Androgen Receptor Gene – CAG Repeats

Lenore K. Beitel, Ph.D.
lenore.beitel@mcgill.ca
Functional Polymorphism of the hAR

Average AR CAG tract

Shorter  African-American

White

Longer  Asian

Prevalance of prostate cancer (NIH):
African-American > White > Asian

Functional Polymorphism of the hAR

% 0 5 10 15 20

CAGs 15 17 19 21 23 25 27 29

- caucasian
- black
- oriental
BIOCHEMICAL GENETICS

BPH and AR-CAG Length

<table>
<thead>
<tr>
<th>((CAG)_n)</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 25</td>
<td>75</td>
<td>95</td>
<td>1.0</td>
</tr>
<tr>
<td>22-24</td>
<td>141</td>
<td>161</td>
<td>1.26</td>
</tr>
<tr>
<td>20-21</td>
<td>139</td>
<td>124</td>
<td>1.67</td>
</tr>
<tr>
<td>≤ 19</td>
<td>96</td>
<td>69</td>
<td>1.92</td>
</tr>
</tbody>
</table>

\[P(\text{trend}) = 0.0002\]

Giovannucci et al. Urology 53: 121, 1999

Laser Capture Microdissection (LCM)

1. Place cap on tissue
2. Pulse laser at target cells
3. Remove cap with adhered target cells
4. Extract molecules from target cells

Prostate section

http://www.moleculardevices.com
CAG Repeat in Prostate Cancer

- Epidemiologic studies suggest correlation (shorter CAG repeat, higher risk)
- CaP is multifocal and heterogeneous
- *in situ* somatic shortening of CAG repeat observed by laser capture microdissection (LCM) & sequencing

Composite Results – Shorter AR CAG Tracts in Prostate Cancer Samples

<table>
<thead>
<tr>
<th>Patient</th>
<th>20592</th>
<th>5273</th>
<th>7344</th>
<th>10033</th>
<th>2184</th>
</tr>
</thead>
<tbody>
<tr>
<td># LCM Samples</td>
<td>14</td>
<td>13</td>
<td>17</td>
<td>24</td>
<td>4</td>
</tr>
</tbody>
</table>

CAGs

Androgen receptor CAG repeat length contraction in diseased and non-diseased prostatic tissues


Diseases Associated with AR CAG Repeat Length Variation

**Table 2. Comparison of matched blood with prostate AR CAG repeat lengths**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>21</td>
<td>21</td>
<td>22 20 17</td>
</tr>
<tr>
<td>L</td>
<td>20</td>
<td>20</td>
<td>22 21</td>
</tr>
<tr>
<td>M</td>
<td>21</td>
<td>26.11</td>
<td>12 0</td>
</tr>
</tbody>
</table>

**SBMA**

CAG tract ≥ 38

Androgen sensitivity Decreased

Gain of function—possible causes

Symptoms

1. Adult onset motor neuropathy of proximal members of hip and shoulder
2. Hypogonadism results in gonadotrophin and testicular atrophy

Reference: Pliskin et al. (2002)

**Prostate cancer**

Shorter

Increased

1. Ethnicity
2. Family history

Inconclusive studies—possible somatic alterations

Reference: Ferro et al. (2002)

**Male infertility**

Longer

Reduced

1. Ethnicity
2. BRCA1 mutation carriers

Inconclusive studies—possible somatic alterations

Reference: Casella et al. (2003)

**Endometrial cancer**

Longer

Reduced

Selective growth advantage—somatic alterations

Reference: Fratantoni et al. (2001)

**Colon cancer**

Shorter

Increased

Inconclusive studies—except in African males

Reference: Sasaki et al. (2000)

**Esophageal cancer**

Shorter

Increased

1. Ethnicity

Reference: Dietrich et al. (2003)

Relative length of CAG tract compared with control populations.

Gottlieb B et al. *Hum Mutat* 23:527-33, 2004
Longer polylglutamine tracts in the androgen receptor are associated with moderate to severe undermasculinized genitalia in XY males

H.N. Lim1,2, H. Chen1, M. McBride2, A.M. Dunning3, R.M. Nixon4, I.A. Hughes1 and J.R. Hawkins1,3

1Department of Paediatrics, University of Cambridge, B2G 1OG, Addenbrooke’s Hospital, Cambridge CB2 2QG, UK, 2Department of Paediatrics, Pamela Youde Nethersole Eastern Hospital, Lai King Road, Choi Wan, Hong Kong, 3CRC Human Cancer Genetics Group, University of Cambridge, Norwich Research Institute, Milton, Cambridge CB1 3RE, UK, 4MRC Statistical Unit, Institute of Public Health, University of Cambridge, Robinson Way, Cambridge CB2 2SR, UK. 5Pharmacia Europe, 214 Cambridge Science Park, Cambridge CB4 0WA, UK.

Received: 12 December 1999. Revised and Accepted: 14 January 2000.

The androgen receptor (AR) is essential to the normal development of the male internal and external genitalia. Consequently, impairment of AR function can result in undermasculinized genitalia that vary from a completely female appearance to isolated hypospadias. Since in vitro studies demonstrate that AR function is reduced by expansion of the polylglutamine tract within the receptor [AR(Gln)], this introduces uncertainty regarding the role of AR-Gln in the development of undermasculinization in these patients.

INTRODUCTION

The formation of the male reproductive system is a complex multi-step process that begins during embryogenesis and continues through puberty. In this process, it is the androgen receptor (AR) that mediates the actions of testosterone and androsterone. After its synthesis and transport to the nucleus, AR-Gln protein may undergo post-translational modifications, which affect AR function. The androgen receptor is essential for normal male development, and mutations result in androgen insensitivity syndrome (AIS). AIS patients may exhibit a spectrum of clinical features ranging from complete feminization to mild undermasculinization. The polylglutamine tract within the androgen receptor (AR-Gln) has been shown to be involved in the regulation of AR function, and is associated with the development of AIS.

LIT-81502

- 11 months old
- born prematurely
- ambiguous genitalia
- 46, XY
- PAIS?? uncertain
- DNA sequenced
- Exons 1-8: normal except for 37 Gln (N=11-36)
- Mother: 23 & 37 Gln
- However, normal brother also had 37 Gln
AR CAG Repeat Sequencing - Antisense

BQ-8800 - 25 Gln codons

Mother - 25 + 26 Gln codons (heterozygote)

AR Polyglutamine Length Variation

Increasing polyglutamine length

Increasing androgenicity

Prostate cancer
BPH

Normal

Increased risk of defective spermatogenesis & breast cancer

SBMA (Kennedy's Disease)

LKB 2002/10/25
### Disease Associations With Variations in the AR Glutamine Repeat

<table>
<thead>
<tr>
<th>Shortened (CAG)$_n$</th>
<th>Increased (CAG)$_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate cancer</td>
<td>Above normal range</td>
</tr>
<tr>
<td>Ovarian hyperandrogenism</td>
<td>SBMA (Kennedy's disease)</td>
</tr>
<tr>
<td>Androgenetic alopecia</td>
<td>Hypospadias (one reported case)</td>
</tr>
<tr>
<td>Aspects of Klinefelter phenotype</td>
<td></td>
</tr>
<tr>
<td>Response to androgen treatment</td>
<td>Within normal range</td>
</tr>
<tr>
<td>Central obesity</td>
<td>Male infertility</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>Gynecomastia</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Hypospadias</td>
</tr>
<tr>
<td>Coronary artery disease severity</td>
<td>Aspects of Klinefelter phenotype</td>
</tr>
<tr>
<td></td>
<td>Bone density</td>
</tr>
<tr>
<td></td>
<td>Breast cancer</td>
</tr>
</tbody>
</table>

### Transactivation by AR is inversely related to Gln tract length

**Relative growth hormone activity**

![Graph showing relative growth hormone activity with different mibolerone concentrations and glutamine tract lengths.](image)
Effect of PolyGln Tract Length on AR Transcriptional Activation

COS-1 cells co-transfected with pSVhAR 0, 12, 20, 40 or 50 Gln and pMMTV-GH (ARE-reporter) incubated w/ 3.5 nM MB (solid line) or 0.4 nM MB (dashed line). (MB is a synthetic androgen)

Spinobulbar Muscular Atrophy (SBMA)

www.kennedysdisease.org

Spinobulbar Muscular Atrophy

Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy.
La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH
Nature. 352:77-9, 1991
**Intron/Exon Structure and Functional Domains of the Human Androgen Receiver**

Gene (Xq11-12)

Protein

- **Ligand-dependent transcription factor**
- **Essential for normal male sexual development**

**Human Androgen Receiver (hAR)**

**CAG Repeats and Spinobulbar Muscular Atrophy**

<table>
<thead>
<tr>
<th>CAG repeats</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>SBMA</td>
</tr>
<tr>
<td>38</td>
<td>Normal</td>
</tr>
<tr>
<td>37</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>hAR</th>
<th>(Gln)$_n$</th>
<th>DNA-binding</th>
<th>Ligand-binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Gly)$_n$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Exon 1

~ 919 aa

Transcription Modulation

DNA Androgen Binding Domains
Spinobulbar Muscular Atrophy (SBMA): Signs and Symptoms in Men

**Neurologic:**
- Dysarthria (talking) & dysphagia (swallowing)
- Lower muscle weakness & wasting

**Muscular:**
- Fasciculations (twitching)
  - of tongue & perioral muscles
- Muscle cramps & atrophy

**Endocrine:**
- Gynecomastia
- Reduced fertility
- Testicular atrophy
- Retain hair on head
- Sparse facial hair

Harding AE et al. 1982

---

**BIOCHEMICAL GENETICS**

Meiotic stability and genotype – phenotype correlation of the trinucleotide repeat in X-linked spinal and bulbar muscular atrophy

Albert R. La Spada¹, Daniel B. Rosing¹, Anita E. Harding¹, Carolyn L. Warner¹, Roland Spiegel², Irena Hausmanowa-Petrusewicz³, Ween-Chue Yee³, & Kenneth H. Fischbeck¹

Expansion of the trinucleotide repeat (CAG), in the first exon of the androgen receptor gene is associated with a rare motor neuron disorder, X-linked spinal and bulbar muscular atrophy. We have found that expanded (CAG) alleles undergo alteration in length when transmitted from parent to offspring. Of 45 meioses examined, 12 (27%) demonstrated a change in CAG repeat number. Both expansions and contractions were observed, although their magnitude was small. There was a greater rate of instability in male meioses than in female meioses. We also found evidence for a correlation between disease severity and CAG repeat length, but other factors seem to contribute to the phenotypic variability in this disorder.
# Neurodegenerative Diseases Caused by Polyglutamine Repeat Expansion

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Protein</th>
<th>No. of Gln Normal</th>
<th>No. of Gln Mutant</th>
<th>Chr. / Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBMA (Spinobulbar muscular atrophy)</td>
<td>Androgen receptor</td>
<td>11-37</td>
<td>38-62</td>
<td>Xq / X-linked</td>
</tr>
<tr>
<td>HD (Huntington's disease)</td>
<td>Huntingtin</td>
<td>11-34</td>
<td>40-120</td>
<td>4p / AD</td>
</tr>
<tr>
<td>SCA 1 (Spino-cerebellar ataxia 1)</td>
<td>Ataxin 1</td>
<td>25-36</td>
<td>41-81</td>
<td>6p / AD</td>
</tr>
<tr>
<td>SCA 2</td>
<td>Ataxin 2</td>
<td>15-24</td>
<td>35-59</td>
<td>12q / AD</td>
</tr>
<tr>
<td>SCA 3 (Machado-Joseph disease, MJD)</td>
<td>Ataxin 3</td>
<td>13-36</td>
<td>62-82</td>
<td>14q / AD</td>
</tr>
<tr>
<td>SCA 6</td>
<td>Ataxin 6</td>
<td>4-16</td>
<td>21-27</td>
<td>19p / AD</td>
</tr>
<tr>
<td>SCA 7</td>
<td>Ataxin 7</td>
<td>7-35</td>
<td>37-130</td>
<td>3p / AD</td>
</tr>
<tr>
<td>DRPLA (Dentatorubro-pallidolusysian atrophy)</td>
<td>Atrophin 1</td>
<td>7-25</td>
<td>49-85</td>
<td>12p / AD</td>
</tr>
</tbody>
</table>
Selective Neuronal Loss in Polyglutamine Diseases

antior horn
cranial motor
nuclei
dorsal root
ganglia
Ross CA Neuron 15:493-6, 1995

SBMA (KENNEDY)

HD

striatum (caudate/putamen)
cerebral cortex, etc.

BIOCHEMICAL GENETICS

SBMA AND THE (CAG)\textsubscript{n}-EXPANDED AR

FACT: No other AR mutation is motor neuronopathic, even if AR is completely deleted

RESOLUTION. A polyGln-expanded AR loses something that it needs to effect full androgen sensitivity, but gains something that is somehow selectively motor neuronopathic
Nuclear Inclusions (NIs) or Aggregates

- NIs found in affected neurons but also in scrotal skin, dermis, kidney, heart, testis
- Not correlated with cell death
- NIs shown to contain heat shock proteins, proteasome components, mitochondria, ubiquitin, SRC-1, CBP

Ab: $\alpha$ AR $\alpha$ Ubiquitin

Motor neurons from SBMA patient

Endocrine Findings - 22 SBMA patients

- No genital ambiguity or hypospadias
- Normal penis and prostate size
- 11/22 (50%) mild symptoms of hypoandrogenicity
  - $\downarrow$ sexual interest, erectile dysfunction, $\downarrow$ facial hair
- 4 sterile (oligospermia), 3 late onset sterility
  - but 14/22 (63%) had children
- 16/22 (73%) had gynecomastia (breast enlargement)
- 3 treated for hypertension, 2 for dyslipidemia
- Only 4/22 (18%) had normal lipid findings

Dejager S et al. JCEM 87:3893-3901, 2002
Age of Onset of Gynecomastia vs. CAG Repeat Length

Dejager S et al. JCEM 87:3893-3901, 2002
Correlation of AR CAG repeat number and age at milestone in SBMA

Brain (2006), 129, 1446-1455

A. Hand tremor
B. Muscular weakness
C. Requiring a handrail when going up stairs

Gain of Function $\geq$ Gain of Structure

PolyGln expansion results in changes in:
- AR conformation
- resistance to proteases (caspases, trypsin)
- interaction with p160 co-activators, other proteins
Normal and polyGln-expanded AR differentially activate or repress gene expression

Figure 3. Androgen-responsive genes in cells expressing the Q24 or Q65 androgen receptor. Cells were treated with either R1881 or vehicle control for 24 h, and androgen-responsive genes were identified using oligonucleotide arrays. The first and second columns show the GeneBank number and gene name. The third and fourth columns show androgen-responsive genes in Q24 and Q65 cells, respectively. The fifth column shows a comparison of baseline gene expression levels in mutant versus wild-type cells. Increased expression is shown in red, decreased in green and no change in black. Expression differences called in 44 comparisons are shown by dark shades, and those called in 34 comparisons are shown by light shades.
SBMA Mouse Model:
Males vs. Females


SBMA Mouse Model:
Males vs. Castrated Males

Polyglutamine-Expanded AR: The Big Picture

**LOSS OF FUNCTION**
- transactivational incompetence
- reduced expression

**GAIN OF FUNCTION**
- normal
- novel

**ANDROGEN INSENSITIVITY**
- ↓ spermatogenesis
- ↓ gynecomastia
- ↓ masculinization
- ↓ CaP
- ↓ BPH

**SELECTIVE MOTORNEURONOPATHY (SBMA)**
- Misfolding
- Truncation
- Aggregation
- Sequestration
- Proteosome Inhibition
- Mitochondrial Dysfunction
- Ligand-dependent polyGln disease

Support and info: www.kennedysdisease.org

Diseases Associated with AR CAG Repeat Length Variation

<table>
<thead>
<tr>
<th>Direct association</th>
<th>CAG tract</th>
<th>Androgen sensitivity</th>
<th>Gain of function—possible causes</th>
<th>Symptoms</th>
<th>Reference</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Indirect association</th>
<th>Relative length of tract</th>
<th>Associated risk factors</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate cancer</td>
<td>Shorter</td>
<td>Increased</td>
<td>Inconclusive studies—possible somatic alterations</td>
<td>Ferro et al. [2002]</td>
</tr>
<tr>
<td>Male infertility</td>
<td>Longer</td>
<td>Reduced</td>
<td>BRCAl mutation carriers</td>
<td>Casolla et al. [2003]</td>
</tr>
<tr>
<td>Female breast cancer</td>
<td>Longer</td>
<td>Reduced</td>
<td>Inconclusive studies—possible somatic alterations</td>
<td>Filipi et al. [2003]</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Longer</td>
<td>Reduced</td>
<td>Somatic alterations</td>
<td>Sasaki et al. [2000]</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Shorter</td>
<td>Increased</td>
<td>Selective growth advantage—somatic alterations</td>
<td>Ferro et al. [2002]</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>Shorter</td>
<td>Increased</td>
<td>Inconclusive studies—except in African males</td>
<td>Dietrich et al. [2003]</td>
</tr>
</tbody>
</table>

Relative length of CAG tract compared with control populations.

Gottlieb B et al. Hum Mutat 23:527-33, 2004
**What We Do**

**Loss of Function AR**

- Clinical History & Physical Exam
  - X-linked or not? / Family History
- Diagnostics: Functional Studies
  - Genital Skin Fibroblasts - primary cell cultures
  - Receptor Studies (T, DHT, MT, MB)
  - $K_d$ affinity constant
  - $k_{off}$ dissociation/off rates
  - $B_{max}$ total binding
  - upregulation studies
  - Southern Blot - AR large deletions
  - Western Blots - AR expression levels
  - Competitive RT-PCR - mRNA abnormalities

---

discussed in previous lectures
What We Do
Loss of Function AR

Molecular Studies
Genomic Studies
- PCR DNA/RNA amplification
- Direct PCR sequencing

Mutation analysis may reveal:
- deletions (small: in frame/ out of frame, large)
- stop (nonsense mutations)
- splicing
- missense single amino substitution
  - N-terminal domain, DBD, LBD

- PCR-directed mutagenesis (AR in expression vector)

What We Do
Loss of Function AR

- PCR-directed Mutagenesis (AR in expression vector)

Functional Transfection Studies
Expression Studies
- Ligand binding assays
- Transactivation assays
  - MMTV, PSA, PB promoters
- DNA binding assays (EMSA)

- N-C terminal interaction assay
- AR coactivator interaction assay
- Western blot analysis
What We Do
Loss of Function AR

Possible Results of Functional Transfection Studies:
- LBD: no/abnormal ligand binding
- DBD: defective DNA binding
- Defective transactivation
- Abnormal N/C terminal interaction
- Abnormal AR coactivator interaction
- Abnormal AR stability

Overall Goal: Understand how AR mutation (genotype) correlates with patient’s phenotype

Steroid Hormone Receptors/
Steroid Hormone Biosynthesis