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THE EXPANDED POLY-Q LENGTH WITHIN AR AND AR COREGULATOR AIB1 AND THEIR CLINICAL IMPLICATIONS

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INTRODUCTION

The cloning of the androgen receptor gene in 1988 (Chang *et al.*, 1988) and identification of androgen receptor (AR) coactivators have greatly improved our understanding of intracellular androgenic action and the role of androgenicity in many androgen-dependent disorders.

AR is a member of the steroid family of nuclear transcription factors, which also includes the estrogen, glucocorticoid, mineralocorticoid, progesterone, vitamin D, thyroid, and retinoic acid receptors (Tsai *et al.*, 1994). Like all nuclear receptors, AR binds to its ligands, mainly the androgen steroids testosterone (T) and 5 α -dihydrotestosterone (DHT), with a high affinity to control a number of biological processes and exert major effects on the reproductive system, prostate, testes, muscles, liver, skin, nervous system, and immune system (Culig *et al.*, 2000a). Because of the significance of AR in androgenicity, AR has been linked to a number of androgen-sensitive disorders. Specifically, the polyglutamine (poly-Q) length in the AR protein (encoded by the CAG repeat length in the androgen receptor gene *AR*) has been implicated in both progressive neurodegenerative diseases and androgen-dependent disorders (Brinkmann *et al.*, 2001). This review provides a summary of the clinical significance of the CAG repeat length in both the *AR* and *AIB1* (amplified in breast cancer 1) genes.

Structure of the AR protein and the AR gene

The AR protein is composed of 919 amino acids, with a molecular size of 98.8 kDa. It has three domains: the well-conserved ligand-binding, the DNA-binding, and the variable *N*-terminal domains (Figure 1). The AR protein is encoded by the *AR* gene located on the short arm of chromosome X (Xq11-12). The *AR* gene is composed of 8 exons separated by large intronic segments. It contains four main functional domains: the amino-terminal transcription activation (transactivation) domain (*N*-terminal domain), the DNA-binding domain (DBD), a hinge region, and the carboxy-terminal ligand-binding domain (LBD) (Culig *et al.*, 2000a) (Figure 1).

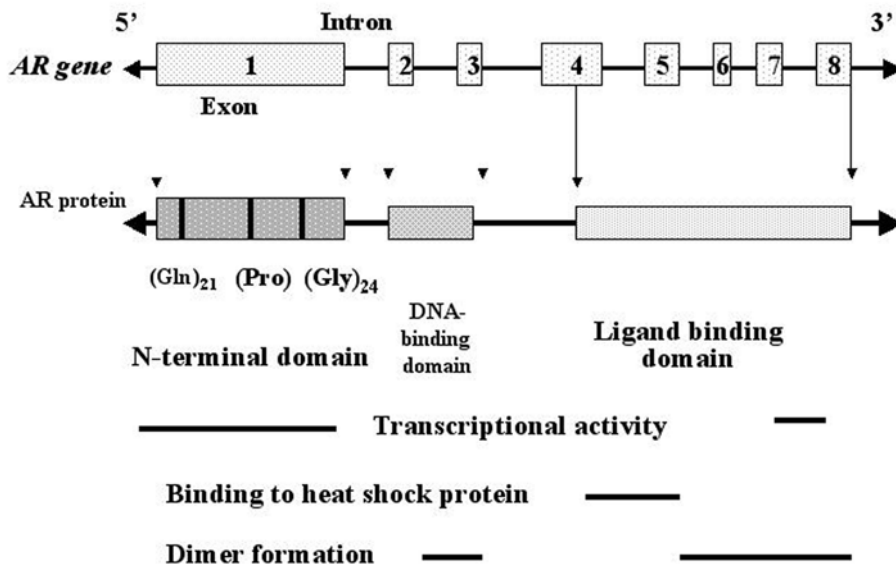


Figure 1: Genomic organization of the human androgen receptor gene and functional domain structure of the androgen receptor protein. The androgen receptor gene, located on X-chromosome q11-q12, consists of eight exons that encode for a 919 amino acid androgen receptor protein.

The transactivation domain in exon 1 encodes the *N*-terminal domain (the transactivation domain) of the AR protein. Exons 2 and 3 contain sequences for the DNA-binding domain, which is involved in receptor dimerization. A part of exon 4 contains information for the hinge region that

is located between the DNA and the ligand-binding domains. The ligand-binding domain is encoded by a part of exon 4 and entire exons 5-8. In exon 1, within the transactivation domain, there are three microsatellite trinucleotide repeats, two of which are polymorphic in length, namely the upstream CAG (encoding a polyglutamine region) and the downstream GGN trinucleotide (encoding a polyglycine region). Between these two regions is a non-polymorphic stretch of nine proline residues. The variability in the reported molecular size of the AR protein is due, in part, to the variable size of the glutamine and glycine repeats that are encoded by the CAG and GGN repeat lengths in exon 1 of the *AR* gene. Among healthy men, the CAG repeat length ranges from 13 to 40, while in most populations the GGN repeat length clusters around 23 (Hsing *et al.*, 2000; Platz *et al.*, 2000).

Figure 2. Androgenic Action within the Prostate

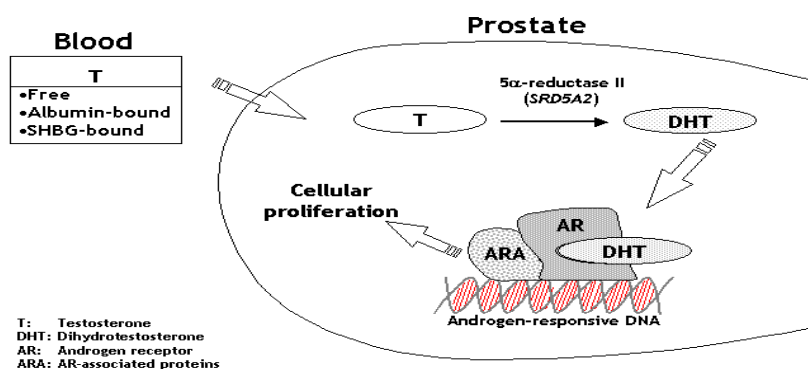


Figure 2. Within the prostate testosterone is metabolized into dihydrotestosterone by the enzyme 5α-reductase. DHT binds to the androgen receptor (AR) and AR coactivators. The intracellular complex is then translocated to the nucleus and binds to the androgen response element of the prostatic gene to induce cellular proliferation.

Function and mechanism

The AR is the vehicle through which androgens exert their regulation of cellular proliferation and differentiation. The unliganded AR is distributed throughout the cytoplasm and nucleus and is complexed with heat-shock proteins (HSP) 90, 70, and 56 to prevent constitutive activation of the receptor. Ligand-binding leads to the dissociation of heat-shock proteins and causes a typical conformational change in the ligand-binding domains of the

AR protein (Culig *et al.*, 2000a). The AR-DHT complex then translocates to the nucleus and binds to specific chromosomal DNA sequences (androgen response elements) in the regulatory regions (promoters/enhancers) of the *AR*-regulated gene (Figure 2).

It is well documented that the CAG repeat length in the *AR* gene is inversely related to the transactivation activity. Both *in vitro* and *in vivo* studies have shown that even within the normal range (between 13 and 40), a shorter CAG repeat length in the *AR* gene is related to increased AR transactivation activity and that deletion of the AR polyglutamine tract increases the AR transactivation activity without affecting hormone binding capacity. It has been reported that for each decrement of 10 in the CAG repeat length there is a 5-10% increase in *in vitro* transactivation (Chamberlain *et al.*, 1994).

AR AND CLINICAL DISORDERS

The AR protein and variations in the CAG trinucleotide repeat length in the *AR* gene have been implicated in various disease processes and hereditary traits, including several cancers (prostate, breast, male breast, and liver), hyperandrogenic disorders (male pattern baldness, hirsutism, and acne), benign prostatic hyperplasia (BPH), androgen insensitivity syndrome (AIS), polycystic ovarian syndrome (PCOS), and progressive neurodegenerative diseases, such as spinal bulbar muscular atrophy (SBMA, also called Kennedy's disease) (Table 1) (Casella *et al.*, 2001).

Table 1. The *AR* CAG repeat length and clinical disorders

<u>AR-related disorders</u>	<u>AR CAG repeat length</u>
<i>Confirmed</i>	
X-linked spinal bulbar muscular atrophy (SBMA) also called Kennedy's disease	>40
Male infertility	Longer
Androgen insensitivity	Longer
<i>Possible</i>	
Prostate cancer	Shorter

Liver cancer	Shorter
<i>Needs further investigation</i>	
Ovarian cancer	Shorter
Endometrial cancer	Longer
Female breast cancer	Longer
Male breast cancer	Longer
Testicular cancer	Longer
Hirsutism, acne	Shorter
Male pattern baldness	Shorter
Polycystic ovarian syndrome	Shorter
Benign prostatic hyperplasia	Shorter

Prostate cancer

The growth and maintenance of the prostate is dependent on androgens, in particular T and DHT. Unbound T in circulation diffuses and enters the prostate. Within the prostate, DHT, the principal nuclear androgen, binds to the AR with a high affinity to form an intracellular DHT-AR complex, which then binds to the androgen-response elements in the prostate DNA, ultimately inducing DNA synthesis and cellular proliferation (Hsing *et al.*, 2002a; Culig *et al.*, 2000a).

The development and progression of prostate tumors are thought to be androgen-dependent (Hsing, 2001a; Hsing *et al.*, 2002a). However, in late-stage prostate cancer, the tumors frequently become androgen independent (androgen refractory) due to mutations of androgen receptors. There are several lines of evidence to suggest that somatic mutation and amplification of the *AR* gene play a key role in the progression and prognosis of prostate cancer (Culig *et al.*, 2000).

Because of the importance of androgenic action in prostate carcinogenesis, in addition to *AR* mutation and amplification, the *AR* CAG repeat length in prostate cancer susceptibility has been investigated in more than a dozen studies (Table 2) (Modugno *et al.*, 1995; Hardy *et al.*, 1996; Stanford *et al.*, 1997; Giovannucci *et al.*, 1997; Edwards *et al.*, 1999; Platz *et al.*, 1998; Sartor *et al.*, 1999; Bratt *et al.*, 1999; Correa-Cerro *et al.*, 1999;

Lange *et al.*, 2000; Nam *et al.*, 2000; Xue *et al.*, 2000; Hsing *et al.*, 2000; Lantil *et al.*, 2001; Beilin *et al.*, 2001; Panz *et al.*, 2001; Modugno *et al.*, 2001). The results are inconsistent. For example, four studies showed that men with a shorter CAG repeat length were at higher risk of prostate cancer (Stanford *et al.*, 1997; Giovannucci *et al.*, 1997; Hsing *et al.*, 2000; Xue *et al.*, 2000) whereas others did not confirm these findings (Edwards *et al.*, 1999; Lange *et al.*, 2000; Beilin *et al.*, 2001). Differences in study population, study design, selection of study subjects, and methods of analysis may account for some of the discrepancy in results across studies. In all of these studies, however, the length of CAG repeats corresponded to racial variation in prostate cancer risk: African-Americans, who have a high risk of prostate cancer, had a shorter CAG repeat length (usually around 19); Caucasians had an intermediate repeat length (between 20 and 21); and Asian men, who have a much lower risk of prostate cancer, had a longer repeat length (between 22 and 24) (Hsing *et al.*, 2002a). These data suggest that androgenicity may play a role in the substantial racial difference in prostate cancer risk across populations (Hsing *et al.*, 2001b).

Other Cancers

AR has also been implicated in several other cancers that have a hormonal basis, including cancers of the liver, male and female breast, endometrium, and ovary.

Liver cancer. Both epidemiological and laboratory studies suggest that androgen is involved in the pathogenesis of hepatocellular carcinoma (HCC). Castration of male mice decreased the incidence of chemically-induced liver tumors (Toh *et al.*, 1981), whereas chronic administration of T increased the risk of spontaneous or chemically-induced HCC in laboratory animals (Ostrowski *et al.*, 1998). Epidemiologic observations suggest that the incidence of primary liver cancer, in particular HCC, is 2-3 times higher among men than women in most countries, leading to the proposal that androgens may be involved in the pathogenesis of liver cancer. A nested case-control study conducted in Taiwan, a hyperendemic area with chronic infection by the hepatitis B virus (HBV), showed that higher serum levels of T at baseline were associated with subsequent risk of HCC, further suggesting a role of androgens in HCC (Yu *et al.*, 2001). More recently, a study of 285 HBV carriers with HCC and 349 HBV carriers without HCC showed that independent of other liver cancer risk factors, a shorter CAG repeat length (<20 vs. >24) was associated with a 2-fold risk of HCC (Yu *et al.*, 2000). HBV carriers with an *AR* CAG repeat length of 20 or less and

higher T levels had a 4-fold increase in HCC risk compared with those with more than 23 repeats and T in the lowest tertile.

Breast cancer. The AR is expressed in normal breast tissue and in some breast tumors (Hall *et al.*, 1996). Because androgens can inhibit the growth of certain breast cancer cell lines, and because ectopic expression of AR leads to the inhibition of breast tumor cell proliferation in response to androgen, it has been suggested that a longer CAG repeat length in the AR gene may confer a higher risk of female breast cancer. Five studies have examined the role of AR CAG repeat length in female breast cancer, with inconsistent results. One study reported that a longer CAG repeat length is associated an increased risk of breast cancer among postmenopausal women (Giguere *et al.*, 2001). Two studies reported no association between AR CAG repeat length and breast cancer in premenopausal or young women (age<40) (Dunning *et al.*, 1999; Spurdle *et al.*, 1999). One study reported that a longer CAG repeat length is associated with less aggressive breast tumors (Yu *et al.*, 2000), and one study reported that breast cancer patients who are also carriers of the BRCA1 mutation had a longer CAG repeat length (Rebbeck *et al.*, 1999). However, this last observation was not confirmed in a study conducted in subjects of Ashkenazi origin (Kadouri *et al.*, 2001). Mutations in the AR gene have also been observed in patients with male breast cancer, but epidemiologic data on polymorphisms are limited.

Ovarian and Endometrial cancers. As androgenicity is associated with the length of AR CAG repeat, and androgen exposure is a risk factor for cancer of the ovary, a shorter CAG repeat length has been hypothesized to increase the risk of ovarian cancer. Thus far, epidemiologic evidence for an association between AR CAG repeat length and cancers of the ovary and endometrium is limited and conflicting. Although some studies have suggested that a shorter CAG repeat length is associated with a younger age of onset of ovarian cancer (Levine *et al.*, 2001), others reported no association (Spurdle *et al.*, 2000).

Table 2. CAG repeat length in the AR gene in relation to prostate cancer

<u>Author</u>	<u>Study design</u>	<u>Results</u>
<i>AR (Xq11-12)</i> Irvine <i>et al.</i> , 1995	CAG repeat Prevalence survey 45 African Americans 39 non-Hispanic whites 39 Asians	<22 repeats: 25% excess

	68 prostate cancer cases (U.S. whites)	
Hardy <i>et al.</i> , 1996	Cross-sectional 109 U.S. White cases	A shorter repeat was associated with younger age at diagnosis
Stanford <i>et al.</i> , 1997	Case-control U.S. whites 302 cases/277 controls	<22: 23% excess risk (0.88 to 1.73)
Giovannucci <i>et al.</i> , 1997	Nested case-control U.S. Whites 587 cases/588 controls	18 vs. 26: 52% excess risk (0.92 to 2.49)
Edwards <i>et al.</i> , 1999	Case-control	No association
Sartor <i>et al.</i> , 1999	Prevalence (no cancer) 65 U.S. Blacks & 130 Whites	Mean CAG repeat length: Whites: 21; blacks: 19
Bratt <i>et al.</i> , 1999	Case-control Swedes 190 cases/186 controls	A shorter repeat length was associated with earlier age at diagnosis and high-grade, high-stage tumors
Correa-Cerro <i>et al.</i> , 1999	Case-control French & German Whites 132 cases/105 controls	<22: 20% excess risk (0.7 to 2.0)
Lange <i>et al.</i> , 2000	Case-control U.S. Whites 270 subjects	>21: 15% reduction in risk (0.53 to 1.35)
Nam <i>et al.</i> , 2000	Case-series 318 Canadian men	<18: 8-fold risk (2.02 to 32.2)
Xue <i>et al.</i> , 2000	Case-control	<20: 97% excess risk (1.05 to 3.72)
Hsing <i>et al.</i> , 2000	Case-control Chinese 189 cases/301 controls	<23: 65% excess risk (1.14 to 2.39)
Latil <i>et al.</i> , 2001	Case-control	≤20 vs. 24: 10% excess

	French 268 cases/156 controls	risk (0.60 to 2.02)
Modugno <i>et al.</i> , 2001	Case-control U.S. Whites 449 cases/558 controls	<23: 75% excess risk (1.05-2.94)
Beilin <i>et al.</i> , 2001	Case-control Australia 448 cases/456 controls	Every 5 additional CAG repeats: 0.98 (0.84 to 1.15)
Panz <i>et al.</i> , 2001	Case-control South Africa 40 Africans and 40 Whites	Cases had a shorter repeat length than controls (20 vs. 23)

Non-cancer disorders

X-linked spinal bulbar muscular atrophy. The link between SBMA and the abnormal expansion of the CAG repeat length (between 40 and 62) in the *AR* gene is well established. SBMA is a severe, rare form of an X-linked adult-onset neurodegenerative syndrome characterized by progressive muscle weakness and atrophy, dysphagia (difficulty in swallowing), and fasciculations (twitch). The disease is complicated by bulbar muscle involvement. Affected males usually have normal male development but may have gynecomastia (enlarged breast in men), testicular atrophy, and oligospermia (an abnormally low concentration of spermatozoa in the semen), suggesting a defect in AR function. An inverse correlation between age of onset and number of CAG repeats has been reported (Doyu *et al.*, 1994). In addition, *in vivo* studies have shown that transgenic mice with highly expanded CAG repeat lengths (>239) developed progressive neurological phenotypes of muscular weakness and shortened life span (Adachi *et al.*, 2001).

The exact molecular mechanism underlying SBMA is unknown. Several hypotheses have been proposed to explain the pathogenesis of SBMA. Thus far, none of these is related to the classic role of AR in androgenicity. The first hypothesis holds that because polyglutamine can form stable β -pleated sheets that enable protein-protein interaction, it is possible that the expanded polyglutamine repeat in the AR protein might lead to aberrant transcriptional regulation of target genes, resulting in motor-neuron death (Brooks, 1995). An alternative hypothesis suggests that the

expansion of the polyglutamine repeat could heighten a normal interaction of novel protein complexes that is toxic to neurons. A third hypothesis is that the expanded polyglutamine may link up with other proteins, making it difficult for cells to degrade, and the protein may then accumulate over time intracellularly, leading to disruption of normal cellular function (Brooks 1995). These proposed mechanisms are not limited to CAG expansion in the AR protein. CAG expansion in several other genes has also been linked to other neurodegenerative disorders, such as Huntington's disease (Yong *et al.*, 2000; Lieberman *et al.*, 2000).

Androgen insensitivity syndrome. Androgen insensitivity syndromes (AIS) include gynecomastia, testicular atrophy, oligospermia, azospermia (absence of sperm in semen), and elevated serum gonadotropins (MacLean *et al.*, 1995). In AIS, androgen cannot complete male genital development due to mutations in the *AR* gene. Typically, there is increased feminization or undermasculinization of the external genitalia at birth, abnormal secondary sexual development in puberty, and infertility (Abdullah *et al.*, 1998). The incidence of AIS is 1 in 200,000 to 1 in 65,000. Carrier women have a 50% chance of transmitting the *AR* gene mutation in each pregnancy. AIS can be further subdivided into complete androgen insensitivity syndrome (CAIS), partial androgen insensitivity syndrome (PAIS), and mild androgen insensitivity syndrome (MAIS). The differentiation of these subcategories is based on clinical findings, laboratory evaluations, and family history of X-linked inheritance (Gottlieb 1999).

A number of types of *AR* mutations, including single base mutations, complete or partial gene deletions, intron deletions, and insertions, are related to various forms and severity of AIS (Gottlieb *et al.*, 1999). Longer CAG repeat lengths may be partially responsible for the androgen insensitivity phenotype (Brickmann *et al.*, 2001), although the evidence is inconclusive.

Male infertility. It has been shown that some patients with defective spermatogenesis have a significantly longer CAG repeat length than healthy men, suggesting a possible role of AR in male infertility due to lower functional activity associated with a longer polyglutamine (Wieacker *et al.*, 1998). The epidemiologic evidence for an association with longer CAG repeat length with male infertility is inconsistent. In some studies conducted in American, Swedish, and German patients, no association was found for CAG repeat length (Dadze *et al.*, 2000) However, in other studies conducted in Chinese, Belgian, and Australian patients, a longer *AR* CAG repeat length was associated with infertility (Legius *et al.*, 1999). In general, the longer

the *AR* CAG repeat length, especially over 28, the greater the risk of impaired spermatogenesis and the more severe the defect (Yong *et al.*, 2000).

Benign Prostatic Hyperplasia. Like prostate cancer, BPH is a disease of older men. Age and functioning testes are the only known risk factors for BPH. Because BPH is also an androgen-dependent disease, *AR* has been implicated in its etiology. *AR* mutations in BPH are uncommon, but CAG repeat length has been investigated. Giovannucci *et al.*, in two separate studies found a shorter CAG repeat length to be associated with obstructive symptoms or with BPH that requires surgical intervention (Giovannucci *et al.*, 1999a; Giovannucci *et al.*, 1999b). They reported that every 6-repeat decrease in CAG repeat length resulted in a 3.6-fold risk of symptomatic BPH. However, others found that the length of the CAG repeat was not related to BPH risk or prostate size (Bousema *et al.*, 2000).

Dermatologic disorders. Because hyperandrogenic dermatologic disorders, including alopecia (balding), hirsutism (defined as an increase in amount and/or coarseness of hair distributed in male pattern in a female), and acne are androgen-dependent diseases it has been suggested that variation in CAG repeat length may be linked to these cutaneous disorders (Sawaya *et al.*, 1998; Vottero *et al.*, 1999). Epidemiologic evidence for the role of CAG repeat length in these disorders is still limited.

Polycystic ovarian syndrome. PCOS is probably the most common hormonal abnormality in women of reproductive age and the leading cause for infertility. PCOS is an endocrine disorder in premenopausal women characterized by irregular menstruation, anovulatory infertility, and excess androgen (Hickey *et al.*, 2002). The cause of PCOS is unknown. Women with PCOS produce higher levels of insulin, which in turn signals the body to release T. As a result, women with PCOS are more likely to develop hyperandrogenic skin disorders such as male pattern baldness, hirsutism, and acne (Scarpitta *et al.*, 2000). Two studies have investigated the role of CAG repeat length in PCOS. Among Australian women a longer CAG repeat length was associated with PCOS in women with higher serum levels of T (Hickey *et al.*, 2002), while in a study in Singapore, lower levels of T and a shorter CAG repeat length were associated with PCOS (Mifsud, 2000).

Cardiovascular disease and rheumatoid arthritis. Because increased *AR* CAG repeat length has been linked to higher serum levels of high density lipoprotein cholesterol (HDL), apoA-I, and vasodilatation, it is possible that

the AR protein may affect the risk of cardiovascular disease (Zitzmann *et al.*, 2001). Although the results are inconclusive, data exist to suggest that genes involved in HDL metabolism may be regulated by AR (Zitzmann *et al.*, 2001). Because it is well known that estrogens and androgens have an effect on cardiovascular diseases and because of the significance of AR in androgenicity, the role of CAG repeat length on cardiovascular disease warrants further investigation.

A shorter CAG repeat length has also been shown to be associated with younger age of onset of rheumatoid arthritis, suggesting the possible role of androgens as a modulating factor (Kawasaki *et al.*, 1999).

AR COACTIVATORS

It has become evident that activities of AR depend not only on the levels of expression of the receptor protein itself but also on those of coregulatory proteins. After binding androgen, the AR-DHT complex targets gene promoters. This process is facilitated by a set of proteins, the coactivators. These coactivators are usually large nuclear proteins that bridge the receptors to the pre-initiation complex. In some cases, the coactivators change the structure of the nucleosome, making the DNA more accessible to transcription factors. Interaction of the AR protein with an array of coactivators have been reported (Yeh *et al.*, 2000). Such an interaction usually leads to a ligand-dependent enhancement of AR activity by 10- to 30-fold (Yeh, 1996; Kang, 1999). To date, a series of AR coactivators (ARAs), have been identified, including ARA70, ARA54, ARA55, ARA24, ARA160, AIB1 (Amplified in Breast Cancer 1), BRCA1, and Rb (Culig *et al.*, 2000; Grossmann *et al.*, 2001; Yeh *et al.*, 2000) (Figure 3). The interactions between these proteins with the AR protein are complex and not well understood.

Previous studies have shown that three coactivators, including RAC3, SRC-1, and transcription intermediary factor 2) (TIF-2), are up-regulated in prostate cancer (Gnanapragasam *et al.*, 2001; Gregory *et al.*, 2001). Because of their significance in AR transactivation, the role of these coactivators in androgen-dependent diseases and in certain progressive neurodegenerative disorders should be examined in future studies.

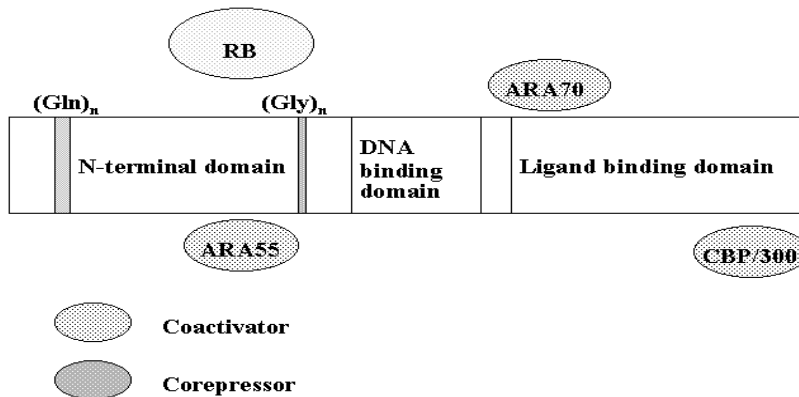


Figure 3. Interaction between the androgen receptor protein and its coactivators. An array of proteins, including RB, ARA70, and ARA55, can interact with the AR protein to increase androgenic action at the target site.

Amplified in Breast Cancer 1

One of the AR coactivators is Amplified in Breast Cancer 1 (*AIB1*). Like the AR protein, *AIB1* contains glutamine-rich and polyglutamine regions in its C-terminal domain. Therefore, it is suggested that glutamine-rich regions in the *AIB1* protein may mediate protein-protein interaction and that variation in the polyglutamine region of *AIB1* could influence its interaction with AR or the basal transcriptional machinery, thereby contributing to certain clinical disorders. The *AIB1* protein is encoded by the *AIB1* gene on chromosome 20. This gene is amplified in 10% of estrogen-receptor positive breast cancers (Anzick *et al.*, 1997). Thus far, two epidemiologic studies have investigated the role of *AIB1* in prostate cancer; one found a positive association between *AIB1* CAG repeat length and prostate cancer (Hsing *et al.*, 2002b), and the other reported no association (Platz *et al.*, 1999).

FUTURE RESEARCH

Basic science

Although the structure and functional importance of the *AR* gene are better understood than some other nuclear receptors, the exact molecular process by which CAG expansion modulates the risk of diseases is unclear. An important area for future research is to clarify the molecular mechanisms so that better therapeutic approaches can be developed to treat AR poly-Q-related diseases. Molecular characterization of structural domains and function of AR coactivators will also help to provide a better understanding of the protein-protein interaction. Another area of focus for future research is likely to be on why different lengths of AR poly-Q, together with their different AR transcriptional activities, contribute to the progress of cancer or SMBA (Kennedy's disease). In addition, whether protease degradation rates can be different among different poly-Q ARs may become a focus in this field. It is likely that different poly-Q lengths within the AR protein may result in different conformations in AR's tertiary structure, which in turn may interact differentially with various AR coregulators or progress into different protein degradation pathways.

Epidemiologic studies

Epidemiologic studies investigating the etiology of hormone-dependent diseases, such as prostate cancer, have focused on comparing circulating levels of T in patients and in healthy men. The role of receptors is rarely evaluated in population-based epidemiologic studies due largely to the difficulty in obtaining tissue for receptor measurement from patients. It is important to bear in mind that serum levels of androgens are only a small and transitory step in the cascade of hormone action from production to biological effect. With the advances in molecular techniques, more recent epidemiologic studies have begun to investigate polymorphic markers and the risk of disease. For CAG repeat length, most studies to date have focused on the role of *AR* CAG repeat length in prostate cancer. Because of its profound effect in androgenicity, the role of AR in a number of androgen-dependent diseases, such as the ones mentioned earlier, warrants further investigation in future epidemiologic studies.

The advent of DNA microchips provides an unusual opportunity for the rapid assessment of multiple genetic variants simultaneously. In future studies, it will be important to evaluate the effect of *AR* CAG repeat length in conjunction with genetic variants of other coactivators to provide a more complete picture of the role of AR. Like the variation in *AR* CAG repeat in

various racial groups, it is possible that racial differences exist in the polymorphisms of these AR coactivators. Whether genetic variants in these coactivators may help explain the large racial differences in the risk of certain diseases, such as cancer of the prostate, needs further clarification. The relationships between circulating and tissue levels of androgens and *AR* CAG repeat length and their roles in these clinical disorders should also be clarified.

CLINICAL APPLICATIONS

Given the importance of AR in androgenicity and the biological effect of *AR* CAG repeat length on AR function, polymorphic variation of the CAG repeat length in the *AR* gene probably contributes to the observed individual phenotype differences in androgenization. Thus, for certain androgen-related disorders, it seems important that assessment of androgen levels, *AR* CAG repeat length, and AR function should be made to provide a more comprehensive clinical assessment in order to make better clinical decisions.

AR CAG repeat length may also be involved in determining the magnitude of cellular responses to carcinogenic events resulting in the development or progression of clinical disorders, such as prostate cancer. It is likely that *AR* CAG repeat length contributes to susceptibility of these disorders in a subset of high-risk population. Thus, it may be useful in the future to identify individuals at high risk of developing severe diseases at an earlier stage through molecular characterization, thereby improving survival and prognosis.

A better understanding of the molecular structure of AR and AR coactivators and their interactions will also ultimately help us to better understand intracellular androgen mechanisms, which will lead to more effective therapy through development of better antiandrogens or gene-directed therapy.

REFERENCES

- Abdullah, A., Trifiro, M.A., Panet-Raymond, V., Alvarado, C., de Turreil, S., Frankel, D., Schipper and H.M., Pinsky, L. Spinobulbar muscular atrophy: polyglutamine-expanded androgen receptor is proteolytically resistant in vitro and processed abnormally in transfected cells. *Hum Mol Genet* 1998; 7: 379-384.
- Adachi, H., Kume, A., Li, M., Nakagomi, Y., Niwa, H., Do, J., Sang, C., Kobayashi, Y., Doyu, M. and Sobue, G. Transgenic mice with an expanded CAG repeat controlled

- by the human AR promoter show polyglutamine nuclear inclusions and neuronal dysfunction without neuronal cell death. *Hum. Mol. Genet.* 2001; 10: 1039-1048.
- Anzick, S.L., Kononen, J., Walker, R.L., Azorsa, D.O., Tanner, M.M., Guan, X.Y., Sauter, G., Kallioniemi, O.P., Trent, J.M. and Meltzer, P.S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* 1997; 277: 965-968.
- Beilin, J., Harewood, L., Frydenberg, M., Mameghan, M., Martyres, R.F., Farish, S.J., Yue, C., Deam, D.R., Byron, K.A. and Zajac, J.D. A case-control study of the androgen gene CAG repeat polymorphism in Australian prostate carcinoma subjects. *Cancer* 2001; 92: 941-949.
- Bratt, O., Borg, A., Kristoffersson, U., Lundgren, R., Zhang, Q.X. and Olsson, H. CAG repeat length in the androgen receptor gene is related to age at diagnosis of prostate cancer and response to endocrine therapy, but not to prostate cancer risk. *Br. J. Cancer* 1999; 81: 672-676.
- Brinkmann, A.O. Molecular basis of androgen insensitivity. *Mol. Cell. Endocrinol.* 2001; 179: 105-109.
- Brooks, B.P. and Fischbeck, K.H. Spinal and bulbar muscular atrophy: a trinucleotide-repeat expansion neurodegenerative disease. *Trends Neurosci.* 1995; 18: 459-461.
- Casella, R., Maduro, M.R., Lipshultz, L.I. and Lamb, D.J. Significance of the polyglutamine tract polymorphism in the androgen receptor. *Urology* 2001; 58:651-656.
- Chamberlain, N.L., Driver, E.D. and Miesfeld, R.L. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res.* 1994; 22: 3181-3186.
- Chang, C.S., Kokontis, J. and Liao, S.T. Molecular cloning of human and rat complementary DNA encoding androgen receptors. *Science* 1988; 240: 324-326.
- Correa-Cerro, L., Wohr, G., Haussler, J., Berthon, P., Drelon, E., Mangin, P., Fournier, G., Cussenot, O., Kraus, P., Just, W., Paiss, T., Cantu, J.M. and Vogel, W. (CAG)_nCAA and GGN repeats in the human androgen receptor gene are not associated with prostate cancer in a French-German population. *Eur. J. Hum. Genet.* 1999; 7: 357-362.
- Culig, Z., Hobisch, A., Bartsch, G. and Klocker, H. Androgen receptor—an update of mechanisms of action in prostate cancer. *Urol. Res.* 2000a; 28: 211-219.
- Culig, Z., Hobisch, A., Bartsch, G. and Klocker, H. Expression and function of androgen receptor in carcinoma of the prostate. *Microsc. Res. Tech.* 2000b; 51: 447-55.
- Dadze, S., Wieland, C., Jakubiczka, S., Funke, K., Schroder, E., Royer-Pokora, B., Willers, R. and Wieacker, P.F. The size of the CAG repeat in exon 1 of the androgen receptor gene shows no significant relationship to impaired spermatogenesis in an infertile Caucasoid sample of German origin. *Mol. Hum. Reprod.* 2000; 6: 207-214.
- Dunning, A.M., McBride, S., Gregory, J., Durocher, F., Foster, N.A., Healey, C.S., Smith, N., Pharoah, P.D., Luben, R.N., Easton, D.F. and Ponder, B.A. No association between androgen or vitamin D receptor gene polymorphisms and risk of breast cancer. *Carcinogenesis* 1999; 20: 2131-2135.
- Doyu, M., Sobue, G., Kimata, K., Yamamoto, K. and Mitsuma, T. Androgen receptor mRNA with increased size of tandem CAG repeat is widely expressed in the neural and nonneural tissues of X-linked recessive bulbospinal neuronopathy. *J. Neurol. Sci.* 1994; 127: 43-47.
- Edwards, S.M., Badzioch, M.D., Minter, R., Hamoudi, R., Collins, N., Ardern-Jones, A., Dowe, A., Osborne, S., Kelly, J., Shearer, R., Easton, D.F., Saunders, G.F., Dearnaley, D.P. and Eeles, R.A. Androgen receptor polymorphisms: association with prostate cancer risk, relapse and overall survival. *Int. J. Cancer.* 1999; 84: 458-465.
- Giguere, Y., Dewailly, E., Brisson, J., Ayotte, P., Laflamme, N., Demers, A., Forest, V.I.,

- Dodin, S., Robert, J. and Rousseau, F. Short polyglutamine tracts in the androgen receptor are protective against breast cancer in the general population. *Cancer Res.* 2001; 61: 5869-5874.
- Giovannucci, E., Stampfer, M.J., Krithivas, K., *et al.* The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. *Proc. Natl. Acad. Sci. USA* 1997; 94: 3320-3323.
- Giovannucci, E., Stampfer, M.J., Chan, A., Krithivas, K., Gann, P.H., Hennekens, C.H. and Kantoff, P.W. CAG repeat within the androgen receptor gene and incidence of surgery for benign prostatic hyperplasia in U.S. physicians. *Prostate* 1999; 39: 130-134.
- Giovannucci, E., Platz, E.A., Stampfer, M.J., Chan, A., Krithivas, K., Kawachi, I., Willett, W.C. and Kantoff, P.W. The CAG repeat within the androgen receptor gene and benign prostatic hyperplasia. *Urology* 1999; 53:121-125.
- Gnanapragasam, V.J., Leung, H.Y., Pulimood, A.S., Neal, D.E. and Robson, C.N. Expression of RAC 3, a steroid hormone receptor co-activator in prostate cancer. *Br. J. Cancer* 2001; 85: 1928-1936.
- Gnanapragasam, V.J., McCahy, P.J., Neal, D.E. and Robson, C.N. Insulin-like growth factor II and androgen receptor expression in the prostate. *Br. J. Urol. Int.* 2000; 86: 731-735.
- Grossmann, M.E., Huang, H. and Tindall, D.J. Androgen receptor signaling in androgen-refractory prostate cancer. *J. Natl. Cancer Inst.* 2001; 93: 1687-1697.
- Gottlieb, B., Pinsky, L., Beitel, L.K. and Trifiro, M. Androgen insensitivity. *Am. J. Med. Genet.* 1999; 89: 210-217.
- Gregory, C.W., He, B., Johnson, R.T., Ford, O.H., Mohler, J.L., French, F.S. and Wilson, E.M. A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. *Cancer Res.* 2001; 61: 4315-4319.
- Hall, R.E., Aspinall, J.O., Horsfall, D.J., Birrell, S.N., Bentel, J.M., Sutherland, R.L. and Tilley, W.D. Expression of the androgen receptor and an androgen-responsive protein, apolipoprotein D, in human breast cancer. *Br. J. Cancer*, 1996; 74: 1175-1180.
- Hardy, D.O., Scher, H.I., Bogenreider, T., Sabbatini, P., Zhang, Z.F., Nanus, D.M. and Catterall, J.F. Androgen receptor CAG repeat lengths in prostate cancer: correlation with age of onset. *J. Clin. Endocrinol. Metab.* 1996; 81: 4400-4405.
- Hickey, T., Chandy, A. and Norman, R.J. The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 2002; 87:161-165.
- Hsing, A.W. Hormones and prostate cancer: What's next? *Epidemiol. Rev.* 2001a; 23: 42-58.
- Hsing, A.W., Gao, Y-T., Wu, G., Wang, X., Deng, J., Sesterhenn, I.A., Mostofi, K.F., Benchiou, J. and Chang, C. Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. *Cancer Res.* 2000; 60: 5109-5114.
- Hsing, A.W. and Devesa, S.S. Trends and patterns in prostate cancer risk: what do they suggest? *Epidemiol. Rev.* 2001b; 23: 3-13.
- Hsing, A.W., Reichardt J., K. and Stanczyk, F.Z. Hormones and prostate cancer: current perspectives and future directions *The Prostate* 2002a; (in press)
- Hsing, A.W., Chokkalingam, A.P., Gao, Y.T., Wu, G., Wang, X., Deng, J., Cheng, J., Sesterhenn, I.A., Mostofi, F.K., Chiang, T., Chen, Y.L., Stanczyk, F.Z. and Chang C. Polymorphic CAG/CAA repeat length in the AIB1/SRC-3 gene and prostate cancer risk: a population-based case-control study. *Cancer Epidemiol. Biomarkers Prev.* 2002b; 11:337-341.
- Irvine, R.A., Yu, M.C., Ross, R.K. and Coetzee, G.A. The CAG and GGC microsatellites of

- the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. *Cancer Res.* 1995; 55: 1937-1940.
- Kadouri, L., Easton, D.F., Edwards, S., Hubert, A., Kote-Jarai, Z., Glaser, B., Durocher, F., Abeliovich, D., Peretz, T. and Eeles, R.A CAG and GGC repeat polymorphisms in the androgen receptor gene and breast cancer susceptibility in BRCA1/2 carriers and non-carriers. *Br. J. Cancer* 2001; 85: 36-40.
- Kantoff, P., Giovannucci, E. and Brown, M. The androgen receptor CAG repeat polymorphism and its relationship to prostate cancer. *Biochim Biophys Acta* 1998; 1378: C1-5.
- Kawasaki, T., Ushiyama, T., Ueyama, H., Inoue, K., Mori, K., Ohkubo, I. and Hukuda, S. Polymorphic CAG repeats of the androgen receptor gene and rheumatoid arthritis. *Ann. Rheum. Dis.* 1999; 58: 500-502.
- King, B.L., Peng, H.Q., Goss, P., Huan, S., Bronson, D., Kacinski, B.M. and Hogg, D. Repeat expansion detection analysis of (CAG)_n tracts in tumor cell lines, testicular tumors, and testicular cancer families. *Cancer Res.* 1997; 57: 209-214.
- Lange, E.M., Chen, H., Brierley, K., Livermore, H., Wojno, K.J., Langefeld, C.D., Lange, K. and Cooney, K.A The polymorphic exon 1 androgen receptor CAG repeat in men with a potential inherited predisposition to prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* 2000; 9: 439-442.
- Latil, A.G., Azzouzi, R., Cancel, G.S., Guillaume, E.C., Cochon-Priollet, B., Berthon, P.L. and Cussenot, O. Prostate carcinoma risk and allelic variants of genes involved in androgen biosynthesis and metabolism pathways. *Cancer* 2001; 92: 1130-1137.
- Legius, E., Vanderschueren, D., Spiessens, C., D'Hooghe, T. and Matthijs, G. Association between CAG repeat number in the androgen receptor and male infertility in a Belgian study. *Clin. Genet.* 1999; (6): 166-167.
- Levine, D.A. and Boyd, J. The androgen receptor and genetic susceptibility to ovarian cancer: results from a case series. *Cancer Res.* 2001; 61: 908-911.
- Lieberman, A.P. and Fischbeck, K.H. Triplet repeat expansion in neuromuscular disease. *Muscle Nerve* 2000; 843-850.
- MacLean, H.E., Chu, S., Joske, F., Warne, G.L. and Zajac, J.D. Androgen receptor binding studies on heterozygotes in a family with androgen insensitivity syndrome. *Biochem. Mol. Med.* 1995; 55: 31-37.
- Mifsud, A., Ramirez, S. and Yong, E.L. Androgen receptor gene CAG trinucleotide repeats in anovulatory infertility and polycystic ovaries. *J. Clin. Endocrinol. Metab.* 2000; 85: 3484-3488.
- Modugno, F., Weissfeld, J.L., Trump, D.L., Zmuda, J.M., Shea, P., Cauley, J.A., Ferrell, R.E. Allelic variants of aromatase and the androgen and estrogen receptors: toward a multigenic model of prostate cancer risk. *Clin. Cancer Res.* 2001; 7: 3092-3096.
- Mononen, N., Syrjakoski, K., Matikainen, M., Tammela, T.L., Schleutker, J., Kallioniemi, O.P., Trapman, J. and Koivisto, P.A Two percent of Finnish prostate cancer patients have a germ-line mutation in the hormone-binding domain of the androgen receptor gene. *Cancer Res.* 2000; 60: 6479-6481.
- Montgomery, J.S, Price, D.K. and Figg, W.D. The androgen receptor gene and its influence on the development and progression of prostate cancer. *J. Pathol.* 2001; 195: 138-146.
- Munoz de Toro, M.M., Maffini, M.V., Kass, L. and Luque, E.H. Proliferative activity and steroid hormone receptor status in male breast carcinoma. *J. Steroid Biochem. Mol. Biol.* 1998; 67: 333-339.
- Nam, R.K., Elhaji, Y., Krahn, M.D., Hakimi, J., Ho, M., Chu, W., Sweet, J., Ostrander, E.A. and Stanford, J.L Genetics of prostate cancer: too many loci, too few genes. *Am. J. Hum. Genet.* 2000; 67: 1367-1375.

- Ostrowski, J., Florio, S.K., Denis, G.V., Suzuki, H. and Bomsztyk, K. Stimulation of p85/RING3 kinase in multiple organs after systemic administration of mitogens into mice. *Oncogene* 1998; 16: 1223-1227.
- Panz, V.R., Joffe, B.I., Spitz, I., Lindenberg, T., Farkas, A. and Haffejee, M. Tandem CAG repeats of the androgen receptor gene and prostate cancer risk in black and white men. *Endocrine* 2001; 15: 213-216.
- Platz, E.A., Giovannucci, E., Dahl, D.M., Krithivas, K., Hennekens, C.H., Brown, M., Stampfer, M.J. and Kantoff, P.W. The androgen receptor gene GGN microsatellite and prostate cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 1998; 7: 379-384.
- Platz, E.A., Giovannucci, E., Brown, M., Cieluch, C., Shepard, T.F., Stampfer, M.J. and Kantoff, P.W. Amplified in breast cancer-1 glutamine repeat and prostate cancer risk. *Prostate J.* 2000; 2: 27-32.
- Rebbeck, T.R., Kantoff, P.W., Krithivas, K., Neuhausen, S., Blackwood, M.A., Godwin, A.K., Daly, M.B., Narod, S.A., Garber, J.E., Lynch, H.T., Weber, B.L. and Brown, M. Modification of BRCA1-associated breast cancer risk by the polymorphic androgen-receptor CAG repeat. *Am. J. Hum. Genet.* 1999; 64: 1371-1377.
- Sartor, O., Zheng, Q. and Eastham, J.A. Androgen receptor gene CAG repeat length varies in a race-specific fashion in men without prostate cancer. *Urology* 1999; 53: 378-380.
- Sawaya, M.E. and Shalita, A.R. Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia, hirsutism, and acne. *J. Cutan. Med. Surg.* 1998; 3: 9-15.
- Spurdle, A.B., Dite, G.S., Chen, X., Mayne, C.J., Southey, M.C., Batten, L.E., Chy, H., Trute, L., McCredie, M.R., Giles, G.G., Armes, J., Venter, D.J., Hopper, J.L. and Chenevix-Trench G. Androgen receptor exon 1 CAG repeat length and breast cancer in women before age forty years. *J. Natl. Cancer Inst.* 1999; 91: 961-966.
- Spurdle, A.B., Webb, P.M., Chen, X., Martin, N.G., Giles, G.G. Hopper, J.L., and Chenevix-Trench, G.. Androgen receptor exon 1 CAG repeat length and risk of ovarian cancer. *Int. J. Cancer* 2000; 87: 637-643.
- Stanford, J.L., Just, J.J., Gibbs, M., Wicklund, K.G., Neal, C.L., Blumenstein, B.A. and Ostrander, E.A. Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. *Cancer Res.* 1997; 57: 1194-1198.
- Stanford, J., and Ostrander, E. Familial prostate cancer. *Epidemiol. Rev.* 2001; 23: 19-23.
- Scarpitta, A.M. and Sinagra, D. Polycystic ovary syndrome: an endocrine and metabolic disease. *Gynecol. Endocrinol.* 2000; 4: 392-395.
- Toh, Y.C. Effect of neonatal castration on liver tumor induction by N-2-fluorenylacetyamide in suckling BALB/c mice. *Carcinogenesis* 1981; 2: 1219-1221.
- Trachtenberg, J., Jewett, M.A. and Narod, S.A. Significance of the CAG repeat polymorphism of the androgen receptor gene in prostate cancer progression. *J. Urol.* 2000; 164: 567-572.
- Tsai, M.J. and O'Malley, B.W. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.* 1994; 63: 451-486.
- von Eckardstein, S., Syska, A., Gromoll, J., Kamischke, A., Simoni, M. and Nieschlag, E. Inverse correlation between sperm concentration and number of androgen receptor CAG repeats in normal men. *J. Clin. Endocrinol. Metab.* 2001; 86: 2585-2590.
- Vottero, A., Stratakis, C.A., Ghizzoni, L., Longui, C.A., Karl, M. and Chrousos, G.P. Androgen receptor-mediated hypersensitivity to androgens in women with nonhyperandrogenic hirsutism: skewing of X-chromosome inactivation. *J. Clin. Endocrinol. Metab.* 1999; 84: 1091-095.
- Wieacker, P.F., Knoke, I. and Jakubiczka, S. Clinical and molecular aspects of androgen receptor defects. *Exp. Clin. Endocrinol. Diabetes* 1998; 106: 446-453.

- Xue, W., Irvine, R.A., Yu, M.C., Ross, R.K., Coetzee, G.A. and Ingles, S.A. Susceptibility to prostate cancer: interaction between genotypes at the androgen receptor and prostate-specific antigen loci. *Cancer Res.* 2000; 60: 839-841.
- Yeh, S., Chang, H.C., Miyamoto, H. Differential induction of the androgen receptor transcriptional activity by selective androgen receptor coactivators. *Keio J. Med.* 1999; 48: 87-92.
- Yeh, S., Sampson, E.R., Lee, D.K., Kim, E., Hsu, C.L., Chen, Y.L., Chang, H.C., Altuwajiri, S., Huang, K.E. and Chang, C. Functional analysis of androgen receptor N-terminal and ligand binding domain interacting coregulators in prostate cancer. *J. Formos. Med. Assoc.* 2000; 99: 885-894.
- Yeh, S., Hu, Y.C., Rahman, M., Lin, H.K., Hsu, C.L., Ting, H.J., Kang, H.Y. and Chang, C. Increase of androgen-induced cell death and androgen receptor transactivation by BRCA1 in prostate cancer cells. *Proc. Natl. Acad. Sci. USA* 2000; 97: 11256-11261.
- Yeh, S., Kang, H.Y., Miyamoto, H., Nishimura, K., Chang, H.C., Ting, H.J., Rahman M, Lin, H.K., Fujimoto, N., Hu, Y.C., Mizokami, A., Huang, K.E. and Chang, C. Differential induction of androgen receptor transactivation by different androgen receptor coactivators in human prostate cancer DU145 cells. *Endocrine* 1999; 11: 195-202.
- Yong, E.L., Lim, L.S., Wang, Q., Mifsud, A., Lim, J., Ong, Y.C. and Sim, K.S. Androgen receptor polymorphisms and mutations in male infertility. *J. Endocrinol. Invest.* 2000; 23: 573-577.
- Yong, E.L., Lim, J., Qi, W., Ong, V. and Mifsud, A. Molecular basis of androgen receptor diseases. *Ann. Med.* 2000; 32: 15-22.
- Yu, M.W., Yang, Y.C., Yang, S.Y., Cheng, S.W., Liaw, Y.F., Lin, S.M. and Chen, C.J. Hormonal markers and hepatitis B virus-related hepatocellular carcinoma risk: a nested case-control study among men. *J. Natl. Cancer Inst.* 2000; 93: 1644-51.
- Yu, M.W., Cheng, S.W., Lin, M.W., Yang, S.Y., Liaw, Y.F., Chang, H.C., Hsiao, T.J., Lin, S.M., Lee, S.D., Chen, P.J., Liu, C.J. and Chen, C.J. Androgen-receptor gene CAG repeats, plasma testosterone levels, and risk of hepatitis B-related hepatocellular carcinoma. *J. Natl. Cancer Inst.* 2001; 92: 2023-2028.
- Yu, H., Bharaj, B., Vassilikos, E.J., Giai, M. and Diamandis, E.P. Shorter CAG repeat length in the androgen receptor gene is associated with more aggressive forms of breast cancer. *Breast Cancer Res. Treat.* 2000; 59: 153-161.
- Zitzmann, M., Brune, M., Kornmann, B., Gromoll, J., von Eckardstein, S., vonEckardstein, A. and Nieschlag, E. The CAG repeat polymorphism in the AR gene affects high density lipoproteincholesterol and arterial vasoreactivity. *J. Clin. Endocrinol. Metab.* 2001; 86: 4867-4873.