

A new de novo mutation (A113T) in HMG box of the SRY gene leads to XY gonadal dysgenesis

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Abstract

We describe a new point mutation in the SRY gene of a Chinese XY female with gonadal dysgenesis (Swyer syndrome). Using the double stranded DNA cycle sequencing method, a single nucleotide substitution of G→A was identified at codon 113 of the patient's SRY gene, resulting in a conservative amino acid change from alanine (A) to threonine (T) at a residue that lies within the putative DNA binding motif. With this mutation, one *MnII* recognition site is abolished and a new *BsmAI* site is present in the DNA sequence of the SRY gene; therefore, it is easily detected by analysis of the digestion of the amplified SRY DNA fragment on an electrophoretic agarose gel. In situ hybridisation to the XY female's chromosomes showed that her mutant SRY gene was indeed located on the short arm of her Y chromosome. The SRY mutation in the XY female reported here occurred de novo, as sequence analysis showed that it was not present in her father or other family members.

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The development of a eutherian mammal as a male is the consequence of testis formation in the embryo, which is thought to be initiated by a gene on the Y chromosome, known as TDF (testis determining factor) in humans.^{1,2} Recently, this gene has been found to be close to the pseudoautosomal boundary of the Y chromosome and has been termed SRY, for sex determining region of the Y.³⁻⁵ SRY encodes a small protein with homology to a conserved DNA binding motif known as the HMG (high mobility group) box. Direct genetic evidence for SRY being the TDF was obtained from the analysis of de novo mutations of SRY in XY females with gonadal dysgenesis.^{6,7} Further evidence derives from experiments with XX mice, transgenic for mouse Sry, leading to testicular development and a subsequent male phenotype.⁸

In this article, we describe a new point mutation in the HMG box motif of SRY in a Chinese XY female with gonadal dysgenesis (Swyer syndrome). This mutation resulted in a conservative amino acid change from alanine to threonine at a residue that lies within the putative DNA binding motif. This mutation was not present in the patient's father, sister, or uncle. Thus, the mutation occurred de novo. The results strongly suggest that this de novo mutation in the SRY gene was associated with sex inversion in the XY female.

Materials and methods

CASE REPORT

The XY female patient was referred to Shanghai Institute of Medical Genetics at the age of 19 with primary amenorrhoea owing to hypergonadotrophic hypogonadism and absence of secondary sexual characteristics. She was 170 cm in height (taller than the mean height of Chinese females) and her external genitalia were those of a normal female. Cytogenetic analysis of peripheral lymphocytes showed a 46,XY karyotype with no evidence of mosaicism. C banding confirmed a Y chromosome. Endocrinological studies showed: FSH > 80 mIU/ml, LH 37.3 mIU/ml, PRL 15.5 ng/ml, E2 < 20 pg/ml, testosterone 0.37 ng/ml, T3 1.1 ng/ml, T4 12.9 ng/ml. Streak gonads and an aplastic uterus were diagnosed at laparotomy. Histological examination of the right streak gonad disclosed gonadoblastoma. The patient was diagnosed as having XY gonadal dysgenesis (Swyer syndrome).

Her parents were healthy and unrelated with normal karyotypes. She had a healthy 13 year old sister. Paternity in the family was confirmed by RBC antigen and HLA typing.

METHOD

Genomic DNA was extracted from the leucocytes of the XY female, her parents and sister, as well as 10 normal male and female controls. Referring to the nucleotide sequence of the human SRY gene, two sets of primers, named A, B and C, D were designed within the SRY open reading frame, amplifying 217 bp and 226 bp fragments, respectively. The primer sequences are: A: 5'-CCATGAACGCATTCATCGTGTG-3', B: 5'-GCCTTCCGACGAGGTCGATACT-3', C: 5'-GAGATCAGCAAGTAGCTGGGA-3', D: 5'-ACAACCTGTTGTCCAGTTGC-3', which were synthesised in an Applied Biosystems DNA synthesiser. The modification of DNA amplification by PCR with *Taq* DNA polymerase (Boehringer Mannheim) was performed according to a method described elsewhere.^{9,10}

The PCR products of the XY female, her father, and normal males were subjected to direct sequencing of the SRY DNA sequence using a double strand (ds) DNA cycle sequencing method¹¹ according to the protocol recommended by BRL Life Technologies Inc. In brief, 1 pmol of the sequencing primer (5'-CCATGAACGCATTCA-TCGTGTG-3') was end labelled with γ -³²P ATP (> 5000 Ci/mmol, ICN) by 1 U of T4 polynucleotide kinase (BRL) at 37°C for 30 minutes and then at 55°C for five minutes to stop the reaction. Then

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50 fmol of template DNA were prereacted with 1 pmol of labelled primer, 4.5 μ l of 10 \times sequencing buffer (Tris-HCl, pH 9.0, 300 mmol/l, MgCl₂, 50 mmol/l, KCl 300 mmol/l, W-1 0.5%), and 2.5 U of *Taq* DNA polymerase (Cetus). The total volume of the pre-reaction mixture was adjusted to 36 μ l with distilled water and 8 μ l of the above pre-reaction mixture was individually distributed into four 0.5 ml Eppendorf tubes containing ddNTP termination mixes and covered with 10 μ l of mineral oil. Thirty PCR cycles were then performed in a Perkin-Elmer Cetus DNA Thermal Cycler: denaturing at 90°C for one minute, annealing at 55°C for one minute, and extending at 70°C for two minutes. The reaction tubes were carefully removed from the incubation chamber and the reactions terminated by adding 5 μ l of stop solution (95% v/v) formamide, 10 mmol/l EDTA (pH 8.0), 0.1% (w/v) bromophenol blue, 0.1% (w/v) xylene cyanol to each tube. The reaction tubes were heated at 90°C for five minutes and then subjected to 8% polyacrylamide/TEB-urea gel electrophoresis at 27 mA for three hours. Autoradiography of the dried gels was performed for 24 hours at room temperature without an intensifying screen.

For further confirmation of the specific mutation in the SRY DNA sequence, 15 μ l of the amplified SRY DNA fragments from the XY female, her father, and normal males were digested with restriction endonuclease *Mnl*I or *Bsm*AI (New England Biolabs) according to the manufacturer's recommendation.

Results

SRY sequences were amplified by PCR from DNA of the XY female, her father, and normal

male controls. Specific amplified DNA bands of 217 bp or 226 bp were shown on the electrophoretic gels when using primer A+B or C+D, respectively, while samples from the mother, sister, and normal female controls showed no corresponding amplified fragment. Direct sequence analysis of the SRY HMG box motif of the patient showed that a single nucleotide substitution of G→A had occurred at position nt747 of the SRY gene in the XY female (fig 1), resulting in a conservative amino acid change from alanine (A) to threonine (T) at codon 113 (A113T), a residue that lies within the HMG box motif. This is a new, previously undescribed SRY mutation, which occurred de novo, as sequence analysis showed that it was not shared by her father or other family members.

While the A113T mutation, one *Mnl*I recognition site was abolished and a new *Bsm*AI site was present in the mutant SRY DNA sequence. The results of *Mnl*I and *Bsm*AI mapping of the amplified DNA are shown in fig 2 and fig 3, respectively. In situ hybridisation to the XY female's chromosomes indicated that her SRY gene was located on the short arm of the Y chromosome (data not shown).

Discussion

The initiation of human male or female sexual development depends on the presence or absence of the Y chromosome,^{12,13} more specifically on a gene on the short arm of the Y chromosome encoding a testis determining factor (TDF). By exploring detailed maps of

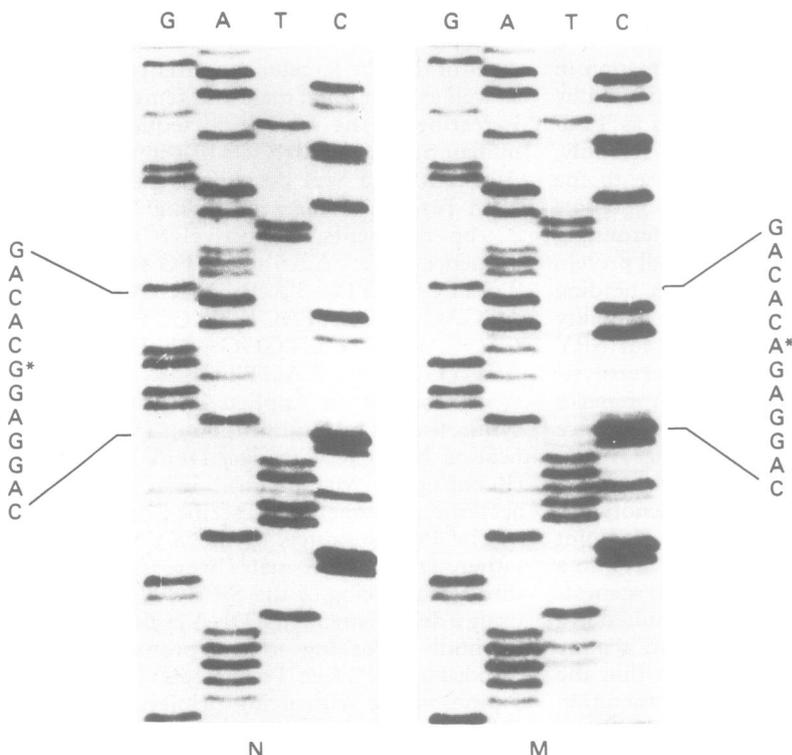


Figure 1 Sequence analysis of human SRY gene using double strand DNA cycle sequencing method. The section involving the mutation in the sequence from the XY female's mutant allele (M) is compared with the corresponding section in the sequence from the normal allele (N). * indicates the mutant site.

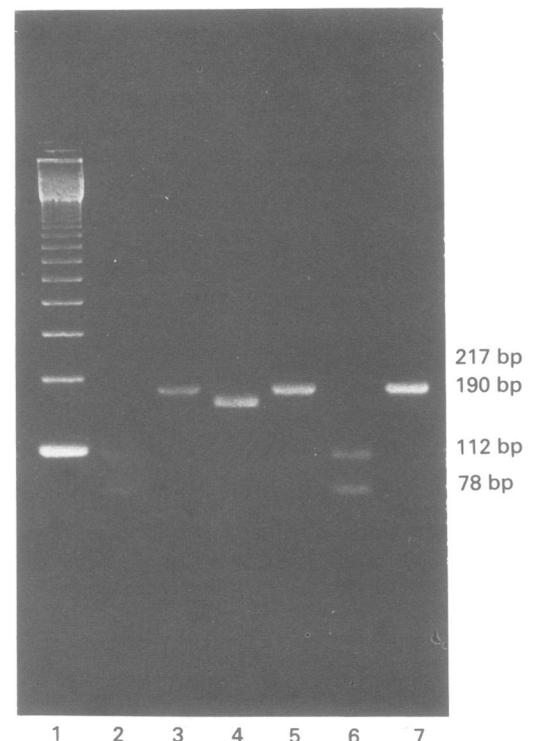


Figure 2 Analysis of the amplified SRY DNA fragments digested with *Mnl*I on electrophoretic agarose gel. (1) 123 bp DNA ladder marker, (2) father, after *Mnl*I digestion (112 + 78 + 27 bp), (3) father, without *Mnl*I digestion (217 bp), (4) patient, after *Mnl*I digestion (190 + 27 bp), (5) patient, without *Mnl*I digestion (217 bp), (6) normal male, after *Mnl*I digestion (112 + 78 + 27 bp), (7) normal male, without *Mnl*I digestion (217 bp).

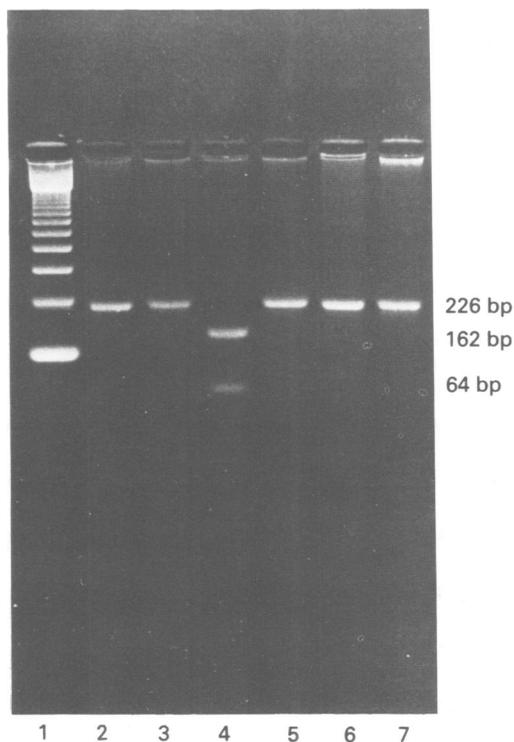


Figure 3 Analysis of the amplified SRY DNA fragments digested with *BsmAI* on electrophoretic agarose gel. (1) 123 bp DNA ladder marker, (2) father, after *BsmAI* digestion (226 bp), (3) father, without *BsmAI* digestion (226 bp), (4) patient, after *BsmAI* digestion (162 + 64 bp), (5) patient, without *BsmAI* digestion (226 bp), (6) normal male, after *BsmAI* digestion (226 bp), (7) normal male, without *BsmAI* digestion (226 bp).

the sex determining region of the human Y chromosome, the gene SRY and its mouse equivalent *Sry* have been cloned.⁵ A number of observations have shown that SRY and mouse *Sry* have many of the predicted properties of TDF and provide strong evidence for SRY being the TDF.^{4,6-8} As predicted as a regulatory gene, SRY encodes a protein containing a DNA binding motif.^{3,14} Recently, it was proven that SRY protein was able to bind to the same core sequence AACAAAG which is recognised by TCF-1. The results suggested that the DNA binding activity of SRY is required for sex determination.¹⁵

Sex reversal in XY females is caused by failure of the testis determining or differentiation pathways. To date, over 10 mutations have been found in SRY and these mutations, apart from a deletion 5' to the SRY open reading frame, all lie within the region that encodes the HMG box.^{6,7,15-20} The majority of mutations that have been tested are de novo and are not shared by the fathers of these XY daughters. Recently, SRY sequences derived from six XY females were cloned into a bacterial expression vector. SRY protein was produced from five of the six mutant genes, but the sixth point mutation, a frameshift mutation, was unable to produce SRY protein. When equal amounts of wild type and mutant SRY protein were subjected to electrophoretic mobility shift assays with the SRY oligonucleotide probe, no or reduced DNA binding activity was detected

with these point mutations.¹⁵ SRY mutations encoded by XY females lose this DNA binding activity, which implies that this activity is necessary for testicular development.

In the present paper, we have identified a new point mutation in the SRY gene in a Chinese XY female, a G to A substitution at codon 113 (A113T) that causes a conservative change from alanine to threonine within the HMG box motif. This mutation leads to complete sex inversion, that is, XY gonadal dysgenesis, in the patient. Since her father and other family members do not carry the same mutation, it must have occurred de novo. This de novo SRY mutation, together with the data on other mutations in SRY, strongly suggest that SRY mutations are associated with sex reversal syndrome, contributing to our understanding of the molecular basis of XY females.

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