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Expression Analysis of Androgen Receptor Transcript Variant 2 (AR2) in Beagle Dog Tissues

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Abstract: An N-terminal truncated Androgen Receptor transcript variant 2 (AR2), lacking transcription activation domain has recently been identified in human and old world monkeys. The present study was designed to investigate whether corresponding AR2 variant mRNA is expressed in canine tissues. Alignment of human and canine genome AR sequences revealed 81% identity of the AR2 variant specific exon 1B in the canine genome sequence. A conserved 20 bp primer was designed for PCR amplification of canine AR2. By using Reverse Transcription-PCR analysis of RNA from multiple beagle dog tissues, researchers demonstrated the AR2 expression in dogs. Canine AR2 variantm RNA expression was observed in liver, kidney, heart and skeleton muscle suggesting a greater importance to investigate the role of AR2 in the physiological and pathological processes.

Key words: Androgen receptor, transcript variant, alternative splicing, beagle dog, liver

INTRODUCTION

Androgen Receptor (AR) is a ligand dependent protein that belongs to the nuclear receptor super family. AR is encoded by a single gene over 90 kb long located on chromosome X at position q12 in humans. It is divided over eight exons and has a promoter region of at least 1400 bp long. At least 4 protein coding transcript variants produced by alternative splicing have been found in human (Flicek *et al*., 2013). The most widely distributed Androgen Receptor transcript variant 1 (AR1) encodes a 98.7 kD protein. As in other steroid hormone receptors, AR1 is composed of ahypervariable Transcription Activation Domain (TAD) in the N-terminal end, a DNA-Binding Domain (DBD) which contains two highly conservedzinc fingers that interacts with a specific DNA site upstream of the target gene and modulates the rate of transcriptional initiation, a large Ligand-Binding Domain (LBD) in the C-terminal end and a short hinge region linking DBD and LBD. The sequence encoding TAD is present in the largest exon 1 (Kuiper *et al*., 1989) which contains trinucleotide repeat sequence, CAG. A linear decrease of transactivation function is related to progressive expansion of the CAG repeats (Chamberlain *et al*., 1994). AR is essential for both male and female reproduction development and function (Walters *et al*., 2010). Androgens bind to LBD and therefore regulate AR function through initiating sequential conformational changes that affect receptor-DNA interactions. However, recent study showed the non-genomic effects of androgens through AR functioning in the cytoplasm to induce the MAPK signal cascade (Heinlein and Chang, 2002; Fix *et al*., 2004).

Human and rogen receptor transcript variant 2 (AR2 also known as AR45) encodes a special N-terminal truncated 45 kD protein (Ahrens-Fath *et al*., 2005). Both human AR1 and AR2 are encoded by 8 exons within the human androgen receptor gene. The sequences of mRNA exon 2 through 8 are identical between the two transcript variants. However, in place of exon 1, AR2 possesses a unique exon 1B, located between normal exon 1 and 2 which encoded an N-terminal 7 amino acid residue. This difference possibly attributed to transcriptional control by a novel promoter upstream of exon 1B or an alternative splicing event. *In vitro* study showed the dominant-negative inhibiting effects of AR2 on AR1 function through formation of AR1-AR2 heterodimers and over expression of AR2 caused repression of androgen-dependent cells proliferation (Ahrens-Fath *et al*., 2005). In another *in vitro* study, a

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negative correlation between AR2 transcript level and RNA Binding Motif gene on the Y chromosome (RBMY) expression was demonstrated indicating the oncogenic mechanism of RBMY might be linked to its regulatory roleon AR1 trans-activation activity by the inhibitory isoform AR2 (Tsuei *et al*., 2011). On the other hand when the transcriptional co-activator TIF2 or the oncogene β-catenin overexpressed, AR2 by itself showed activation of androgen-dependent promoters in presence of Dihydrotestosterone (DHT) (Ahrens-Fath *et al*., 2005). In addition, co-transfection of AR2 and the Human Ether-a-go-go-Related Gene (HERG) in HEK293 cells or CHO cells demonstrated that the stimulation of AR2 could stabilize HERG potassium channel protein via activation of Extracellular signal Regulated Kinase (ERK)1/2 (Wu *et al*., 2008).

Expression analysis studies concluded that human and Non-Human Primate (NHP) AR2 was predominately expressed in heart and the mRNA AR2/AR1 ratio in heart was highest indicating the modulating effects of AR2 might be essential in heart (Ahrens-Fath *et al*., 2005; Weiss *et al*., 2007). Gender difference in the incidence of Cardiovascular Diseases (CVD) has drawn a lot of attention through these years. And the role of androgen receptor in several CVDs has been documented by many studies. Activation of AR by DHT can be associated with increased human monocyte adhesion to endothelial cells a proatherogenic effect (McCrohon *et al*., 1999; Death *et al*., 2004), elevated blood pressure (Morano *et al*., 1990; Reckelhoff *et al*., 2000) and promoted myocardial hypertrophy (Morano *et al*., 1990; Marsh *et al*., 1998; Muller *et al*., 2000). Therefore, it might be necessary to figure out the function of AR2 in the cardiovascular system.

In a genome sequence analysis study, AR2 was predicted to be expressed in dogs (Weiss *et al*., 2007). Beagle dog has been considered as the most suitable large animal model for cardiovascular safety evaluation including cardio toxicity and safety pharmacology studies (Herman and Ferrans, 1998; Gralinski, 2003). It might be a decent animal model for functional study of AR2. But the existence of this particular transcript variant has not been demonstrated in canine tissues yet. In this study, researchers described comparison of human and canine genome AR sequence, primer design to amplify canine AR2 sequence and demonstrated the expression of AR2 in multiple tissues by RT-PCR analysis.

MATERIALS AND METHODS

Comparison between human and canine genome AR sequence: Sequencesof *Homo sapiens* androgen receptor gene (Chromosome X: 66, 764, 465-66, 950, 461), *Canis lupus* familiaris androgen receptor gene (Chromosome X: 51, 969, 785-52, 151, 852), *Homo sapiens* androgen receptor transcript variant 1 mRNA (NM_000044.3), *Homo sapiens* androgen receptor transcript variant 2 mRNA (NM_001011645.2) and *Canis lupus* familiaris androgen receptor mRNA (NM_001003053.1) were obtained from Genbank (Benson *et al*., 2013) and Ensembl (Flicek *et al*., 2013). Sequences were then aligned through the nucleotide blast web interface of Genbank (Benson *et al*., 2013).

Design of canine AR2 primers: In order to confirm the existence of the predicted canine AR2 mRNA which is transcripted from exon 1B and exon 2-8 of AR, PCR primer design was based on the homologous locus of human AR exon 1B in canine genome sequence. The AR2 forward primer was directly against the specific locus which encodes the 7 unique amino acids distinguished from AR1 while and the AR2 reverse primer recognized exon 4.

Animals and tissues: Healthy adult male beagle dogs were sacrificed by phenobarbital overdose. Liver, kidney, skeleton muscle, testis and apex cordis tissue samples were collected and snap-frozen in liquid nitrogen and stored in -80°C until use.

RT-PCR and sequencing analysis of AR2 mRNA expression in multiple beagle dog tissues: Total RNA was extracted with RNeasy® Plus Mini kit or RNeasy® Lipid Tissue Mini kit (QIAGEN) following the manufacturer's instructions. Total RNA was analyzed by Nano Drop® ND-1000 Spectrophotometer for quantity and Agilent 2100 Bio analyzer for purity and quality. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was performed with GeneAmp® RNA PCR Core kit on GeneAmp® PCR System 9700. Additional reaction time was used to obtain full length cDNA. Reaction conditions were: 60 min at 42°C, 5 min at 99°C. Same quantity (1 μg) of total RNA was added to the PCR System to ensure relative expression across different tissues. Specific PCR was then performed with primers described in Table 1 (Integrated DNA Technologies, Inc.).

Table 1: Primers used for expression analysis of transcript variants of androgen receptor

Primers name	Sequence	Product size
AR Forward	5'-AAG CGG CAG CTT ATC AAA GTC GAG-3'	424
AR Reverse	5'-CTA GGA CTT GGA AAG GGC ACT CTG-3'	
AR2 Forward	5'-ATG ATA CTC TGG CTT CAC AG-3'	519
AR2 Reverse	5'-AAG GCT AGA GAG CAA GGC TGC AAA GGA GTC-3'	

Reaction conditions were: 105 sec at 95°C; 35 cycles of 15 sec at 95°C, 30 sec at 60°C; 7 min at 72°C. The PCR products were analyzed by using pre-cast E-Gel® EX 2% Agarose (Invitrogen) Gel System. After electrophoresis, the bands were cut and DNA was extracted by using QIA guick® Gel Extraction kit (QIAGEN) and used for sequencing. The sequence identity of the amplified DNA was confirmed by BLAST analysis.

RESULTS AND DISCUSSION

Genome sequence of exon 1B is homologous in human and canine: Androgen receptor is encoded by a single gene located on chromosome X in both human and canine genome. The most commonly expressed mRNA, AR1 is composed of 8 exons in both species with similar transcription patterns. Comparison of 182 bp exon 1B sequence of human AR2 from Genbank database (NM_001011645.2) with canine AR genome sequence identified putative AR2 specific coding region between exons 1 and 2 of canine *AR* gene (NW_003726126.1). Alignment analysis revealed 81% identity between two species and a sequence stretch of 20 bp open reading frame showed complete identity (Fig. 1a). With first base pair of exon 2 taken into consideration, the unique 7 amino acid residue in canine AR2 was predicted to be the same with human AR2 (MILWLHS). The predicted canine AR exon 1B located about 20.8 kb downstream of exon 1, 80.4 kb upstream of exon 2. Despite the position difference between human and predicted canine exon 1B, the existence of canine AR2 was considered to be possible (Fig. 1b).

Confirmation of AR2 existence in beagle dog hearts: When human AR2 was first identified with mRNA expression analysis, it was found mainly expressed in heart (Ahrens-Fath *et al*., 2005). RT-PCR was carried out with total RNA from apex cordis (heart) of three adult male beagle dogs. Primers pair corresponding to the known AR1 transcript (Table 1) (Lu *et al*., 2001) amplified a ~400 bp product and sequence confirmed as AR1 mRNA (Fig. 2a). PCR with the primers designed for AR2 (Table 1) amplified a ~500 bp DNA fragment (Fig. 2b). DNA sequence of this 500 bp fragment was compared with canine genome sequence and (AR2 forward primer excluded) showed 99% identity to canine AR sequence as shown in Fig. 2c. Thus, the presence of AR2 transcript variant in beagle dog heart tissue was confirmed.

Tissue-specific expression of AR2 in beagle dogs: RT-PCR analysis was performed in multiple beagle dog tissues to determine the expression pattern of two AR transcript variants. Samples from three individual beagle dogs were collected for each tissue and AR and AR2 specific primers were used (Table 1). The results indicated canine AR2 had relatively higher transcription level in liver and kidney followed by testis and heart. The weakest signal was observed in skeleton muscle. Interestingly, similar amount of AR2 transcript level was detected in testis and heart while the primary AR transcript showed significantly higher expression level in testis than in heart. In addition, AR2 presented much lower transcription level in all tissues than AR in general (Fig. 3).

The N-terminal truncated alternative transcript variant AR2 lacks the entire normal N-terminal domain

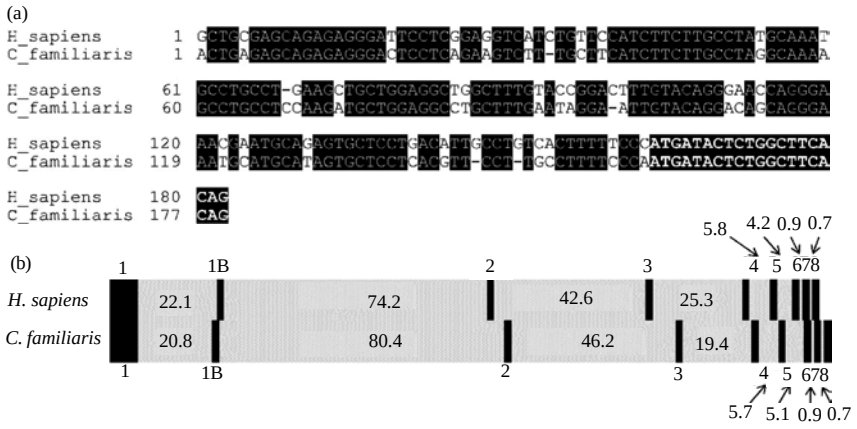

Fig. 1: Comparison of the sequence and location of genomic regions containing AR2 specific exon 1B between human and canine; a) Sequence alignment analysis of genomic regions exon 1B between species. Conserved sequences are in black boxes. The open reading frames are shown in bold letters; b) Location comparison of genomic regions exon 1B between species. Exons are shown in black boxes with numbers above or under; introns are shown in grey boxes with sizes in kb

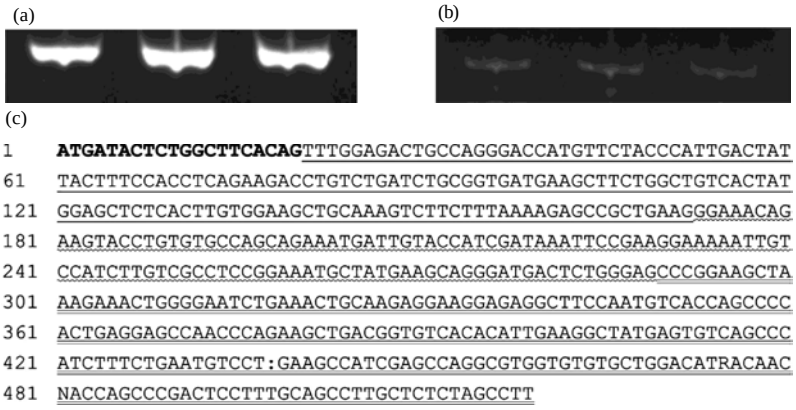

Fig. 2: RT-PCR analysis of the expression of AR2 transcript variant in beagle dog heart tissues; a) Agarose gel analysis of AR1RT-PCR products from 3 different RNA samples; b) Agarose gel analysis of AR2 RT-PCR products from 3 different RNA samples; c) DNA sequenceof AR2 RT-PCR product. Bold: exon 1B; underlined: exon 2; curvy underlined: exon 3; double underlined: partial exon 4

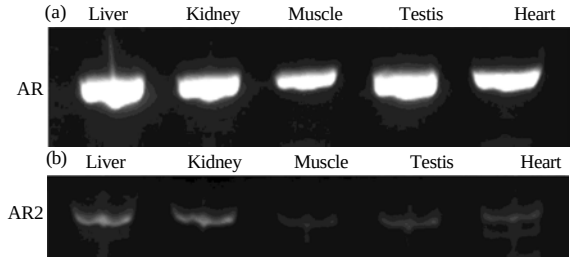

Fig. 3: Expression analysis of both androgen receptor transcript variant mRNA in multiple beagle dog tissues. Total RNA from the indicated organs was used as template for PCR amplification. Same amount of total RNA was added to the PCR System (1 μg) to ensure the comparability among different tissues; a) AR and b) AR2

(also known as Transcription Activation Domain, TAD) encoded by AR genome exon 1. There are totally three Transactivation Units (TAUs) in AR1 with Activation Function 1 (AF1), Activation Function 5 (AF5) in the N-terminal domain and Activation Function 2 (AF2) in LBD. Two AF functions are ligand dependent (AF1 and AF2) whereas AF5 operates in a ligand independent way (Brinkmann, 2001). Thus, AF1 and AF5 are absent in AR2. Analysis of AR deletion mutants revealed almost the entire N-terminal domain to be essential for the receptor function (Palvimo *et al*., 1993). AF1 was required for full ligand activated transcriptional activity while AF5 was sufficient for full constitutive transcriptional activity (Jenster *et al*., 1995). Though the biological activity of androgens is thought to occur predominantly through AR that functions as a ligand-activated transcription factor, non-genomic effects of androgens may occur in the cytoplasm to induce the MAPK signal cascade (Heinlein and Chang, 2002). Researchers can therefore infer that put aside the dominant-negative inhibiting effects of AR2 on AR1, activation of AR2 by androgens may cause biological changes by non-genomic mechanisms. Studies have demonstrated the ability of AR2 to alter certain protein degradation profile via activation of ERK1/2 (Wu *et al*., 2008) which was consistent with the signaling pathway of AR1 non-genomic effects. However, AR2 did stimulate androgen-dependent promoters in the presence of cofactor TIF2 or oncoprotein β-catenin (Ahrens-Fath *et al*., 2005). There are still huge gaps in the understanding of AR2 interactions with other proteins in the signaling pathways and transcription processes.

In this study by using sequence analysis of human and canine genomes and RT-PCR analysis of beagle dog heart RNA, researchers demonstrated conservation of AR2 specific region between exon 1 and 2 of canine *AR* gene and confirmed the expression of AR2 in canine heart tissue. Presence of AR2 had been predicted based on sequence analysis in multiple species including chimpanzee, orangutan, macaque, marmoset, elephant, pig and dog (Weiss *et al*., 2007). Absence of AR2 sequence was inferred in rat and mouse due to a stop codon downstream of the potential start codon of exon 1B (Weiss *et al*., 2007). Conservation among many different species indicates AR2 might have significant biological functions in mammals. Since, the most commonly used rodent animal models may not be suitable, beagle dogs as well as anthropogenic cell lines could be employed in the functional study of AR2.

The results show that the expression pattern of AR2 in canine tissues appears to be distinct from human. Heart

and skeleton muscle tissues of human were shown to have higher AR2 expression (Ahrens-Fath *et al*., 2005). Researchers observe relatively higher transcript levels of AR2 in canine liver and kidney tissues. In addition, the relative mRNA levels of AR2 was significantly lower than AR1 in all tissue types, suggestingthe biological function of AR2 in canine might bedifferent fromhuman. Clinical observations and experimental studies have confirmed the impact of androgens in liver and kidney. The structure of renal tubules and various aspects of renal functions have shown gender differences (Sabolic *et al*., 2007). Females show better clinical outcome in the treatment of chronic renal diseases (Kummer *et al*., 2012). Activation of AR appeared to induce podocyte apoptosis that precedes glomerul osclerosis (Doublier *et al*., 2011). Cell-selective knockout of AR in albumin-positive hepatocytes led to the development of insulin resistance and hepatic steatosis (Lin *et al*., 2008). Several studies suggested that the protective effect of AR against renal cell carcinoma. Nuclear AR was found to be the marker of responsiveness to medroxy-progesterone acetate in human renal cell carcinoma (Concolino *et al*., 1981) while an immuno histochemical analysis of over a hundred tumors demonstrated the significant association of AR expression with low stage well or moderately differentiated tumors and a favorable outcome (Langner *et al*., 2004). Similarly in Upper Urinary Tract Urothelial Carcinoma (UUTUC) there was a positive correlation for higher AR expression found in superficial or low-grade UUTUCs (Shyr *et al*., 2013). On the contrary, a systematical literature review implied the contributive role of androgens in Hepatocellular Carcinoma (HCC). They found over expression of AR in liver tumors and strong association between AR levels and intra hepatic recurrence of tumors (Kalra *et al*., 2008). Given the relative high expression level of AR2 in beagle dog kidney and liver and the extensive influence of AR on normal and tumor tissues in these two organs, it is of important to investigate the role of AR2 incanine kidney and liver functions.

CONCLUSION

Researchers identified canine exon 1B in canine genome AR sequence and confirmed the expression of AR2 by RT-PCR analysis of RNA from multiple beagle dog tissues. Expression pattern of AR2 in canine tissues appears to be distinct compared to the AR2 expression pattern reported for human. Further, investigation might be necessary for interpret the pathological role of AR2.

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REFERENCES

Ahrens-Fath, I., O. Politz, C. Geserick and B. Haendler, 2005. Androgen receptor function is modulated by the tissue-specific AR45 variant. FEBS J., 272: 74-84.

Benson, D.A., K. Clark, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell and E.W. Sayers, 2013. GenBank. Nucleic Acids Res., 41: D36-D42.

Brinkmann, A.O., 2001. Lessons to be learned from the androgen receptor. Eur. J. Dermatol., 11: 301-303.

Chamberlain, N.L., E.D. Driver and R.L. Miesfeld, 1994. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res., 22: 3181-3186.

Concolino, G., A. Marocchi, V. Toscano and F. Di Silverio, 1981. Nuclear androgen receptor as marker of responsiveness to medroxyprogesterone acetate in human renal cell carcinoma. J. Steroid Biochem., 15: 397-402.

Death, A.K., K.C.Y. McGrath, M.A. Sader, S. Nakhla, W. Jessup, D.J. Handelsman and D.S. Celermajer, 2004. Dihydrotestosterone promotes vascular cell adhesion molecule-1 expression in male human endothelial cells via a nuclear factor-κB-dependent pathway. Endocrinology, 145: 1889-1897.

Doublier, S., E. Lupia, P. Catanuto, S. Periera-Simon and X. Xia *et al*., 2011. Testosterone and 17κ-estradiol have opposite effects on podocyte apoptosis that precedes glomerulosclerosis in female estrogen receptor knockout mice. Kidney Int., 79: 404-413.

Fix, C., C. Jordan, P. Cano and W.H. Walker, 2004. Testosterone activates mitogen-activated protein kinase and the cAMP response element binding protein transcription factor in sertoli cells. Proc. Natl. Acad. Sci. USA., 101: 10919-10924.

Flicek, P., I. Ahmed, M.R. Amode, D. Barrell and K. Beal *et al*., 2013. Ensembl 2013. Nucleic Acids Res., 41: D48-D55.

Gralinski, M.R., 2003. The dog's role in the preclinical assessment of QT interval prolongation. Toxicol. Pathol., 31: 11-16.

Heinlein, C.A. and C. Chang, 2002. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol. Endocrinol., 16: 2181-2187.

Herman, E.H. and V.J. Ferrans, 1998. Preclinical animal models of cardiac protection from anthracycline-induced cardiotoxicity. Semin. Oncol., 25: 15-21.

Jenster, G., H.A.G.M. van der Korput, J. Trapman and A.O. Brinkmann, 1995. Identification of two transcription activation units in the N-terminal domain of the human androgen receptor. J. Biol. Chem., 270: 7341-7346.

Kalra, M., J. Mayes, S. Assefa, A.K. Kaul and R. Kaul, 2008. Role of sex steroid receptors in pathobiology of hepatocellular carcinoma. World J. Gastroenterol., 14: 5945-5961.

Kuiper, G.G.J.M., P.W. Faber, H.C.J. van Rooij, J.A.G.M. van der Korput and C. Ris-Stalpers *et al*., 1989. Structural organization of the human androgen receptor gene. J. Mol. Endocrinol., 2: R1-R4.

Kummer, S., G. von Gersdorff, M.J. Kemper and J. Oh, 2012. The influence of gender and sexual hormones on incidence and outcome of chronic kidney disease. Pediatrics Nephrol., 27: 1213-1219.

Langner, C., M. Ratschek, P. Rehak, L. Schips and R. Zigeuner, 2004. Steroid hormone receptor expression in renal cell carcinoma: An immunohistochemical analysis of 182 tumors. J. Urol., 171: 611-614.

Lin, H.Y., I.C. Yu, R.S. Wang, Y.T. Chen and N.C. Liu *et al*., 2008. Increased hepatic steatosis and insulin resistance in mice lacking hepatic androgen receptor. Hepatology, 47: 1924-1935.

Lu, B., S.L. Smock, T.A. Castleberry and T.A. Owen, 2001. Molecular cloning and functional characterization of the canine androgen receptor. Mol. Cell. Biochem., 226: 129-140.

Marsh, J.D., M.H. Lehmann, R.H. Ritchie, J.K. Gwathmey, G.E. Green and R.J. Schiebinger, 1998. Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation, 98: 256-261.

McCrohon, J.A., W. Jessup, D.J. Handelsman and D.S. Celermajer, 1999. Androgen exposure increases human monocyte adhesion to vascular endothelium and endothelial cell expression of vascular cell adhesion molecule-1. Circulation, 99: 2317-2322.

Morano, I., J. Gerstner, J.C. Ruegg, U. Ganten, D. Ganten and H.P. Vosberg, 1990. Regulation of myosin heavy chain expression in the hearts of hypertensive rats by testosterone. Circ. Res., 66: 1585-1590.

Muller, J.M., U. Isele, E. Metzger, A. Rempel and M. Moser *et al*., 2000. FHL2, a novel tissue-specific coactivator of the androgen receptor. Embo J., 19: 359-369.

Palvimo, J.J., P.J. Kallio, T. Ikonen, M. Mehto and O.A. Janne, 1993. Dominant negative regulation of trans-activation by the rat androgen receptor: Roles of the N-terminal domain and heterodimer formation. Mol. Endocrinol., 7: 1399-1407.

Reckelhoff, J.F., H. Zhang and K. Srivastava, 2000. Gender differences in development of hypertension in spontaneously hypertensive rats: Role of the renin-angiotensin system. Hypertension, 35: 480-483.

Sabolic, I., A.R. Asif, W.E. Budach, C. Wanke, A. Bahn and G. Burckhardt, 2007. Gender differences in kidney function. Pflug Arch.-Eur. J. Phy., 455: 397-429.

Shyr, C.R., C.C. Chen, T.F. Hsieh, C.H. Chang and W.L. Ma *et al*., 2013. The expression and actions of androgen receptor in Upper Urinary Tract Urothelial Carcinoma (UUTUC) tissues and the primary cultured cells. Endocrine, 43: 191-199.

Tsuei, D.J., P.H. Lee, H.Y. Peng, H.L. Lu and D.S. Su *et al*., 2011. Male germ cell-specific RNA binding protein RBMY: A new oncogene explaining male predominance in liver cancer. PLoS ONE, Vol. 6. 10.1371/journal.pone.0026948.

Walters, K.A., U. Simanainen and D.J. Handelsman, 2010. Molecular insights into androgen actions in male and female reproductive function from androgen receptor knockout models. Hum. Reprod. Update, 16: 543-558.

Weiss, B., H. Faus and B. Haendler, 2007. Phylogenetic conservation of the androgen receptor AR45 variant form in placental mammals. Gene, 399: 105-111.

Wu, Z.Y., K. Chen, B. Haendler, T.V. McDonald and J.S. Bian, 2008. Stimulation of N-terminal truncated isoform of androgen receptor stabilizes human ether-a-go-go-related gene-encoded potassium channel protein via activation of extracellular signal regulated kinase 1/2. Endocrinology, 149: 5061-5069.