PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Androgen receptor mutation in Kennedy'sdisease

Kenneth H. Fischbeck, Andrew Lieberman, Christine K. Bailey, Annette Abel and Diane E. Merry

Phil. Trans. R. Soc. Lond. B 1999 **354**, 1075-1078
doi: 10.1098/rstb.1999.0461

THE ROYAL SOCIETY

Androgen receptor mutation in Kennedy's disease

Kenneth H. Fischbeck[1], Andrew Lieberman[1], Christine K. Bailey[2], Annette Abel[1] and Diane E. Merry[2]

[1]*Neurogenetics Branch, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD 20892, USA*
[2]*Neurology Department, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA*

Kennedy's disease is an X-linked form of motor neuron disease caused by an expanded polyglutamine repeat in the androgen receptor. While the expansion mutation causes some loss of transcriptional activity by the androgen receptor, the predominant effect of expansion is probably a toxic gain of function, similar to the mechanism of other polyglutamine expansion diseases. Features of the neurodegenerative phenotype of Kennedy's disease have now been reproduced in transgenic animals and neuronal cell culture. Nuclear inclusions of mutant androgen receptor protein are found in these model systems and in autopsy samples from patients with Kennedy's disease.

Keywords: polyglutamine disease; motor neuron disease; androgen receptor

1. INTRODUCTION

X-linked spinal and bulbar muscular atrophy (SBMA) was the first repeat expansion disease gene to be discovered. This review covers what we have come to learn of the disease mechanism of SBMA, and how it relates to the other polyglutamine expansion neurodegenerative diseases.

2. KENNEDY'S DISEASE

Although there were earlier reports, particularly in the Japanese literature, SBMA often goes by the name 'Kennedy's disease' after a description of the X-linked pattern of inheritance published by William Kennedy and his colleagues 30 years ago (Kennedy *et al*. 1968). SBMA is a chronic, progressive neuromuscular disorder, characterized by proximal muscle weakness, atrophy, and fasciculations. Affected males may show signs of androgen insensitivity, including gynaecomastia, reduced fertility, and testicular atrophy. The cause of the disease is expansion of a trinucleotide repeat in the androgen receptor gene on the X chromosome at Xq11–12 (La Spada *et al*. 1991).

The principal pathological manifestation of SBMA is the loss of motor neurons in the spinal cord and brainstem (Sobue *et al*. 1989). There is also a subclinical loss of sensory neurons in the dorsal root ganglia.

The causative defect is an expanded CAG repeat in the first exon of the androgen receptor gene, near the 5′ end of the gene. It encodes a run of glutamine residues near the amino terminus of the protein, separate from the DNA and hormone binding domains and close to the transcriptional activation domain. In normal individuals the repeat averages about 20 CAGs, with a range of 11–33 CAGs. In patients with SBMA, the repeat is two to three times its normal length, about 38–62 CAGs. As with the other repeat expansion diseases, the longer the repeat the earlier the onset of the disease (La Spada *et al*. 1992).

3. ANDROGEN RECEPTOR FUNCTION

The androgen receptor is a nuclear receptor, a member of the steroid and thyroid hormone receptor family. The members of this family are intracellular receptors with well defined interactions. The androgen receptor is produced in the cytoplasm, where it is phosphorylated, binds stoichiometrically to heat shock proteins, and is transported to the nucleus, where it is actively taken up through nuclear pores. In the presence of ligand (testosterone or dihydrotestosterone), it dissociates from the heat shock proteins and is free to dimerize and bind DNA at specific regulatory elements. It then functions as a ligand-dependent transcription factor; that is, it functions by up- or down-regulating target genes, through interactions with other proteins in the transcriptional activation complex.

We sought to determine whether the normal function and interactions of the androgen receptor protein are altered by the polyglutamine expansion (Brooks *et al*. 1997). We found that the receptor binds ligand normally. The full-length protein also has normal intracellular localization in cultured motor neuron–neuroblastoma hybrid cells. In the absence of ligand the localization is primarily cytoplasmic, and in the presence of ligand primarily nuclear. This is a good indication that most of the normal interactions of the androgen receptor protein are unaffected by the polyglutamine expansion. We did find, as have others (Mhatre *et al*. 1993), that target gene transactivation by the mutant receptor is reduced, probably because of decreased levels of the receptor protein

(Choong *et al*. 1996). This may account for the signs of androgen insensitivity often seen in patients with SBMA, and may also contribute to the motor neuron degeneration that occurs in the disease.

Androgens have long been known to affect muscle strength, and at least some of this effect may take place at the level of the motor neuron rather than the muscle. Androgens promote the survival and dendritic arborization of sexually dimorphic motor neurons, and they have effects on non-dimorphic motor neurons, as well. An early study of ligand binding in the central nervous system showed the highest levels of binding to spinal and bulbar motor neurons (Sar & Stumpf 1977), the same cells that are prone to degeneration in SBMA. Also, androgens lead to increased survival of brainstem motor neurons after cranial nerve section (Yu 1989). Thus it is possible that the loss of androgen receptor function contributes to the motor neuron degeneration and progressive weakness of SBMA. This is consistent with anecdotal clinical reports and the results of one controlled study (Mendell *et al*. 1996), which showed increased strength in SBMA patients treated with exogenous androgen. It is not clear, however, that this effect is specific to the disease. It may be that anyone treated with androgens will get stronger.

We are currently attempting to identify the targets of androgen receptor action that are responsible for the trophic effects of androgens in motor neurons. For this purpose, we are using a cell culture system that recapitulates some of the effects of androgens *in vivo* (Brooks *et al*. 1998). In this system, androgen treatment leads to altered morphology and increased cell survival. Preliminary results indicate that among the genes induced by androgen treatment in this system is the mammalian homologue of *tra-2*, a factor responsible for sexual differentiation in *Drosophila*. Thus the mechanism for development of sexual dimorphism, including sexual differentiation of motor neurons, could be broadly conserved across species.

4. TOXIC GAIN OF FUNCTION

While loss of androgen receptor function may be a factor in the motor neuron dysfunction and degeneration of SBMA, this is not likely to be the primary effect of polyglutamine expansion. Other patients are known to have mutations that cause a loss of androgen receptor function, and these patients have a different phenotype (androgen insensitivity or testicular feminization syndrome), with feminization but no weakness due to motor neuron degeneration. Since loss of androgen receptor function has a different effect, we have long suspected that polyglutamine expansion instead produces a gain of function, that is, it alters the structure of the receptor protein so that it becomes toxic to motor neurons.

Further evidence that androgen receptor polyglutamine expansion causes a toxic gain of function in the gene product comes from the finding of similar mutations in other disorders. The other polyglutamine expansion diseases are all dominantly inherited neurodegenerative diseases with similar age of onset and rate of progression. They are all caused by the same kind of mutation in widely expressed genes, and in each case there is a correlation between repeat length and age of onset. That these disorders involve a toxic gain of function mechanism is indicated by the finding that for several of them loss of gene function leads to a different phenotype, yet transgenic expression of the mutant protein in mice recapitulates features of the human disease.

Table 1. *Androgen receptor transgenic mice*

(AR, androgen receptor; gln, glutamine; Mx, interferon-inducible Mx promoter; NSE, neuron-specific enolase; MLC, myosin light chain; NFL, neurofilament light chain; PRP, prion protein promoter.)

promoter	AR construct	repeat length	phenotype
Mx NSE MLC	full length	45 gln	normal
NSE NFL	full length	66 gln	normal
PRP	truncated	112 gln	gait difficulty, tremor, circling, foot clasping

5. TRANSGENIC MODELS

Over the past several years we have made various lines of transgenic mice with mutant versions of the androgen receptor gene (see table 1). Mouse lines with expression of full-length androgen receptor driven by the interferon-inducible Mx, myosin light chain, neurofilament light chain, neuron-specific enolase, and human androgen receptor promoters all failed to show an abnormal phenotype, despite repeat expansions of up to 66 glutamines (longer than the longest repeat observed in SBMA patients) and up to twice endogenous expression levels in the spinal cord (Bingham *et al*. 1995; Merry *et al*. 1996; La Spada *et al*. 1998). Recently, we produced mice with a truncated androgen receptor containing 112 glutamines driven by the prion protein promoter, and these animals have a striking neurological phenotype, with progressive gait difficulty, circling behaviour, tremor, and seizures (Abel *et al*. 1998). This finding is consistent with results reported in huntingtin and ataxin-3 transgenics, where truncated protein has a particularly pronounced effect (Ikeda *et al*. 1996; Mangiarini *et al*. 1996). It is not clear at this point whether the severe phenotype we find in transgenic mice with the truncated expanded protein is due to the truncation, the expression level, or the extent of repeat expansion in the construct used to make these, as compared to the earlier lines of androgen receptor transgenic mice. Further transgenic experiments with full-length protein containing 112 repeats and driven by the same promoter should help to resolve this issue. It may be noted that the truncated version of the androgen receptor expressed in the transgenic mice with the neurological phenotype is similar (although not identical) to the fragment produced by caspase cleavage (Ellerby *et al*. 1999; Kobayashi *et al*. 1998), consistent with the hypothesis that

an androgen receptor cleavage product is more toxic than the full-length protein *in vivo*.

Recently, polyglutamine neurotoxicity has been reproduced in *Drosophila* (Warrick *et al*. 1998). As with other polyglutamine transgenes, truncated, expanded androgen receptor produces a neurodegenerative phenotype in flies (N. Bonini, unpublished results), indicating a similar mechanism of action. Available transgenic mouse and fly models of SBMA and the other polyglutamine expansion diseases should allow us to answer the outstanding questions in this field: what accounts for the similarities among these diseases? What accounts for the differences? And most importantly, what can be done to treat them?

6. INCLUSIONS AND AGGREGATES

In 1997, a common pathological feature was discovered to go with the presumed common mechanism of the polyglutamine expansion diseases: nuclear inclusions of polyglutamine-containing protein. These inclusions, which are ubiquitinated and present in neurons that become dysfunctional and die, have been found in most of the polyglutamine diseases and animal models where they have been sought, including the transgenic flies (Davies *et al*. 1997; Paulson *et al*. 1997; DiFiglia *et al*. 1997; Warrick *et al*. 1998). Nuclear inclusions are present in motor neurons in SBMA (Li *et al*. 1998*a*), as well as in the transgenic mice we have produced (Abel *et al*. 1998). Interestingly, nuclear inclusions of ubiquitinated androgen receptor protein are also present in non-neural tissues in SBMA patients (Li *et al*. 1998*b*).

We have been able to reproduce nuclear inclusions with truncated, expanded androgen receptor protein in transiently transfected cells in culture (Merry *et al*. 1998). In Cos cells, the inclusions are primarily cytoplasmic, while in motor neuron-like MN-1 cells the inclusions are nuclear, perhaps indicating a cell-specific mechanism for inclusion formation. The Western blot correlate of the nuclear inclusions is aggregated protein that barely enters the gel. With increasing repeat length, more of the protein becomes aggregated. At the same time, a specific cleavage product appears, indicating that aberrant androgen receptor proteolysis may lead to, or result from, the protein aggregation. The protein cleavage and aggregation are also associated with repeat-length-dependent cellular toxicity. Further experiments to characterize this process in stably transfected, inducible cell lines are in progress.

If protein aggregation and nuclear inclusions are important to the pathogenesis of SBMA and the other polyglutamine expansion diseases, then this suggests several approaches to treatment. One could attempt to block expression of the mutant protein, or inhibit its processing and nuclear uptake, or block the aggregation and downstream effects, including apoptotic cell death. In any event, cell culture and animal models that are now becoming available can serve as useful systems for further elucidation of the disease mechanism and pharmacological screening. In the case of SBMA, it remains to be determined whether ligand effects, which can alter the subcellular distribution and processing of the receptor protein, could provide a handle on effective treatment of the disease.

This work was supported in part by grants from the Muscular Dystrophy Association and the National Institutes of Health (intramural funds and grant number NS32214).

REFERENCES

Abel, A., Taye, A. A., Fischbeck, K. H. & Merry, D. E. 1998 Truncated androgen receptor gene with an expanded CAG repeat causes phenotypic changes in transgenic mice (abstract). *Am. J. Hum. Genet*. **63**, A319.

Bingham, P. M., Scott, M. O., Wang, S., McPhaul, M. J., Wilson, E. M., Garbern, J. Y., Merry, D. E. & Fischbeck, K. H. 1995 Stability of an expanded trinucleotide repeat in the androgen receptor gene in transgenic mice. *Nature Genet*. **9**, 191–196.

Brooks, B. P., Paulson, H. L., Merry, D. E., Salazar-Grueso, E. F., Brinkmann, A. O., Wilson, E. M. & Fischbeck, K. H. 1997 Characterization of an expanded glutamine repeat androgen receptor in a neuronal cell culture system. *Neurobiol. Dis*. **4**, 313–323.

Brooks, B. P., Merry, D. E., Paulson, H. L., Lieberman, A., Kolson, D. & Fischbeck, K. H. 1998 A cell culture model for androgen effects in motor neurons. *J. Neurochem*. **70**, 1054–1060.

Choong, C. S., Kemppainen, J. A., Zhou, Z. & Wilson, E. M. 1996 Reduced androgen receptor gene expression with first exon CAG repeat expansion. *Mol. Endocrinol*. **10**, 1527–1535.

Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L. & Bates, G. P. 1997 Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD muation. *Cell* **90**, 537–548.

DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P. & Aronin, N. 1997 Aggregation of huntingtin in neuronal inranuclear inclusions and dystrophic neurites in brain. *Science* **277**, 1990–1993.

Ellerby, L. M. (and 11 others) 1999 Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. *J. Neurochem*. **72**, 185–195.

Ikeda, H., Yamaguchi, M., Sugai, S., Aze, Y., Narumiya, S. & Kakizuka, A. 1996 Expanded polyglutamine in the Machado Joseph disease protein induces cell death *in vitro* and *in vivo*. *Nature Genet*. **13**, 196–202.

Kennedy, W. R., Alter, M. & Sung, J. H. 1968 Progressive proximal spinal and bulbar muscuar atrophy of late onset: a sex-linked recessive trait. *Neurology* **18**, 671–680.

Kobayashi, Y., Miwa, S., Merry, D. E., Kume, A., Mei, L., Doyu, M. & Sobue, G. 1998 Caspase-3 cleaves the expanded androgen receptor protein of spinal and bulbar muscular atrophy in a polyglutamine length-dependent manner. *Biochem. Biophys. Res. Comm*. **252**, 145–150.

La Spada, A., Wilson, E. M., Lubahn, D. B., Harding, A. E. & Fischbeck, K. H. 1991 Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77–79.

La Spada, A. R., Roling, D., Harding, A. E., Warner, C. L., Spiegel, R., Hausmanowa-Petrusewicz, I., Yee, W. C. & Fischbeck, K. H. 1992 Meiotic stability and genotype–phenotype correlation of the trinucleotide repeat in X-linked spinal and bulbar muscular atrophy. *Nature Genet*. **2**, 301–304.

La Spada, A. R. (and 13 others) 1998 Androgen receptor YAC transgenic mice carrying CAG 45 alleles show trinucleotide repeat instability. *Hum. Mol. Genet*. **7**, 959–967.

Li, M., Miwa, S., Kobayashi, Y., Merry, D. E., Yamamoto, M., Tanaka, F., Doyu, M., Hashizume, Y., Fischbeck, K. H. & Sobue, G. 1998*a* Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. *Ann. Neurol*. **44**, 249–254.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Li, M., Nakagomi, Y., Kobayashi, Y., Merry, D. E., Tanaka, F., Doyu, M., Fischbeck, K. H. & Sobue, G. 1998*b* Non-neural inclusions of androgen receptor protein in spinal and bulbar muscular atrophy. *Am. J. Pathol.* **153**, 695–701.

Mangiarini, L. (and 10 others) 1996 Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87**, 493–506.

Mendell, J. R., Freimer, M. & Kissel, J. T. 1996 Randomized, double-blind crossover trial of androgen hormone deficiency and replacement in X-linked bulbar spinal muscular atrophy (abstract). *Neurology* **46**, A469.

Merry, D. E., McCampbell, A., Taye, A. A., Winston, R. L. & Fischbeck, K. H. 1996 Toward a mouse model for spinal and bulbar muscular atrophy: effect of neuronal expression of androgen receptor in transgenic mice (abstract). *Am. J. Hum. Genet.* **59**, A271.

Merry, D. E., Kobayashi, Y., Bailey, C. K., Taye, A. A. & Fischbeck, K. H. 1998 Cleavage, aggregation and toxicity of the expanded androgen receptor in spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* **7**, 693–701.

Mhatre, A. N., Trifiro, M. A., Kaufman, M., Kazemi-Esfarjani, P., Figlewicz, D., Rouleau, G. & Pinsky, L. 1993 Reduced transcriptional reglatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. *Nature Genet.* **5**, 184–187.

Paulson, H. L., Perez, M. K., Trottier, Y., Trojanowski, J. Q., Subramony, S. H., Das, S. S., Vig, P., Mandel, J. L., Fischbeck, K. H. & Pittman, R. N. 1997 Intranuclear inclusions of expanded polylutamine protein in spinocerebellar ataxia type 3. *Neuron* **19**, 333–344.

Sar, M. & Stumpf, W. E. 1997 Androgen concentration in motor neurons of cranial nerves and spinal cord. *Science* **197**, 77–79.

Sobue, G., Hashizume, Y., Mukai, E., Hirayama, M., Mitsuma, T. & Takahashi, A. 1989 X-linked recessive bulbospinal neuronopathy. A clinicopathological study. *Brain* **112**, 209–232.

Warrick, J. M., Paulson, H. L., Gray-Board, G. L., Bui, Q. T., Fischbeck, K. H., Pittman, R. N. & Bonini, N. M. 1998 Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneraion in *Drosophila*. *Cell* **93**, 939–949.

Yu, W. H. 1989 Administration of testosterone attenuates neuronal loss following axotomy in the brain-stem motor nuclei of female rats. *J. Neurosci.* **9**, 3908–3914.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES