Cell Death and Differentiation (2006) 13, 524–526

www.nature.com/cdd

Letter to the Editor

A neuroendocrine dysfunction, not testicular mutant ataxin-3 cleavage fragment or aggregate, causes cell death in testes of transgenic mice

Cell Death and Differentiation (2006) **13,** 524–526. doi:10.1038/sj.cdd.4401800; published online 11 November 2005

Dear Editor,

Machado–Joseph disease (MJD), or spinocerebellar ataxia type 3 (SCA3), is a hereditary neurodegenerative disorder resulting from 56–84 consecutive glutamines in mutant ataxin-3; normal ataxin-3 has 14–37 consecutive glutamines.[1,2] Mutant ataxin-3 is widely expressed,[3] causes neuronal loss in selective brain regions,[1] and has isoforms such as mjd1a that result from alternative splicing.[2] Neurotoxicity is associated with a critical concentration[4] of a mutant ataxin-3 putative-cleavage fragment containing the polyglutamine expansion.[5] It is controversial whether neurotoxicity is also associated with the formation of neuronal mutant ataxin-3 aggregate and intranuclear inclusions.[3–6] Here, we used a mouse model of mutant ataxin-3 toxicity to examine whether such pathogenic markers occur in dying non-neuronal cells.

Our previously described transgenic mice (Q71-B and Q71-C homozygotes)[4] expressing human mutant ataxin-3 mjd1a under control of the mouse prion promoter have a phenotype resembling MJD and are infertile. Their heterozygous parents do not show MJD-like behavior and breed poorly (approximately 50% of the Q71-B and 25% of the Q71-C heterozygotes set up for breeding with wild-type mice have offspring). Transgenic mice expressing human wild-type ataxin-3 mjd1a (Q20-A) have normal behavior and fertility.[4] We now establish that the infertility in transgenic mice results from gonadal failure secondary to a neuroendocrine dysfunction and is not associated with the formation of mutant ataxin-3 putative-cleavage fragment or aggregate in gonadal cells.

Q71-B homozygotes had testicular atrophy, aspermia, and abundant apoptotic cells (Figure 1a and b). Testes of Q71-B heterozygotes, Q20-A transgenic mice, and wild-type mice were similar (Figure 1a and b), even at 12 months of age.

Mutant ataxin-3 mjd1a appeared localized to the nucleus and cytoplasm in testicular cells of Q71-B homozygotes and Q71-B heterozygotes (Figure 1b). The nuclear staining was mostly diffuse, with no inclusions comparable to those found in neurons of Q71-B homozygotes.[4] Human normal ataxin-3 mjd1a appeared enriched in the cytoplasm of testicular cells.

Homogenates of testes from Q71-B homozygotes and from Q71-B heterozygotes contained full-length mutant ataxin-3 mjd1a but not the cleavage fragment or aggregate previously detected in brain (Figure 1c). The other bands detected in testes of Q71-B homozygotes and heterozygotes were also present in homogenates of testes from Q20-A transgenic mice and therefore correspond to normal ataxin-3 or background bands.

Because the brain pathogenic markers were absent from testis, we examined whether testicular failure was secondary to deficient serum concentrations of gonadotropins: luteinizing (LH) or follicle stimulating (FSH) hormone (Table 1). Both hormone concentrations in serum were low in male Q71-B homozygotes, when compared to wild-type mice. Male Q71-B heterozygotes had significantly low serum concentrations of FSH but not LH. The second line of Q71-B transgenic mice, Q71-C, had higher transgene expression levels,[4] lower fertility, abnormal testes (not shown) and low concentrations of gonadotropins in serum (Table 1).

To determine whether the concentrations of other pituitary hormones in serum were abnormal in male Q71 homozygotes, we analyzed prolactin (PRL), thyroid stimulating hormone (TSH) and growth hormone (GH); only TSH concentrations were significantly lower in Q71 homozygotes than in wild-type mice (Table 1).

Females of both lines of Q71 transgenic mice had fertility problems and homogenates of their ovaries lacked the cleavage fragment and aggregate (not shown). Q71-B homozygotes had low LH, but not FSH, concentrations in serum when compared to wild-type mice (Table 1). The serum concentrations of both hormones in Q71 heterozygotes and wild-type mice were not significantly different. Since the serum concentrations of gonadotropins in females vary with the estrus cycle, we analyzed prolactin (PRL), a hormone relevant to female but not male fertility. The PRL serum concentration was low in Q71 homozygotes, and not significantly different in Q71 heterozygotes, when compared to wild-type mice (Table 1).

In summary, we found that infertile male transgenic mice expressing human mutant ataxin-3 mjd1a had testicular atrophy, aspermia, and abundant apoptotic testicular cells (Q71-B homozygotes) or were poorly fertile and had testes with normal morphology (Q71-B heterozygotes). Q20-A transgenic mice were normal. The testes from Q71-B homozygotes and heterozygotes lacked the mutant ataxin-3 mjd1a cleavage fragment and aggregate previously detected in their brain;[4] results in males of Q71 transgenic mice, Q71-C, were similar. Ovaries from infertile female Q71-B and Q71-C homozygotes also lacked the fragment and aggregate. We conclude that the infertility in Q71 transgenic mice is not

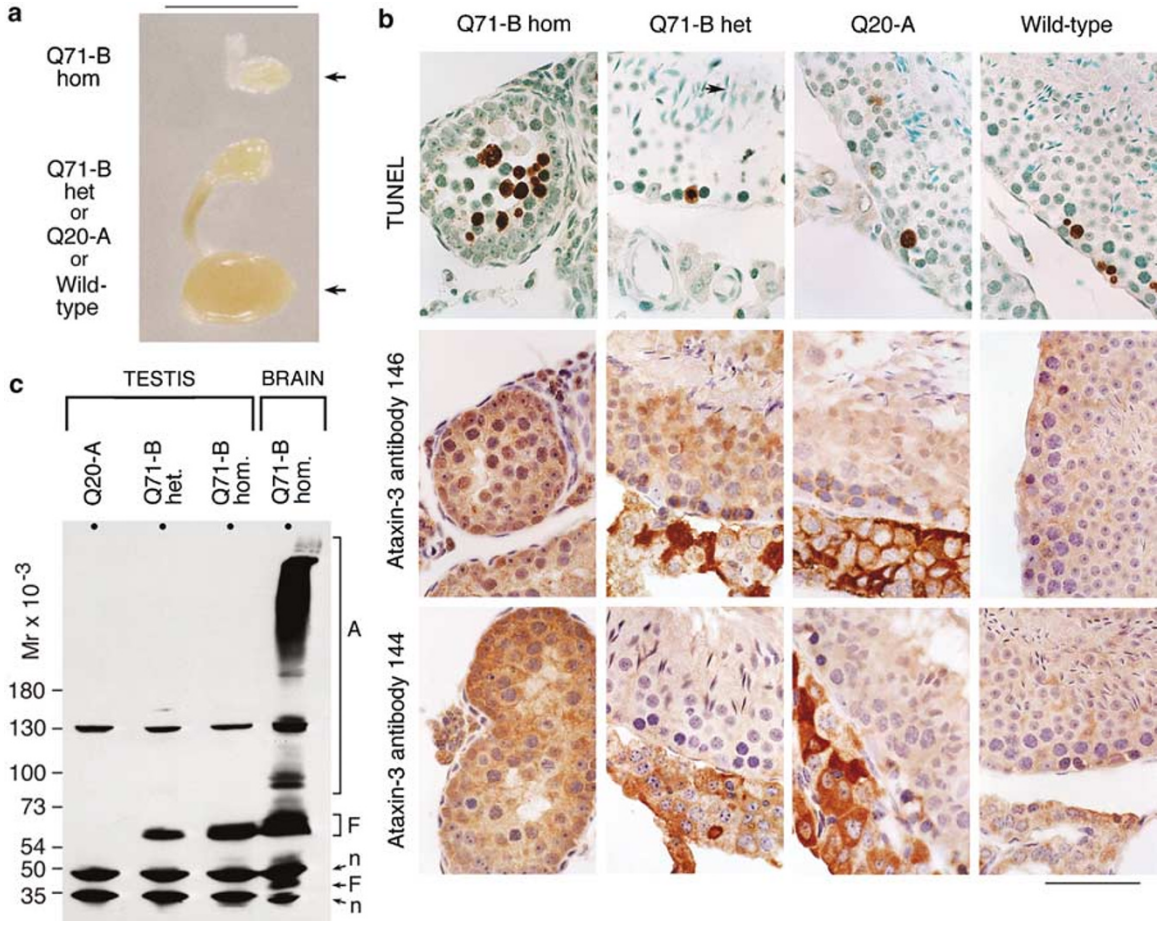

Figure 1 (**a**) Testes (arrow) and epididymides harvested from 4-month-old Q71-B homozygotes (Q71-B hom), their heterozygous parents (Q71-B het), Q20-A transgenic mice, or wild-type mice B6C3F1-J (Jackson laboratory, Bar Harbor, ME) perfused with paraformaldehyde. The magnification of the images is represented with a bar corresponding to 1 cm. **(b)** Cells in sections (6 μm) of paraffin-embedded testes from perfused transgenic or wild-type mice, TUNEL-labeled (Roche Diagnostics, Pleasanton, CA) and counterstained with methyl green, or immunostained for ataxin-3 with antibody 144 (specific for residues 1–14) or 146 (specific for residues 320–334) and counterstained with hematoxylin using previously described procedures.[4] A few spermatozoids are highlighted (arrow). The magnification is the same for all images and is represented with a bar corresponding to 50 μm. **(c)** Western blot of homogenates (320 μg per lane) of testes and of brain from transgenic mice, revealed with ataxin-3 specific antiserum (a gift of Dr. H. Paulson, Department of Neurology, University of Iowa, Iowa City, IA).[3] Human mutant ataxin-3 aggregate (A), full-length form (FL), and cleavage fragment (F) are highlighted, as well as the full-length and smaller form of human normal ataxin-3 (n). Human and murine normal ataxin-3 co-migrated

Table 1 Serum hormone concentrations in transgenic and wild-type mice

	Concentration in serum (ng/ml $\pm$ S.D.)				
	LH	**FSH**	**PRL**	**TSH**	**GH**
Males					
Q71-B homozygotes	$<0.1\pm0.0$*	18 ± 8.0**	40 ± 13	0 ± 0**	3 ± 1.4
Q71-B heterozygotes	0.2 ± 0.2	27 ± 11**	46 ± 24	ND	ND
Q71-C homozygotes	ND	23 ± 3.0**	ND	0 ± 0**	4 ± 0
Q71-C heterozygotes	$<0.1\pm0.0$	29 ± 11**	52 ± 30	ND	ND
Wild-type	0.7 ± 0.6	65 ± 18	31 ± 10	147 ± 34	3.8 ± 2.6
Females					
Q71-B homozygotes	$<0.1\pm0.0$*	16 ± 6.0	54 ± 21**	ND	ND
Q71-B heterozygotes	0.99 ± 0.4	20 ± 7.0	75 ± 43	ND	ND
Q71-C heterozygotes	1.05 ± 0.2	21 ± 6.0	108 ± 43	ND	ND
Wild-type	1.68 ± 1.4	20 ± 10	102 ± 82	ND	ND

Serum hormone concentrations in transgenic and wild-type mice were determined with radioimmunossays by Dr. A.F. Parlow at the National Hormone and Peptide Program (NHPP), Harbor University of California in Los Angeles Medical Center, Torrance, CA. At least three mice 6-months old or younger were used per assay. *Values below detection level of the assay. **Values significantly different from those obtained for the same hormone in wild-type mice (*t*-test; $P<0.05$) ND, not determined

associated with the formation of mutant ataxin-3 mjd1a putative-cleavage fragment or aggregate in gonads.

Q71 transgenic mice had low serum concentrations of several pituitary hormones including gonadotropins, indicating that they have a lesion in the hypothalamic–pituitary axis and their gonadal failure is secondary to this lesion, as reported for hypogonadotropic hypogonadism.[7] Q71-B homozygotes compared to Q71-B heterozygotes have a more severe phenotype and, as previously established, a higher concentration of mutant ataxin-3 mjd1a putative-cleavage fragment in brain cells.[4] Thus, a critical intracellular concentration of such fragment could be associated with neurotoxicity in the hypothalamic–pituitary axis of Q71 transgenic mice, as proposed for other brain regions of these transgenic mice and of MJD patients.[4]

Whereas adenocorticotropic hormone deficiency has been reported in one MJD patient,[8] we found that all pituitary hormone concentrations in the serum of two male MJD patients were normal (unpublished results). Thus, the concentration of hormones in serum should be measured in a larger number of MJD patients to establish whether such patients have a neuroendocrine dysfunction and could benefit from hormonal therapy.

Testicular abnormalities were observed in transgenic mice expressing another polyglutamine protein, mutant huntingtin.[9] The concentrations of gonadotropins in their sera have not been determined. Such hormone measurements could be useful biomarkers of neuronal dysfunction.

Acknowledgements

This work was supported by American Heart Association (AHA) Established Investigator Grant 0140166N, and National Institute of Neurological Disorders and Stroke (NINDS) Grant NS42731 to VFCG. We thank the MJD patients for contributing blood samples, Dale Brown for assistance with mouse maintenance, Michael McElwaine for shooting the testes photographs, Drs. Deborah McClellan and Pamela Talalay for editorial assistance, and Suzanne Fowble and Mary Keyser for budget administration.

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1. Sudarsky L and Coutinho P (1995) Clin. Neurosci. 3: 17–22
2. Kawaguchi Y *et al.* (1994) Nat. Genet. 8: 221–228
3. Paulson HL *et al.* (1997) Ann. Neurol. 41: 453–462
4. Goti D *et al.* (2004) J. Neurosci. 24: 10266–10279
5. Ikeda H *et al.* (1996) Nat. Genet. 13: 196–202
6. Paulson HL *et al.* (1997) Neuron 19: 333–344
7. Silveira LF, MacColl GS and Bouloux PM (2002) Semin. Reprod. Med. 20: 327–338
8. Ogawa K *et al.* (2002) Rinsho. Shinkeigaku. 42: 149–153
9. Leavitt BR *et al.* (2001) Am. J. Hum. Genet. 68: 313–324