

## NEW MUTATION IDENTIFIED IN THE SRY GENE HIGH MOBILITY GROUP (HMG)

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### SUMMARY

Mutations in the SRY gene prevent differentiation of fetal gonads to testes and cause development of a female phenotype, and lead to sex reversal and pure gonadal dysgenesis (Swyer syndrome, OMIM 480000) develops. Different types of mutations identified in the SRY gene are responsible for 15% of gonadal dysgenesis cases. In this study, we report a new mutation (p.Arg132Pro) in the High Mobility Group (HMG) region of SRY gene detected in a patient with 46,XY karyotype who has primary amenorrhea. This mutation leads to replacement of the polar and basic arginine with a nonpolar hydrophobic proline residue at aminoacid 132 in the nuclear localization signal region of the protein. With this case report we would like to emphasize the genetic approach to the patients with gonadal dysgenesis. If Y chromosome is detected during cytogenetic analysis, revealing the presence of SRY gene and identification of its mutations by sequencing analysis is important.

**Key words:** amenorrhea, gonadal dysgenesis, HMG box, SRY gene, Swyer syndrome

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### SRY GENİ HIGH MOBILITY GROUP (HMG) BÖLGESİNDE TANIMLANAN YENİ MUTASYON

#### ÖZET

SRY geninde meydana gelen mutasyonlar gonadların testis yönünde farklanmasını engelleyerek dişi fenotipi ile birlikte XY cinsiyet dönüşümü ve saf gonadal disgenezi (Swyer sendromu, OMIM 480000) gelişimine neden olabilmektedir. Bu gende tanımlanmış farklı mutasyonlar gonadal disgenezi olgularının %15'inden sorumludur. Bu çalışmada, 46,XY karyotipine sahip primer amenoreli bir olguda SRY geni "High Mobility Group" (HMG) bölgesinde saptadığımız yeni bir mutasyonu (p.Arg132Pro) bildiriyoruz. Saptadığımız mutasyon proteinin nükleer lokalizasyon sinyali bölgesinde polar bazik bir aminoasit olan arjinin yerine, nonpolar hidrofobik bir aminoasit olan prolinin geçmesine neden olmaktadır. Bu olgu sunumu ile gonadal disgenezili hastalarda, karyotipte Y kromozomu saptanması durumunda SRY geninin varlığının gösterilmesi ve bu gendeki mutasyonların özellikle dizi analizi yöntemi ile araştırılmasının genetik yaklaşım açısından önemi vurgulanmaktadır.

**Anahtar kelimeler:** amenore, gonadal disgenezi, HMG kutusu, SRY geni, Swyer sendromu

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**INTRODUCTION**

Differentiation to normal male sex begins at 5-6 weeks of gestation and requires information acquired from the Y chromosome<sup>(1)</sup>. In case of absence of the information or lacking the expression from the Y chromosome, ovarian development occurs and female phenotype is observed. Sex determining region (*SRY* gene) which is located on the short arm of Y chromosome, plays role in the development of testes<sup>(1)</sup>. *SRY* gene is known to be the triggering point in the development of the Sertoli cell<sup>(1,2)</sup>. *SRY* is a single-exon-gene which consists of 845 nucleotides encoding 204 amino acids. The protein encoded from *SRY* gene has a *DNA-binding domain high mobility group* (HMG) motif, thus functioning as a transcription factor<sup>(2)</sup>. Mutations both in the promoter region, and exons of this gene have been reported in *The Human Gene Mutation Database*<sup>(3)</sup> and in the literature. *SRY* gene mutations have been reported in patients with XY sex reversal<sup>(4,6)</sup>. Gene mutations are reported as specific single mutations for gonadal dysgenesis and sex reversal cases<sup>(1)</sup>. In this case report, a new mutation identified in the HMG region of the *SRY* gene in an individual who has female phenotype with 46,XY karyotype is discussed with clinical findings.

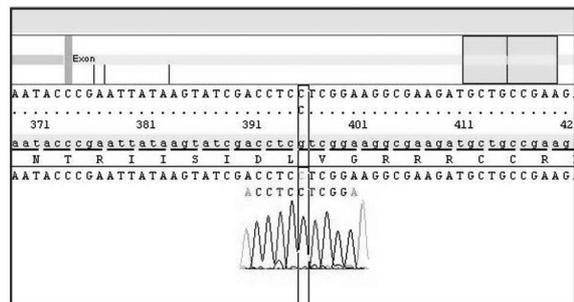
**CASE REPORT**

A sixteen-year-old patient was referred to the gynecology department from another clinic with amenorrhea. The patient did not have spontaneous menstruations. She was reported to have uterus detected by ultrasonography and she had bleeding after using oral contraceptives for a month. It was noted that the uterus and ovaries had been observed smaller than normal size at the pelvic magnetic resonance (MR) imaging. The patient had a 15-year-old brother and a 9 year-old sister. Her parents were not consanguineous. According to physical examination patient had a phenotypically normal female appearance, and the frontal hairline was localized lower than normal. Axillary and pubic hair and breast development were consistent with Tanner stage 2. Pelvic examination revealed that aperture of hymen and the external genital organs were normal. The patient had 172 cm height and 90 kg weight. Mental and motor development was normal. According to the ultrasonography, uterus dimensions

were 46X15X24mm, endometrium was linear, left ovary was 19X6 mm, and right ovary was 19X7mm. Prominent follicle was not observed in ovaries. Blood test results were; FSH:38.8 mIU/ml (2-10 mIU/ml), LH:14.7 mIU/ml (2-15 mIU/ml), estradiol:13 pg/ml (30-119 pg/ml), free testosterone: 1.98 pg/ml (0.04-4.18 pg/ml), DHEA-SO4:5271 ng/ml (651-3680 ng7ml), 17-OH progesterone:1.47 ng/ml (0.30-1 ng/ml), TSH:1.03 UIU/ml (0.40-4.67 UIU/ml), and prolactin:767 mIU/L (33,36-580 mIU/L). Patient was diagnosed as hypergonadotropic hypogonadism and had menstruation after using oral contraceptive.

Lymphocyte culture was prepared from peripheral blood sample after the informed consent was obtained. Giemsa trypsin banding was applied to the harvested metaphases. Karyotype was detected as 46,XY in 30 metaphases. Fluorescence in situ hybridization (FISH) method was performed by using LSI *SRY*/CEPX (Vysis, Abbott Molecular Inc, Des Plaines, IL, ABD) probe to detect the chromosomal localization of the *SRY* gene and to exclude the possible translocations or rearrangements. *SRY* gene signal was detected on the short arm of the Y chromosome. At the same time, the *SRY* gene amplification was detected with polymerase chain reaction, thus the presence of *SRY* gene was confirmed with a second method.

In order to investigate the presence of possible mutations in the *SRY* gene, promoter region and exon of the gene were amplified with two primers set by using polymerase chain reaction. Later, Sanger sequencing was performed and capillary gel electrophoresis was performed on ABI 3130 (Applied Biosystems, Foster City, CA, ABD) instrument. The data was analyzed by using Seqscape program, and compared with the reference sequence given at [www.ensembl.org](http://www.ensembl.org)<sup>(7)</sup>. As a result of this analysis presence of the p.Arg132Pro mutation in the HMG region was detected in our case (Figure 1).



**Figure 1:** Electropherogram image of the p.Arg132Pro mutation detected at the *SRY* gene by using DNA sequence analysis. The mutation is shown between the two vertical lines.

## DISCUSSION

The presence of the *SRY* gene has been reported to be the factor responsible for development of the gonads as testes during mammalian embryogenesis, hence resulting in the development of the embryo with a male sex<sup>(3)</sup>. When *SRY* gene is absent or its expression is impaired, fetal gonads develop as ovaries. XY gonadal dysgenesis is a clinical entity characterized by testicular regression that can be in a pure or partial form. Pure gonadal dysgenesis (Swyer syndrome) can be defined as 46,XY karyotype with female external genitalia, normal Mullerian structures, but with streak gonads<sup>(5)</sup>. Partial gonadal dysgenesis, on the other hand, is usually together with ambiguous genital and partial testicular differentiation. Uni- or bilateral dysgenetic or streak gonads can be observed in these individuals<sup>(3)</sup>.

Male/female sex reversal develops due to *SRY* gene mutations<sup>(8)</sup>. *SRY* gene has a single exon containing a HMG region and its protein both binds to the DNA and bends it, as a result of which, it acts as a transcriptional regulator<sup>(3,9)</sup>. Different mutations of the *SRY* gene are responsible for 15% of gonadal dysgenesis cases. The 41 mutations of *SRY* gene reported up to date cumulate specially in the HMG domain emphasizing the importance of this region in the development of gonadal dysgenesis<sup>(4)</sup>.

The mutation (p.Arg132Pro) detected in our patient involves a change of a nonpolar hydrophobic amino acid proline, instead of a polar basic amino acid arginine. A p.Arg132Gly mutation has been reported at the same point previously in a patient with pure gonadal dysgenesis<sup>(4)</sup>. The detection of this new mutation in our patient supports the information and the importance of HMG domain in the pathogenesis of gonadal dysgenesis. Mutations of this domain affect protein folding, hence cause the disruption of DNA folding and binding functions of protein. The disruption of especially the nuclear localization signal affects the transport of the protein to the nucleus and its function. HMG domain mutations of the *SRY* gene occur usually *de novo*, however during clinical approach, although rare, gonadal mosaicism and lack of penetrance of the mutation should also be taken into account<sup>(10)</sup>.

Our patient was diagnosed as Swyer Syndrome according to the physical examination and laboratory analysis results. There were no other affected members

in the family and one brother and one sister of the patient were both healthy, thus we did not think familial inheritance. Other family members did not give consent for *SRY* gene mutation screening and we could not prove this hypothesis. Genetic counseling was given to the patients' family according to the findings and gonadectomy was suggested due to the increased cancer risk<sup>(4)</sup>.

In cases with a suspected gonadal dysgenesis diagnosis, the presence of the *SRY* gene should be confirmed. Investigation of the mutations of the gene by DNA sequencing analysis is important during genetic approach. Although there are many mutations reported already, new mutations in this gene and the definition of their clinical reflections will enable us to better understand the role of *SRY* gene during sexual differentiation.

## REFERENCES

1. Schäffler A, Barth N, Winkler K, Zietz B, Rümmele P, Knüchel R, Schölmerich J, Palitzsch KD. Identification of a new missense mutation (Gly95Glu) in a highly conserved codon within the high-mobility group box of the sex-determining region Y gene: report on a 46,XY female with gonadal dysgenesis and yolk-sac tumor. *J Clin Endocrinol Metab* 2000; 85(6): 2287- 92.
2. Li B, Phillips NB, Jancso-Radek A, Ittah V, Singh R, Jones DN, Haas E, Weiss MA. *SRY*-directed DNA bending and human sex reversal: reassessment of a clinical mutation uncovers a global coupling between the HMG box and its tail. *J Mol Biol* 2006; 360: 310- 28.
3. HGMD, <http://www.hgmd.org/>
4. Shahid M, Dhillion VS, Jain N, Hedau S, Diwakar S, Sachdeva P, Batra S, Das BC, Husain SA. Two new novel point mutations localized upstream and downstream of the HMG box region of the *SRY* gene in three Indian 46,XY females with sex reversal and gonadal tumour formation. *Mol Hum Reprod* 2004; 10: 521- 6.
5. Salehi LB, Scarciolla O, Vanni GF, Nardone AM, Frajese G, Novelli G, Stuppia L. Identification of a novel mutation in the *SRY* gene in a 46, XY female patient. *Eur J Med Genet* 2006; 49: 494- 8.
6. Cunha JL, Soardi FC, Bernardi RD, Oliveira LE, Benedetti CE, Guerra-Junior G, Maciel-Guerra AT, de Mello MP. The novel p.E89K mutation in the *SRY* gene inhibits DNA binding and causes the 46,XY disorder of sex development. *Braz J*

- Med Biol Res 2011; 44: 361- 5.
7. [http://www.ensembl.org/Homo\\_sapiens/Gene/Summary?db=core;g=ENSG00000184895;r=Y:2654896-2655740;t=ENST00000383070](http://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000184895;r=Y:2654896-2655740;t=ENST00000383070)
  8. Kaur G, Delluc-Clavieres A, Poon IK, Forwood JK, Glover DJ, Jans DA. Calmodulin-dependent nuclear import of HMG-box family nuclear factors: importance of the role of SRY in sex reversal. *Biochem J* 2010; 430(1): 39- 48.
  9. Phillips NB, Jancso-Radek A, Ittah V, Singh R, Chan G, Haas E, Weiss MA. SRY and human sex determination: the basic tail of the HMG box functions as a kinetic clamp to augment DNA bending. *J Mol Biol* 2006; 358: 172- 92.
  10. Filges I, Kunz C, Miny P, Boesch N, Szinnai G, Wenzel F, Tschudin S, Zumsteg U, Heinemann K. A novel missense mutation in the high mobility group domain of SRY drastically reduces its DNA-binding capacity and causes paternally transmitted 46,XY complete gonadal dysgenesis. *Fertil Steril* 2011; 96(4): 851- 5.