J. Steroid Biochem. Molec. Biol. Vol. 43, No. 7, pp. 659–663, 1992
Printed in Great Britain.

0960-0760/92 $5.00 + 0.00

PRENATAL PREDICTION OF ANDROGEN INSENSITIVITY SYNDROME USING EXON 1 POLYMORPHISM OF THE ANDROGEN RECEPTOR GENE

JEAN MARC LOBACCARO,[1] SERGE LUMBROSO,[1] FRANÇOISE CARRÉ PIGEON,[2] JEAN-LOUIS CHAUSSAIN,[3] JEAN-EDMOND TOUBLANC,[3] JEAN-CLAUDE JOB,[3] GEORGES OLEWNICZACK,[4] PIERRE BOULOT[5] and CHARLES SULTAN[1,6*]

[1]Unité de Biochimie Endocrinienne du Développement et de la Reproduction, Hôpital Lapeyronie et Institut National de la Santé Et de la Recherche Médicale, INSERM Unité 58, Montpellier, [2]CREBIOP, CHRU, Reims, [3]Endocrinologie Pédiatrique, Service de Pédiatrie, Hôpital St Vincent de Paul, Paris, [4]Service de Pédiatrie et Médecine Néonatale, CH Soissons, [5]Service de Gynécologie-Obstétrique, Maternité, Montpellier and [6]Endocrinologie et Gynécologie Pédiatriques, Service de Pédiatrie I, Hôpital St Charles, Monpellier, France

(Received 17 May 1992)

Summary—Exon 1 polymorphism of the androgen receptor (AR) gene is characterized by a $(CAG)_n(CAA)$ repeat at position 172 following the translation start codon. The aim of this study was to determine whether AR gene exon 1 polymorphism could be used to perform prenatal diagnosis in high risk families with complete or partial androgen insensitivity syndrome. After enzymatic amplification of a 1 kilobase exon 1 fragment, each DNA was simultaneously digested by MspI and PstI restriction enzymes. After electrophoresis on a 15% acrylamide gel or a 6% Nusieve gel, we measured the size of the obtained fragments and determined the number of CAG repeats since a 282 basepair fragment corresponds to 21 CAG. We previously showed that the number of CAG repeats within the AR gene exon 1 in 23 families with complete or partial androgen insensitivity syndrome was 19 ± 4. By this method, we detected heterozygosity in 50% of the mothers. We present here 2 exclusion prenatal diagnoses using exon 1 polymorphism of the AR gene. Family A presented a boy with a severe form of partial androgen insensitivity syndrome. The mother had 2 uncles with ambiguous genitalia. In family B, the affected child had a complete androgen insensitivity syndrome. In both families, analysis of the AR gene exon 1 polymorphism of the trophoblastic DNA showed the presence of the normal maternal X chromosome. The parents decided to carry on the gestation. In family A, the newborn had normal male external genitalia. In family B, sonography confirmed the presence of normal male external genitalia. These data suggest that exon 1 polymorphism of the AR gene could be prenatally used to predict androgen insensitivity syndrome.

INTRODUCTION

Androgen insensitivity syndromes (AIS) are X-linked disorders which result from defects in the intracellular androgen action on target cells [1]. Phenotypic expression of AIS is quite variable ranging from the complete female phenotype to the apparently normal but infertile male [2]. Complete AIS (CAIS) is characterized clinically by a female phenotype. The clinical expression of partial AIS (PAIS) covers a wide spectrum: from undermasculinized males to apparently normal males with infertility or hypofertility [3]. In both cases, there is increased production of testosterone and luteinizing hormone (LH).

Quantitative and qualitative analysis of androgen receptors (AR) in genital skin fibroblasts from patients with PAIS or CAIS have proven to be a useful method for studying these defects in androgen activity [4]. AR are undetectable in most CAIS [2] and are in low concentration in PAIS [4].

The study of restriction fragment length polymorphism (RFLP) and enzymatic amplification (PCR) of the AR gene from complete or partial AIS patients showed that AR gene deletions are infrequent [5–9]. However, some mutations have been described within the AR gene [7].

Screening of carriers and prenatal diagnosis of AIS in high-risk families is impossible unless

*To whom correspondence should be addressed at: Unité de Biochimie Endocrinienne du Développement et de la Reproduction, Hôpital Lapeyronie, 34059 Montpellier Cedex, France.

the mutation is described. It is thus necessary to look for AR gene polymorphism in order to differentiate the two maternal X chromosomes and characterize the affected allele. Two AR gene polymorphisms have been described. Brown *et al.* [10] showed a moderate frequency HindIII polymorphism which was proved to be useful to differentiate both maternal X chromosomes in a family with PAIS [11]. Lubahn *et al.* [12] described a polymorphic tandem CAG repeat in the first exon of the AR gene. This exon 1 polymorphism is more efficient for carrier diagnosis since it can be used for heterozygosity detection in 50 to 89% of the women [13, 14].

We report here prenatal prediction of AIS using exon 1 polymorphism of the AR gene on the trophoblastic DNA in 2 families.

EXPERIMENTAL

Families

The *family A* (Fig. 1) presented a boy with a PAIS (newborn ambiguous genitalia) with decreased number of AR binding capacity determined on cultured genital skin fibroblasts ($B_{max} = 316$ fmol/mg DNA; $N = 650 \pm 200$ fmol/mg DNA). The mother had 2 uncles with severe ambiguous genitalia. We analyzed the DNA of the affected child, his mother and one of his maternal uncles. The second one was deceased.

In *family B*, the 46,XY affected child presented a CAIS (female phenotype) with negative AR binding capacity. We analyzed the DNA of the child and his mother.

The RFLP of the AR gene of the 2 families were previously studied as family No. 11 and 2, respectively, in [9]. Using the three AR cDNA probes spanning the coding region, no deletion was found within the AR gene. Furthermore, the two mothers were homozygous for the HindIII polymorphism.

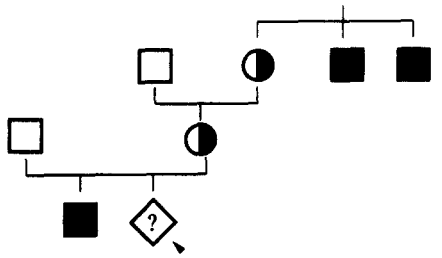

Fig. 1. Family A pedigree. The fetus is indicated by an arrow.

Trophoblasts were biopsed under ultrasound guidance at a gestational age of 14 weeks. The fetal sex determination was rapidly determined using the PCR of the SRY gene as described previously [15]. SRY is known to be equated to the testis determining factor [16].

Exon 1 polymorphism study

Isolation of DNA. Genomic DNA was prepared from peripheral (20–30 ml) white blood cells by LSN lysis (LSN: 0.3 mM lithium acetate, 1 mM Na_2–EDTA, 10 mM Tris–HCl, pH 8.0, 2% SDS), phenol/chloroform extraction, ethanol precipitation and TE (10 mM Tris–HCl pH 8.0, 1 mM EDTA) resuspension [11]. Trophoblastic DNA was extracted with the same method except for overnight digestion with 100 μg/ml of proteinase K (Boehringer Mannheim, Germany). The source of reagents was Carlo Erba (Italy).

Enzymatic amplification. PCRs were performed in a final volume of 50 μl using 500 ng of DNA, 150 ng of each primer (L11, 5′ AGCCAAGCTAAGGATGGAA3′; L18, 5′ GGCCCTGTGAACTTGACGGCA3′) [13] (Fig. 2), 200 μM of each nucleotide (Pharmacia, France), 1.5 mM $MgCl_2$ 10 mM Tris (pH 8.3), 50 mM KCl, 0.01% gelatin and 0.2 U of the enzyme Taq polymerase (Promega, Coger, France). Reactions were cycled for 1 min at 95°C (denaturation), 1 min at 58°C (annealing) and 2 min at 72°C (DNA synthesis) for 35

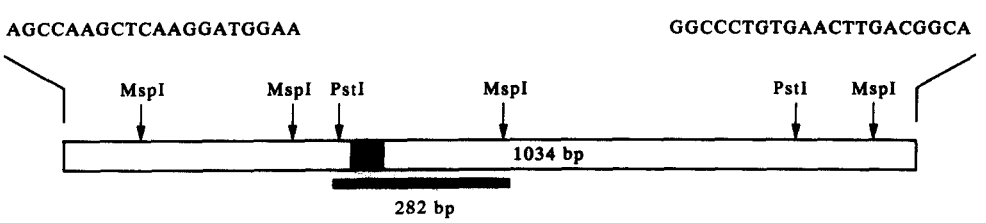

Fig. 2. Schematic representation of the PCR amplification. Digestion of the amplified fragment by PstI and MspI. The fragment containing the CAG repeat is theoretically 282 bp long. Sizes are indicated in basepairs (bp).

cycles. 8 μl of the amplified sample was submitted to electrophoresis on a 1.2% agarose gel stained with ethidium bromide to verify the length of the fragments. Electrophoresis was carried out at 100 volts for 1 h.

Exon 1 polymorphism study. After enzymatic amplification each DNA (10 μl reaction aliquot) was simultaneously digested by MspI and PstI restriction enzymes (Boehringer) to obtain one 535 basepair (bp), one 122 bp, one 40 bp, two 20 bp, one 15 bp and one 282 bp restriction fragment which contains the polymorphic tandem CAG repeat. Fragments were electrophoresed on a 15% polyacrylamide gel in 1 × TBE buffer (5 × TBE: 0.45 M Tris–Borate, 0.01 M Na_2–EDTA, pH 8.0) or on a 6% Nusieve gel (Tébu, France) in 1 × TBE. Acrylamide gel electrophoresis was carried out using a system designed by Biorad with a 23°C recirculating bath for a total of 4500 V.h. The gel was stained with ethidium bromide and photographed under u.v. light to determine the mobility of each DNA fragment.

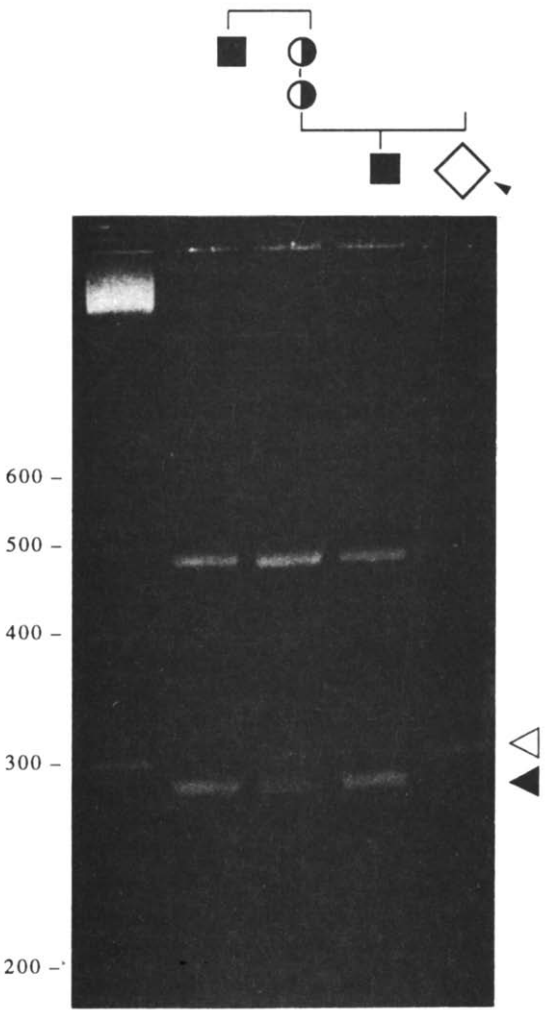

Fig. 3. Acrylamide gel analysis of the digested exon 1 PCR of family A. Sizes are indicated in basepairs (bp). The fetus is indicated by an arrow. Affected allele is indicated by a black arrow.

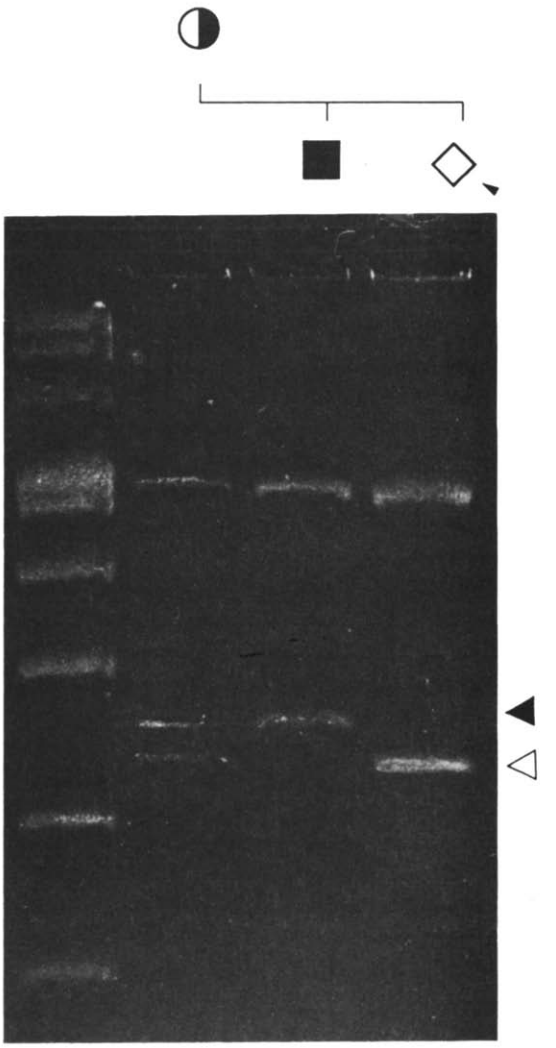

Fig. 4. Acrylamide gel analysis of the digested exon 1 PCR of family B. The fetus is indicated by an arrow. Affected allele is indicated by a black arrow. Unaffected allele is indicated by an open arrow.

RESULTS

After the 4500 V.h electrophoresis, only the 535 and 282 bp fragments remained on the acrylamide gel. It was thus easier to measure the size of the obtained fragments and to determine the number of CAG since, according to Lubahn *et al.* [12], the 282 bp fragment corresponds to 21 CAG. To control the efficiency of our method, the size of the CAG polymorphic fragment in control DNAs were confirmed by DNA sequencing. Heterozygous women are defined by the presence of two separate fragments.

Prenatal diagnosis of AIS

The PCR of the opening reading frame of the SRY gene showed the canonical 648 bp fragment indicating within 24 h that the fetal karyotype was 46,XY.

The exon 1 polymorphism study allowed us to distinguish the two X maternal chromosomes.

In family A, we determined that the hit X chromosome was associated with the higher mobility fragment obtained after PCR and double digestion comparing the affected child's, mother's and uncle's patterns (Fig. 3). Using enzymatic amplification on trophoblastic DNA, we detected the presence of the lower mobility fragment associated with the normal X chromosome. The parents thus decided to carry on the gestation. Four weeks later, sonography confirmed the presence of normal male external genitalia. The mother has just given birth to a male newborn with definitely normal male external genitalia.

In family B, the mother was heterozygous for the exon 1 polymorphism. We determined that the hit X chromosome was associated with the lower mobility fragment (Fig. 4). On the trophoblastic DNA, we showed the presence of the higher mobility fragment suggesting the fetus was not affected. At 22 weeks of age, sonography showed normal male external genitalia (well formed scrotum and a 5 mm length penis).

DISCUSSION

In the first published sequence of the androgen receptor gene, Lubahn *et al.* [12] surprisingly observed one group of repeated amino acids varied in length within the human species. The 21 glutamines, encoded by CAG, in the human AR were observed in two cDNA clones, while there were 25 repeated glutamines at the same location in the genomic clone. Since these clones were isolated from independent libraries prepared from DNA of different individuals, Lubahn *et al.* [12] concluded that the discrepancy in glutamine number represented allelic polymorphism. Faber *et al.* [17] confirmed the presence of this polymorphism. This exon 1 polymorphism was recently used by LaSpada *et al.* [18] to investigate whether the AR gene was a candidate for X-linked spinal and bulbar muscular atrophy. The authors found variation from published control sequence only in the first exon of the gene where Lubahn *et al.* [12] described the polymorphic tandem CAG. This repeat fell within the coding domain of the gene, corresponding to a long tract of glutamine residues beginning at position 58. LaSpada *et al.* observed a mean Gln of 21 ± 2 (mean $\pm$ SD) (range 17–26 Gln) in the 75 studied controls, while the patients affected by X-linked spinal and bulbar muscular atrophy showed a significantly larger number of Gln. Edwards *et al.* [cited in 18] reported similar results in 228 X control chromosomes.

We previously reported [13] the study of the exon 1 polymorphism among 23 families with AIS: no difference between the control X chromosomes and hit X chromosomes (20 ± 5 vs 19 ± 4 gln) and no correlation between the amount of CAG and the clinical expression of AIS were detected. Moreover, the heterozygosity of females was 0.5. Sleddens *et al.* [14] recently described 11 different allele products among 63 chromosomes. The heterozygosity of the females was 0.89. A different ethnical origin of the families could explain this discrepency: our population was mainly of Mediterranean origin (Latins and North-Africans). A difference in the methodology developed to separate two very close alleles could not be ruled out however.

Prenatal diagnosis of AIS in high risk families is theoretically possible by studying the quantity of AR in amniotic fluid cells [19]. Actually molecular diagnosis is much more efficient. As long as the mutation of the AR gene responsible for the AIS is not found, prenatal diagnosis is impossible. Recent demonstration of HindIII polymorphism associated with PAIS [11] or CAIS [8] permits diagnosis of carriers. This HindIII RFLP is time-consuming and expensive and its application is limited because of its low frequency, only 18% of the women being heterozygous. On the other hand we had found that exon 1 polymorphism study could be used for prenatal diagnosis in 50% of the studied families [13]. This only requires PCR, enzymatic digestion and acrylamide running gel electrophoresis and can thus be performed in 48 h, together with the PCR of the SRY gene for sex determination.

In these 2 families, the pregnant mothers were ready to abort if the fetal karyotype was 46, XY. The exon 1 polymorphism permitted us to characterize the hit chromosome and to determine that the fetus carried the normal X chromosome suggesting he was not affected. In family A, the newborn actually had normal

male external genitalia. In family B, sonography indicated a normal male differentiation.

Although recombination of both maternal X chromosomes is theoretically possible and the prenatal prediction requires investigation of a brother's and/or cousin's or uncle's pattern, we suggest that AR exon 1 polymorphism analysis could be applied to define carriers and perform prenatal diagnosis in some high risk families.

REFERENCES

1. Brown T. R. and Migeon C. J.: Androgen receptors in normal and abnormal male sexual differentiation. In *Steroid Hormone Resistance: Mechanisms and Clinical Aspects* (Edited by Chrousos, Loriaux, Lipsett). Plenum Press, New York (1986) pp. 227–256.
2. French F. S., Lubahn D. B., Brown T. R., Simental J. A., Quigley C. A., Yarbrough W. G., Tan J. A., Sar M., Joseph D. R., Evans B. A. J., Hughes I. A., Migeon C. J. and Wilson E. M.: Molecular basis of androgen insensitivity. *Recent Prog. Horm. Res.* **46** (1990) 1–38.
3. Amrheim J. A., Mayer W. J. III, Jones H. W. and Migeon C. J.: Androgen insensitivity in man: evidence for genetic heterogeneity. *Proc. Natn. Acad. Sci. U.S.A.* **73** (1976) 891–894.
4. Sultan Ch., Picard J. Y., Josso N., Migeon C. J.: Incomplete androgen insensitivity syndrome. *Clin. Endocr.* **19** (1983) 565–574.
5. Brinkmann A. O., Kuiper G. G. J. M., Ris-Stalper C., van Rooij H. C. J., Romalo G., Trifiro M., Mulder E., Pinsky L., Schuweikert H. U. and Trapman J.: Androgen receptor abnormalities. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 349–352.
6. DiLauro S. L., Behzadian A., Tho S. P. T. and McDonough P. G.: Probing genomic deoxyribonucleic acid for gene rearrangement in 14 patients with androgen insensitivity syndrome. *Fert. Steril.* **55** (1991) 481–485.
7. Griffin J. E.: Androgen resistance—The clinical and molecular spectrum. *New Engl. J. Med.* **326** (1992) 611–618.
8. Quigley C. A., Friedman K. J., Johnson A., Lafreniere R. G., Silverman L. M., Lubahn D. B., Brown T. R., Wilson E. M., Willard H. F. and French F. S.: Complete deletion of the androgen receptor gene: definition of the null phenotype of the androgen insensitivity syndrome and determination of carrier status. *J. Clin. Endocr. Metab.* **74** (1992) 927–933.
9. Lobaccaro J. M., Belon C., Chaussain J. L., *et al.*: Molecular analysis of the androgen receptor gene in 52 patients with complete or partial androgen insensitivity syndrome: a collaborative study. *Horm. Res.* **37**: (1992) 54–59.
10. Brown C. J., Goss S. J., Lubahn D. B., Joseph D. R., Wilson E. M., French F. S. and Willard H. F.: Androgen receptor locus on the human X chromosome: regional localization to Xq11-12 and description of a DNA polymorphism. *Am. J. Hum. Genet.* **44** (1989) 264–269.
11. Lobaccaro J. M., Belon Ch., Ruiz-Pacheco R., Heinrichs C., van Regemorter N., Terraza A. and Sultan Ch.: Genetic association of HindIII polymorphism with the androgen receptor gene in partial androgen insensitivity syndrome. *Ann. Génét.* **34** (1991) 9–13.
12. Lubahn D. B., Joseph D. R., Sar M., Tan J., Higgs H. N., Larson R. E., French F. S. and Wilson E. M.: The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. *Molec. Endocr.* **2** (1988) 1265–1275.
13. Lobaccaro J. M., Lumbroso S. and Sultan Ch.: Analyse des polymorphismes du gène du récepteur des androgènes: application au dépistage de l'hétérozygotie et au diagnostic anténatal des syndromes d'insensibilités aux androgènes. *C. R. Soc. Biol.* **185** (1992) 422–433.
14. Sleddens H. F. B. M., Oostra B. A., Brinkmann A. O. and Trapman J.: Trinucleotide repeat polymorphism in the androgen receptor gene (AR). *Nucleic Acids Res.* **20** (1992) 1427.
15. Medlej R., Lobaccaro J. M., Berta P., Belon Ch., Leheup B., Toublanc J. E., Weill J., Chevalier C., Dumas R. and Sultan Ch.: Screening for Y derived sex determining gene SRY in 40 patients with Turner syndrome. *J. Clin. Endocr. Metab.* (1992) In press.
16. Berta P., Hawkins J. R., Sinclair A. H., Taylor A., Griffiths B. L., Goodfellow P. N. and Fellous M.: Genetic evidence equating SRY and the testis-determining factor. *Nature* **348** (1989) 448–450.
17. Faber P. W., Kuiper G. G. J. M., van Rooij H. C. J., van der Korput J. A. G. M., Brinkmann A. O. and Trapman J.: The N-terminal domain of the androgen receptor gene is encoded by one large exon. *Molec. Cell. Endocr.* **61** (1989) 257–262.
18. La Spada A. R., Wilson E. M., Lubahn D. B., Harding A. E. and Fischbeck K.: Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352** (1991) 77–79.
19. Sultan Ch., Emberger J. M., Devillier C., Chavis C., Terraza A., Descomps B. and Jean R.: Specific 5α DHT receptor and 5α reductase activity in human amniotic fluid cells. *Am. J. Obstet. Gynec.* **150** (1984) 956–958.