular nests surrounded by connective tissue stroma. The cellular nests contain a mixture of germ cells, with granular cytoplasm and round vesicular nuclei, and immature Sertoli and granulosa cells surrounding small round spaces that resemble Call-Exner bodies. Small foci of calcification are seen in central area. (H&E). Scale bar = 200 µm. (C) The tumour cells of dysgerminoma show positive reactivity with placental alkaline phosphatase (PALP). Scale bar = 200 µm. (D) The tumour cells of gonadoblastoma nests reveal positive reactivity with placental alkaline phosphatase (PALP). Scale bar = 200 µm.

Figure 7. (A) P1: the mutant sequence of case 1 showing change of codon 681 GAG (glutamic acid) to AAG (lysine). The arrow indicates the mutant nucleotide (G→A). (B) N1: the normal sequence.

Figure 8. (A) P2: the mutant sequence of case 2 showing a change of codon 607 CGA (arginine) to CAA (glutamine). The arrow indicates the mutant nucleotide (G→A). (B) N2: the normal sequence.
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soon as CAIS is diagnosed (Shah et al., 1992; Patterson et al., 1994b; Wiener et al., 1997). Testicular carcinoma in situ has been diagnosed in children with AIS (Müller and Skakkebæk, 1984) and invasive germ cell tumour has been reported in pubertal patients with AIS (Hurt et al., 1989). In view of the possibility of providing useful information for therapeutic decisions regarding early orchietomy in infants or young children with high risk AR gene mutations, such as Arg607–Gln in this presentation, we suggest regular molecular genetic analysis of the AR gene in 46,XY females with germ cell tumour and AIS.

References


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