

*Short Report*

## **A case of ectopic scrotum in a dog with Tyr236His substitution in epidermal growth factor receptors**

**Anna Ruszczak<sup>1</sup>, Piotr Jurka<sup>1\*</sup>, Kamil Kacprzak<sup>1</sup>,  
Maciej Wątroba<sup>1,2</sup>, Rafał Parada<sup>2</sup>, Mariusz Sacharczuk<sup>2\*\*</sup>**

<sup>1</sup> Department of Small Animal Diseases with Clinic, University of Life Sciences,  
Nowoursynowska 166, 02-787 Warsaw, Poland

<sup>2</sup> Department of Genomic, Institute of Genetics and Animal Breeding,  
Polish Academy of Sciences, Jastrzebiec, Postępu 36A, 05-552 Magdalenka, Poland

*(Accepted October 27, 2015)*

Ectopic scrotum and ectopic testis are both rare conditions. This report describes a 9-year-old crossbreed male dog with both abnormalities, which also has T721C transition in the gene of epidermal growth factor receptor resulted in a Tyr241His substitution in the peptide chain and the second silent A111G transition. High level of total estrogens (101.0 pg/ml), normal testosterone level (2.15 ng/ml) in blood, prostatic hyperplasia (46x44 mm) and hyperplastic epididymis were observed. Moreover, both testicles had signs of connective tissue hyperplasia in epididymis, vasodilation of pampiniform plexus and impairment of spermatogenesis in the left testicle. Both scrotums consisted of the same layers. This constellation of anatomical and genetic anomalies has not been previously described. We postulated that T721C polymorphism in the *Egfr* gene is one of the genetic factors in polygenic etiology of ectopic scrotum.

**KEY WORDS:** ectopic scrotum / dog / EGF receptor / Tyr236His

Ectopic scrotum (ES) is an extremely rare malformation, described so far only in inbred rats as a polygenic disorder [Ikadi *et al.* 1988]. In human ES was described

---

\*, \*\*Corresponding author: \*piotr\_jurka@sggw.pl; \*\*m.sacharczuk@ighz.pl

in a variety of locations, from the perineum and inguinal canal to the medial thigh, mainly in the inguinal area [Hoar *et al.* 1998, Kumar *et al.* 2002].

The swelling and migration gubernaculum with relaxation of the cranial suspensory ligament in testicular descent allow testes to move normally from the posterior abdominal wall to the internal inguinal ring [Zhao *et al.* 2014]. Several factors, such as Hoxa-10, EGF, calcitonin gene-related peptide (CGRP), and hormones especially androgens and insulin-like factor 3 (INSL-3), have been suggested as regulators of testicular descent [Kaleva and Toppari 2003].

The aim of this study was description and attempt to explain etiology of ES in dog. To authors' knowledge this is the first study, which describes ES in dog.

## Material and methods

### Case presentation

A 9-year-old male mixed breed dog weighing 35 kg presented right ectopic scrotum with testis. ES was localized 3 cm from the left normal scrotum on the line of penis and 6 cm above the opening of the foreskin (Fig. 1).



Fig. 1. Normal (right side) and ectopic scrotum (left side) before castration in the dog.

#### **Biochemistry, hematology and endocrinology**

Blood was collected for biochemical immunoenzymatic tests (Pointe Scientific, Poland), hematological analysis using Coulter method [Kakel 2013] and The Abacus Junior Vet veterinary hematology analyzer (Diatron MI PLC, Budapest, Hungary) and hormonal profiles. Total estrogen (Pointe 2000 apparatus) and testosterone (immunoenzymatic test DPC, USA, Immulite 2000 apparatus) concentrations were measured twice (before castration and 3 months after). An ultrasound scanner (Honda 4000, Japan) with a 5/7.5 MHZ probe was used to examine the reproductive system before castration and 3 months after operation.

#### **Anesthesia and operation**

Anesthesia for castration was induced with intramuscular (i.m.) ketamine 5 mg/kg (Bioketan®, Vetoquinol) and medetomidine 25µg/kg (Cepetor®, Scanvet) injection and maintained with isoflurane (Forane®, Baxter), a nonflammable liquid administered by vaporizing after endotracheal intubation and a non-rebreathing system. Perioperative analgesia was maintained with methadone (Physeptone®, Martindale Pharmaceuticals) at a dose of 0.2 mg/kg i.v. and meloxicam (Metacam®, Boehringer Ingelheim) at a dose of 0.3 mg/kg s.c. Total castration was made and testes were removed together with scrotum. Then wound was closed using absorbable sutures (Polysorb, Vetoquinol) with a standard closure technique (Fig. 2). Material for histopathological examination was taken during surgery, settled in 10% formalin and stained by the Masson Trichrome staining (MTS).



Fig. 2. Ectopic (right side) and normal (left side) testis with hyperplasia of connective tissue in epididymis and vasodilation of pampiniform plexus.

### Genetic evaluation

DNA was isolated from frozen whole blood samples using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following standard protocols and procedures.

Pairs of specific primers were designed using the Primer3 program [Rozen and Skaletsky 2000] for gene amplification and sequencing. Primers were purified using high pressure liquid chromatography. The primer sequences for each gene are shown in Table 1. Additionally, primer properties such as T<sub>m</sub> values, self complementarity, and possible formation of “hairpin” structures were established using the Oligonucleotide Properties Calculator software (<http://www.basic.northwestern.edu/biotools/oligocalc.html>) in order to optimize the amplification process.

**Table 1.** Characteristics of the genes, genomic residue and type of SNPs

Gene	Gene function	GenBank accession number	Chromosome	Exon	Allele	Type of SNP	SNP position	AA coord
<i>Egfr</i>	Projection protein	XM_533073.4	CF18	1	A111G	syn	3	Glu37Glu
				6	T721C	n/syn	1	Tyr241His

PCR was performed in the total reaction volume of 10 µl included 10 ng of genomic DNA, 25 mM dNTPs, 25 mM of each specific primer and 2 U/ml of Taq DNA polymerase (Polgen, Lodz, Poland) in a buffer containing 100 mM Tris-HCl, 20 mM MgCl<sub>2</sub> and 500 mM KCl in injection quality water (Polpharma, Starogard Gdanski, Poland). The following reaction conditions were applied: 3:30 minutes at 94°C followed by 35 amplification cycles (30 seconds at 94°C, 55 seconds at 63°C, 1:30 minutes at 72°C) and a final elongation for 10 minutes at 72°C. Automated sequencing was performed in both directions on an ABI PRISM™ 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned and analysed with Sequencher Demo™ software (Gene Codes Corporation, Ann Arbor, MI, USA). The polymorphism was identified in sequence traces displayed by the software.

Complete nucleotide sequences were translated using a Nucleic Acid Analysis and Manipulation Program from the Colorado State University website (<http://www.vivo.colostate.edu/molkit/index.html>) and then compared with a reference sequence using BLASTP program in order to find mismatches. The results were compared using the SIFT algorithm [Ng and Henikoff 2001, Ng and Henikoff 2003] – Table 2.

**Table 2.** Primer sequences of the *Egfr* gene

Gene	Exon	Left primer	Right primer
<i>Egfr</i>	1	TTTGCTACTTGGCATTTCACG	GCAAAAAGCAAATACACTTCCAAC
	6	CTGCAAGGTGAGCTTTCTGC	TGGACCCAAAGACTACCCGA

## **Results and discussion**

High estradiol (101.0 pg/ml) and normal testosterone (2.15 ng/ml) levels were found. After castration both hormones decreased significantly to <20 pg/ml and <0.5 ng/ml, respectively. In contrast, there were no significant changes in hematological and biochemical blood parameters before and post/following surgery. In the ultrasound – benign prostatic hyperplasia (BPH) (46 x 44 mm) with few cysts, no changes in testes and hyperplastic epididymis (mainly in ectopic testicle (ET)) were present. ET was smaller (19x13x9 mm) compared to the normal one (25x18x15 mm), however epididymis in ET was higher (11x7x5 mm) than normal one (7x5x4 mm).

Histologically both testes showed hyperplasia of connective tissue of epididymis, vasodilation of pampiniform plexus, low numbers of spermatogonia, spermatocytes, spermatids and disappearance of sperm in the left testicle and partial in the right. The lamina propria showed a variable degree of thickening and collagenization and the interstitial tissue was very developed. No neoplastic changes in both testes were found. Both scrotums consisted of the same layers: skin, subcutaneous tissue and thin muscles.

In preliminary genetic evaluation no polymorphic sites were identified in any exon of the genes from Hox and BMP families, calcitonin gene-related peptide (CGRP) and insulin-like factor 3 (INSL-3), potentially engaged in the ectopic scrotum etiology. However, in present studies we found two polymorphic sites in the coding region of the epidermal growth factor (EGF) receptor gene. The first occurred in the form of T721C transition in heterozygote form and resulted in a Tyr241His substitution in the peptide chain. The SIFT score for Tyr241His was 0.69 (amino acids with probabilities <0.05 are predicted to be deleterious). Another polymorphic site in the coding region *Egfr* gene was caused by A111G transition and did not lead to an amino acid substitution in the peptide chain.

Gubernaculum in testicular descent is responsible for correct location of both testis and scrotum [Hoar *et al.* 1998]. However, its role is complicated by subsequent differential growth of labioscrotal folds in which the gubernaculum is stabilized [Kumar *et al.* 2002]. Disturbance of these interactions can cause ES [Hoar *et al.* 1998] and is probably a result of aberrant gubernaculum stabilization. Histologically, in our case both suggest that ES was formed in the same way like normal scrotum (NS), but gubernaculum in ES attached cranially in comparison to NS. Testicular descent depends on the androgens, INSL-3, CGRP and EGF [Douglas *et al.* 1995, Comploj and Pycha 2012].

We determined that this individual carried a functionally relevant T706C transition in the epidermal growth factor gene which resulted in a Tyr241His substitution in the peptide chain. We demonstrated for the first time the importance and phenotypic effect of such transition, which probably resulted in ectopic scrotum and testis with hyperplasia of connective tissue in the epididymis in this dog. Because the T721C transition occurred in heterozygote form, we hypothesized that the lack of compensation from non-affected second allele may have been caused by allele-specific expression. Such

phenomenon in recent studies has provided evidence for non-canonical imprinting effects that are associated with allele-specific expression based at tissue level [Gregg 2014]. Arrest of spermatogenesis in testes in ectopic scrota may be a result of increased temperature in this scrotum. The results of histological and genetic studies suggest that changes observed in the ET were similar to these described in cryptorchid testes. Significant reduction in number of spermatogonia, spermatocytes, spermatids and relatively abundant interstitial tissue are observed in cryptorchid testis comparing to normal testis. [Moon *et al.* 2014] Cryptorchidism changes the histology of the tissue in the testis which results in fertility reduction. [Moon *et al.* 2014] Changes observed in ET suggests that the ectopic scrotum does not fulfill its role. Despite significance of present ES case in a dog, the etiology of ES seems to be polygenic and the results cannot be regarded as universal for other species.

#### REFERENCES

1. COMPLOJ E., PYCHA A., 2012 – Diagnosis and Management of Cryptorchidism. *European Urology* 11, 2-9.
2. DOUGLAS A., HUSMANN M.D., JAY B., LEVY M.D., 1995 – Current concepts in the pathophysiology of testicular undescend. *Urology* 46, 2.
3. FRANZOSI M. R., MATTOS L.S., 2000 – Cryptorchidism in dog. *Ciència Animal* 10, 61-70.
4. GREGG C., 2014 – Known unknowns for allele-specific expression and genomic imprinting effects. *F1000Prime Reports* 6, 75.
5. HOAR R.M., CALVANO C.J., REDDY P.P., BAUER S.B., JANDELL M., 1998 – Unilateral Suprainguinal Ectopic Scrotum: The Role of the Gubernaculum in the Formation of an Ectopic Scrotum. *Teratology* 57, 64-69.
6. IKADAI H., AJISAWA C., TAYA K., IMAMICHI T., 1988 – Suprainguinal ectopic scrota of TS inbred rats. *Journal of Reproduction and Fertility* 84, 701-707.
7. KAKEL S.J., 2013 – The evaluation of traditional and automatic Coulter method in estimation of haematological parameters in adult rats. *Journal of Basic and Applied Sciences* 2, 31-35.
8. KALEVA M., TOPPARI J., 2003 – Genetics and Hormones in Testicular Descent. *Hormones* 2, 211-216.
9. KUMAR V., MARULAI AH M., CHATTOPADHYAY A., RAO P., 2002 – Unilateral inguinal ectopic scrotum with covered exstrophy. *Pediatric Surgery International* 18, 511-513.
10. MOON J.H., YOO D.Y., JO Y.K., KIM G.A., JUNG H.Y., CHOI J.H., HWANG I.K., JANG G., 2014 – Unilateral cryptorchidism induces morphological changes of testes and hyperplasia of Sertoli cells in a dog. *Laboratory Animal Research* 30 (4), 185-189.
11. NG P.C., HENIKOFF S., 2001 – Predicting deleterious amino acid substitutions. *Genome Research* 11, 863-874.
12. NG P.C., HENIKOFF S., 2003 – SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Research* 31, 3812-3814.
13. ROZEN S., SKALETSKY H., 2000 – Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology* 132, 365-386.
14. ZHAO X., ONTERU S., SAATCHI M., GARRICK D., ROTHSCILD M., 2014 – A genome-wide association study for canine cryptorchidism in Siberian Huskies *Animal Breeding and Genetics* 131, 202-209.