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*A cura di Marcella Motta*(†) *e Adele Robbiati Bianchi*

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# THE PHYSIOPATHOLOGICAL ROLES OF ANDROGENS IN MOTONEURONS

ANGELO POLETTI (*)

SUNTO. – Il recettore degli androgeni è stato purificato negli anni '70 e clonato negli anni '80. Questa proteina fa parte della superfamiglia dei recettori steroidi e media i principali effetti degli androgeni nei tessuti dipendenti o sensibili agli androgeni. Diverse funzioni fisiologiche nel cervello sono controllate in modo differenziato nei due sessi e il recettore degli androgeni gioca un ruolo specifico nei processi di differenziazione sessuale ed è coinvolto nel mantenimento del comportamento sessuale maschile in età adulta. Se mutato, il recettore degli androgeni può avere un impatto su molte attività regolate dagli androgeni a causa di una perdita della funzione androgenica nelle cellule bersaglio. Tuttavia, nel caso di un particolare tipo di mutazione, l'allungamento del tratto di poliglutammine normalmente presente nella sua regione N-terminale, il recettore degli androgeni diventa neurotossico e può indurre la morte cellulare di motoneuroni localizzati nel midollo spinale, che esprimono livelli molto elevati di questa proteina. In questo lavoro, discuteremo brevemente le azioni più importanti delle attività androgenica mediata dal recettore degli androgeni nel cervello e i meccanismi attraverso i quali la forma mutata del recettore degli androgeni può portare alla neurodegenerazione nell'atrofia muscolare spinale e bulbare (SBMA).

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ABSTRACT. – The androgen receptor has been purified in the '70s and cloned in the '80s. It is a member of the steroid receptor superfamily and mediated the most important effects of androgen in androgen dependent or sensitive tissues. Several physiological function of the brain are differentially controlled in the two sexes and androgens play specific role in the processes of sexual differentiation and it is involved in the maintenance of male sex behaviour in adulthood. When mutated, the androgen receptor may impact on many of these androgen-regulated activities because of a loss of androgenic

(*) Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Dipartimento di Eccellenza 2018-2022, Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studi di Milano, Milano. Centro InterUniversitario sulle Malattie Neurodegenerative, Università degli Studi di Firenze, Genova, Roma Tor Vergata e Milano, Italia. E-mail: angelo.poletti@unimi.it

function in target cells. However, in the case of a peculiar type of mutation, the elongation of the polyglutamine tract normally present in its N-terminus, the androgen receptor becomes neurotoxic and induces cells death of a number of motoneurons in the spinal cord, which express very high level of this protein. Here, we will briefly discuss the most important actions of androgen receptor-mediated androgen activity in the brain and the mechanisms by which the mutant androgen receptor may lead to neurodegeneration in Spinal and Bulbar Muscular Atrophy (SBMA).

The androgen receptor (AR) is the factor mainly in charge in mediating the masculinizing effects of androgens in target tissue. Specific functions related to sex differences are exerted by androgens in the brain. In this area, the AR is present at high levels in the hypothalamus, particularly in neurons which are localized in the lateromamillary nucleus, in the medial mamillary nucleus, in the diagonal band (horizontal limb) of Broca, in the sexually dimorphic nucleus present in the preoptic area as well as in the paraventricular, suprachiasmatic, ventromedial and infundibular nuclei [1, 2]. AR has also been detected in other hypothalamic nuclei (e.g. nucleus basalis of Meynert, supraoptic and the periventricular nuclei, bed nucleus of the stria terminalis, medial preoptic area, etc.) [1, 2, 3]. By analysing possible differences in AR distribution in male and female brains, it emerged that many of these regions and nuclei are sexually dimorphic [4] with differential expression AR levels in the two sexes. These observation have been used to explain the existence of physiological changes of the organization of the hypothalamic-pituitary-gonadal axis in the two sexes, as well as it has contributed to explain male and female sexual behavior, or the prevalence of some psychiatric and neurological diseases in one of the two sexes [5]. Other brain regions characterized by an intense localization of AR protein are the hippocampus and the temporal cortex [6]; as opposite to the hypothalamus, these structures do not show variation of AR expression in the two sexes [7]. Finally, it has been demonstrated the presence of considerably high level of AR in the motoneurons located in the bulbar region and of the anterior horn in the spinal cord, as well as in sensory neurons present in the dorsal root ganglia, and in the sural nerve [8].

The androgen receptor (AR) gene has been cloned in 1988 [9, 10, 11] and only three years later a mutated version of the AR has been associated to spinal and bulbar muscular atrophy (SBMA) or Kennedy's

disease [12] a neurodegenerative disease affecting motoneurons. The AR gene encodes a protein which acts as a ligand activated transcription factor responsible for most of the biological actions exerted by the androgenic steroids in target tissues. The AR belongs to the nuclear receptor superfamily and similarly to other member of this family control the transcription of specific target genes [13]. Structurally, the AR contains several well characterized domains, which are capable to mediate the interaction between the protein and its ligand (LBD), the protein and the DNA (DBD), and be the bases for protein-protein interactions. This protein-protein interaction is responsible for AR dimerization and AR association with co-factors and co-regulators, as well as for the interaction with molecular chaperones both responsible for the modulation of its biological activity [14, 15]. When AR is not bound to its ligand, the protein remains confined in the cytoplasm where it forms a multi-heteromeric inactive complex with Heat Shock Proteins (Hsp) [16, 17]. Upon binding to testosterone, or other androgens, the AR dissociates from the HSPs dimerizes and translocates into the nucleus. Here, the AR binds the promoter region of androgen responsive genes, typically containing androgen responsive elements (ARE), activating transcription of target genes.

In the past 30 years, several groups have described that many AR gene mutations associate to a variety of human diseases. Most of these mutations are linked to clinical conditions in which a clear loss-of-the AR-function is present. Indeed, the mutation in the gene are translated in a receptor protein with an impaired capability to activate transcription of the androgen target genes. This loss-of-AR-function could be partial or complete, causing a limited or complete androgen insensitivity in males, associated to a wide range of mixed sexual phenotypes [18]. Besides these, other AR mutations are capable to potentiate the normal AR functions, or generate a constitutively activated protein which can cause prostate cancer or other androgen-dependent diseases.

A very interesting situation occurs when the target of the mutation is the CAG (cytosine, adenine, guanine) triplet repeat stretch located in the first coding exon of the AR gene. This mutation consists in an expansion of the length of the CAG repeat, which becomes longer than 36 contiguous CAG codons. In this case, the resulting encoded AR protein contains an aberrantly long polyglutamine (polyQ) tract in its N-terminal

domain, which surprisingly confers neurotoxic properties to the mutant AR (ARpolyQ). These neurotoxic properties of the ARpolyQ are particularly manifested on motoneurons located in the anterior horns of the spinal cords, in the bulbar region of the brainstem and on sensory neurons located in the dorsal root ganglia. The death of motoneurons has the consequence to induce muscle atrophy. Individual carrying this mutant AR gene are affected by the peculiar form of motoneuron disease (MNDs), previously mentioned, the SBMA [12, 19] in which both motor and sensory functions are altered. Of note, the same type of CAG expansion encoding for the elongated polyQ tract has been reported in other genes and the coded proteins cause different types of neurodegenerative diseases [12, 20, 21, 22], which are: the Huntington's disease (HD), different types of spinal-cerebellar ataxias (SCAs), and the dentatorubral and pallidoluysian atrophy (DRPLA) [21, 23]. Singularly, these are very rare diseases, but all together constitute the most frequent class of inherited neurodegenerative diseases in human. It is likely that common mechanisms of neurotoxicity are exerted when the polyQ tract is present in a given protein [23, 24, 25].

The mutant ARpolyQ which causes SBMA is characterized by a partial loos-of-function (LOF), since its transcriptional competence is reduced when compared to wtAR [26, 27, 28, 29]. This probably accounts for the endocrine alteration typically reported in most SBMA patients, like gynecomastia and hypogonadism hypogonadic [19, 30]. Notably, we found that in immortalized moto neurons ARpolyQ has a lower transcriptional competence than wtAR when the target on promoter is a classical androgen responsive element (ARE) on which AR activates transcription mediating a positive androgenic control. Conversely, ARpolyQ and wtAR present very similar inhibitory activities if an alternative promoter region is involved, such as the AR promoter/5'-UTR activation which undergoes to a feedback mechanism exerted by the AR itself on its own promoter [26]. Indeed, we identified two opposite mechanism of the androgenic control of AR expression in moto neurons: a negative feedback on the AR promoter and a positive feed-forward activity on a region located in the AR ORF [26]. These two different androgenic modulations of AR gene could involve different regulatory elements and factors.

The fact that ARpolyQ loose part of its activity on classical ARE-

promoters suggests that some androgenic response may be lost in motoneurons of SBMA patients. Therefore, this may have impact on the physiological regulation exerted by androgens in these cells in which, for example, androgens control the development and adult maintenance of moto neurons of the spinal nucleus of the bulbocavernosus (SNB) system at different stages [31]. Most of these androgenic activities seem to take place during development, especially around birth, or in the process of sexual differentiation when androgens exert different priming activities in the brain. Indeed, androgens modulate synapses formation at neuromuscular junctions and may regulate the growth and arborization of dendritic branches. Androgens act also in adulthood by preserving the moto neurons size [32] and the lack of androgens correlates with a reduction of moto neuron size and of the extension of dendrite [2, 33, 34]. These effects may be due to testosterone itself or to its more potent 5 alpha-reduced derivative dihydrotestosterone (DHT) that can be formed directly "in loco". In fact, spinal cord motoneurons have been found to express very high levels of the enzyme 5 alpha-Reductase type 2 responsible for this conversion and the production of DHT in most androgen dependent tissues [35]. Some of these effects can be mediated at a molecular levels by the protein neuritin (or CPG15), that is known to activate neurite outgrowth and we found to be controlled by the androgenic activation of AR in moto neurons [36, 37, 38, 39, 40, 41, 42].

Despite all these observation, so far no data seems to indicate that the LOF of the ARpolyQ may have impact on the androgenic activities in motoneuronal cells in these brain districts.

Most of the data obtained so far in SBMA strongly suggest that motoneuron death in this disease is due to neurotoxic properties which the elongated polyQ confers to the AR. This gain-of-unction (GOF) is mediated by several different mechanisms, but seems to be triggered by testosterone which is capable to induce aberrant conformations to the mutant ARpolyQ [3]. Indeed, being SBMA a disease associated to a GOF, it is surprising that only male are affected in SBMA, and female that carry the mutant gene are protected from disease manifestation. It has been initially postulated that this may involves the process of random X-chromosome inactivation; this could preserve about half of motoneurons from GOF of ARpolyQ. Surprisingly, while women heterozygous for expanded CAG repeat in exon 1 of AR gene do not

develop SBMA [43, 44], also two women homozygous for SBMA were identified and none of them showed clinical manifestation of SBMA [45]. Moreover, all mice models developed so far, present disease symptomatology only in male, even when the transgene carrying the polyQ is located randomly into the genone (and not in the X-chromosome). In these mice, castration in male ameliorates the phenotype, while the treatment with testosterone in females induces SBMA symptoms [46, 47, 48, 49, 50, 51, 52, 53, 54]. Similar data were obtained in fly models of SBMA [55, 56]. Thus, the male risk to develop SBMA is due to circulating testosterone.

At the molecular levels, we found that ARpolyQ binding to testosterone induces its aggregation [57, 58, 59]. Our data suggest that the aggregates initially sequester misfolded ARpolyQ protecting from its neurotoxicity [3], but at later stages they may become toxic by impairing important intracellular pathways (e.g.: axonal transport or mitochondria distribution [57]. In addition, ARpolyQ seems to be particularly cytotoxic at nuclear levels and cytoplasmic retention ameliorate survival of SBMA motoneurons in culture and of SBMA mouse models [60]. The neurotoxicity triggered by testosterone on ARpolyQ is likely due to the fact that this process involves the release of AR from accessory chaperones and the activation process of the AR. This requires conformational rearrangements to reach the active status of the transcription factor and the expanded polyQ might alter the correct protein folding. Of note, this process is counteracted by selected anti-androgens, like Cyproterone acetate, Flutamide or Bicalutamide [61, 62, 63, 64]. In particular, Bicalutamide has the property to act as a type I antagonist against AR and to slow down its nuclear translocation allowing ARpolyQ cytoplasmic retention and improved autophagic degradation [61, 62, 65, 66]. We combined the use of bicalutamide with that of an effective autophagic activator, the trehalose, and found a potent synergic effect of the two compounds because of the enhanced cytopasmic degradation of ARpolyQ in these conditions [65].

Once activated by testosterone, the mutant ARpolyQ can thus generate misfolded species that must be removed from cell in order to prevent aggregate accumulation and possible damages of the degradative pathways. This process is generally regulated by specific chaperones, including the heat shock proteins (HSPs), which are overex-

pressed upon different cell stresses, including proteotoxic stresses [67]. Chaperones are a large family with more than 150 members (subgrouped in: small HSPs, HSP40s, HSP60s, HSP70s, HSP90s and HSP100) [68]) and work often in conjunction with co-chaperones (e.g.: nucleotide exchange factors (NEFs), such as BCL2-associated athanogene (BAG) family of proteins [69]).

It has been reported that several of these chaperones/co-chaperones are linked, when mutated, to neurodegenerative diseases of other disorders in which neurons are affected [70]. The function of chaperones is to promote protein folding, counteracting misfolding and aggregation. If this process fails, they are able to direct misfolded proteins to the degradative systems. Among these, the most important are: the ubiquitin-proteasome system (UPS) and the autophagic pathway, which are finely tuned by specific chaperones and co-chaperones [71, 72, 73, 74, 75]. We found that some smallHSP (also named HSPBs), like HSPB8, are able to fully counteract ARpolyQ aggregation under several different circumstances [76]. This occurs by facilitating a peculiar form of autophagy, named chaperone-assisted selective autophagy (CASA), which is based on the activity of the CASA complex. Indeed, this complex includes two molecules of HSPB8 interacting with BAG3, HSP70 and CHIP/STUB1. Once the CASA complex has recognized mutant ARpolyQ, it interacts with SQSTM1/p62, an autophagy receptor which recognizes both the ubiquitinated proteins and the lipidated form of LC3 (LC3-II) associated to the autophagosome membranes to target the misfolded proteins of the CASA complex to degradation [77]. Alternatively, the complex HSP70-CHIP/STUB1 and SQSTM1/p62 can works in conjunction with BAG1 to target ARpolyQ to UPS [75]. When this equilibrium is imbalanced, ARpolyQ accumulated into intracellular aggregates into motoneurons.

HSPB8 is widely distributed in most human tissues, and it is upregulated in SBMA [61, 78, 79, 80]. When mutated it causes Charcot-Marie-Tooth type 2L disease, hereditary distal motor neuropathy type II (dHMN-II) or distal myopathy [81, 82, 83]. HSPB8 is highly expressed in anterior horn motoneurons [80] and in skeletal muscle, two tissues potentially affected in SBMA [66]. In addition HSPB8 becomes overexpressed during disease progression, thus contributing to the removal of misfolded ARpolyQ. When expressed in cells, HSPB8 facilitates ARpolyQ clearance via autophagy removing the autophagy flux blockage which characterize this disease [61, 65, 75,

84]. HSPB8 also removes other misfolded proteins responsible for neuronal death in other neurodegenerative diseases [61, 75, 80, 85, 86, 87, 88, 89, 90, 91], suggesting that this factor can be a potential target to counteract proteotoxicity in a wide variety of disorders affecting the brain.

In conclusion, androgens have several role in the brain and most of the effects on neuronal cells are mediated by the AR. Reduced function of AR may impact on the sex and aggressive behavior in male and may be linked to depression, while aberrant functions associated to the presence of the elongated polyQ tract may cause death of motoneurons and their target muscle cells. There are several strategies potentially useful to be use to counteract the toxicity of the mutant ARpolyQ, including the approaches aimed to prevent its activation, but that unfortunately cause heavy side effects at endocrine levels. We have identified a factor, HSPB8 which when activated protect against mutant ARpolyQ toxicity and may be an important target for future therapeutic approaches in SBMA.

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The Author wish to dedicate this manuscript to the memory of his

Master of Science and Mentor professor Luciano Martini who founded the Institute of Endocrinology, and thanks to his endless support, greatly contributed to the development of the scientific vocations of many of his fellows, allowing them to become independent investigators.

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Both will always be remembered by all their fellows.

## REFERENCES

1. Matsumoto A, Micevych P, Arnold P. Androgen regulates synaptic input to motoneurones of the adult rat spinal cord. J Neurosci. 1988;8:4168-4176.
2. Matsumoto A. Hormonally induced neuronal plasticity in the adult motoneurons. Brain research bulletin. 1997;44(4):539-547.
3. Poletti A. The polyglutamine tract of androgen receptor: from functions to dysfunctions in motor neurons. Front Neuroendocrinol. 2004 Apr;25(1):1-26. doi:10.1016/j.yfrne.2004.03.001.
4. Fernandez-Guasti A, Kruijver FP, Fodor M, et al. Sex differences in the distribution of androgen receptors in the human hypothalamus. J Comp Neurol. 2000;425:422-435.
5. Swaab DF, Chung WCJ, Kruijver FPM, et al. Structural and Functional Sex Differences in the Human Hypothalamus. Horm Behav. 2001;40:93-98.
6. Puy L, MacLusky NJ, Becker L, et al. Immunocytochemical detection of androgen receptor in human temporal cortex characterization and application of polyclonal androgen receptor antibodies in frozen and paraffin-embedded tissues. J Steroid Biochem Mol Biol. 1995;55:197-209.
7. Beyenburg S, Watzka M, Clusmann H, et al. Androgen receptor mRNA expression in the human hippocampus. Neurosci Lett. 2000;294:25-28.
8. Li M, Sobue G, Doyu M, et al. Primary sensory neurons in X-linked recessive bulbospinal neuronopathy: histopathology and androgen receptor gene expression. Muscle Nerve. 1995;18:301-308.
9. Chang CS, Kokontis J, Liao ST. Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science. 1988;240(4850):324-326.
10. Lubahn DB, Joseph DR, Sullivan PM, et al. Cloning of human androgen receptor complementary DNA and localization to the X chromosom. Science. 1988;240:327-330.
11. Trapman J, Klaassen P, Kuiper GGJM, et al. Cloning, structure and expression of a cDNA encoding the human androgen receptor. Biochem Biophys Res Comm. 1988;153:241-248.
12. La Spada AR, Wilson EM, Lubahn DB, et al. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature. 1991;352(6330):77-79.

13. Gelmann EP. Molecular biology of the androgen receptor. J Clin Oncol. 2002;20(13):3001-3015.
14. Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol. 2002;16(10): 2181-2187.
15. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev. 2002;23(2):175-200.
16. Georget V, Terouanne B, Nicolas JC, et al. Mechanism of antiandrogen action: key role of hsp90 in conformational change and transcriptional activity of the androgen receptor. Biochemistry. 2002;41(39):11824-11831.
17. Fliss AE, Rao J, Melville MW, et al. Domain requirements of DnaJ-like (Hsp40) molecular chaperones in the activation of a steroid hormone receptor. J Biol Chem. 1999;274:34045-34052.
18. Brinkmann AO. Molecular basis of androgen insensitivity. Mol Cell Endocrinol. 2001;179(1-2):105-109.
19. Kennedy WR, Alter M, Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset. A sex-linked recessive trait. Neurology. 1968;18:671-680.
20. Fischbeck KH. Kennedy disease. J Inher Metab Dis. 1997;20(2):152-158.
21. Fischbeck KH. Polyglutamine expansion neurodegenerative disease. Brain Res Bull. 2001;56:161-163.
22. Fischbeck KH. Past, present, and future of polyglutamine expansion disease. Clin Neurol. 2011 Nov;51(11):825.
23. Taylor JP, Hardy J, Fischbeck KH. Toxic proteins in neurodegenerative disease. Science. 2002;296:1991-1995.
24. Ross CA. Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. Neuron. 2002;35:819-822.
25. Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. Nature Med. 2004 Jul;10 Suppl:S10-7.
26. Vismara G, Simonini F, Onesto E, et al. Androgens inhibit androgen receptor promoter activation in motor neurons. Neurobiol Dis. 2009 Mar;33(3):395-404. doi: 10.1016/j.nbd.2008.11.007.
27. Beitel LK, Alvarado C, Mokhtar S, et al. Mechanisms mediating spinal and bulbar muscular atrophy: investigations into polyglutamine-expanded androgen receptor function and dysfunction. Front Neurol. 2013;4:53. doi: 10.3389/fneur.2013.00053.
28. Beitel LK, Prior L, Vasiliou DM, et al. Complete androgen insensitivity due to mutations in the probable alpha-helical segments of the DNA-binding domain in the human androgen receptor. Hum Mol Genet. 1994 Jan;3(1):21-7.
29. Mandrusiak LM, Beitel LK, Wang X, et al. Transglutaminase potentiates ligand-dependent proteasome dysfunction induced by polyglutamine-expanded androgen receptor. Hum Mol Genet. 2003;12:1497-1506.
30. Sobue G, Hashizume Y, Mukai E, et al. X-linked recessive bulbospinal neuronopathy. A clinicopathological study. Brain 1989;112:209-232.
31. Goldstain LA, Sengelaub DR. Timing and duration of dihydrotestosterone treatment affect the development of motoneuron number and morphology in a sexually dimorphic rat spinal nucleus. J Comparat Neuro. 1992;326:147-157.
32. Watson NV, Freeman LM, Breedlove SM. Neuronal size in the spinal nucleus of

the bulbocavernosus: direct modulation by androgen in rats with mosaic androgen insensitivity. J Neurosci. 2001;21:1062-1066.
33. Brooks BP, Paulson HL, Merry DE, et al. Characterization of an expanded glutamine repeat androgen receptor in a neuronal cell culture system. Neurobiol Dis. 1997;3(4):313-323.
34. Brooks BP, Merry DE, Paulson HL, et al. A cell culture model for androgen effects in motor neurons. J Neurochem. 1998;70(3):1054-1060.
35. Pozzi P, Bendotti C, Simeoni S, et al. Androgen 5-alpha-reductase type 2 is highly expressed and active in rat spinal cord motor neurones. J Neuroendocrinol. 2003 Sep;15(9):882-7.
36. Naeve GS, Ramakrishnan M, Kramer R, et al. Neuritin: a gene induced by neural activity and neurotrophins that promotes neuritogenesis. Proc Natl Acad SciUSA. 1997;94(6):2648-2653.
37. Di Giovanni S, Faden AI, Yakovlev A, et al. Neuronal plasticity after spinal cord injury: identification of a gene cluster driving neurite outgrowth. FASEB J. 2005 Jan;19(1):153-4.
38. Marron TU, Guerini V, Rusmini P, et al. Androgen-induced neurite outgrowth is mediated by neuritin in motor neurones. J Neurochem. 2005 Jan;92(1):10-20. doi: 10.1111/J.1471-4159.2004.02836.X.
39. Tetzlaff JE, Huppenbauer CB, Tanzer L, et al. Motoneuron injury and repair: New perspectives on gonadal steroids as neurotherapeutics. J Mol Neurosci 2006;28(1):53-64.
40. Cappelletti G, Galbiati M, Ronchi C, et al. Neuritin (cpg15) enhances the differentiating effect of NGF on neuronal PC12 cells. J Neurosci Res. 2007 Sep;85(12):2702-13. doi: 10.1002/jnr.21235.
41. Fargo KN, Alexander TD, Tanzer L, et al. Androgen regulates neuritin mRNA levels in an in vivo model of steroid-enhanced peripheral nerve regeneration. J Neurotrauma. 2008 May;25(5):561-6. doi: 10.1089/neu.2007.0466.
42. Zito A, Cartelli D, Cappelletti G, et al. Neuritin 1 promotes neuronal migration. Brain Struct Funct. 2014 Jan;219(1):105-18. doi: 10.1007/s00429-012-0487-1.
43. Nance M. Clinical aspects of CAG repeat diseases. Brain Pathol. 1997;7:881-900.
44. Paradas C, Solano F, Carrillo F, et al. Highly skewed inactivation of the wild-type X-chromosome in asymptomatic female carriers of spinal and bulbar muscular atrophy (Kennedy's disease). J Neurol. 2008 Jun;255(6):853-7. doi: 10.1007/s00415-008-0766-1.
45. Schmidt BJ, Greenberg CR, Allingham–Hawkins DJ, et al. Expression of X-linked bulbospinal muscular atrophy (Kennedy disease) in two homozygous women. Neurology. 2002;59:770-772.
46. Katsuno M, Adachi H, Kume A, et al. Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron. 2002 Aug 29;35(5):843-54.
47. Adachi H, Katsuno M, Minamiyama M, et al. Heat shock protein 70 chaperone overexpression ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model by reducing nuclear-localized mutant androgen receptor protein. J Neurosci. 2003;23:2203-2211.
48. Katsuno M, Adachi H, Doyu M, et al. Leuprorelin rescues polyglutamine-

dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. Nature Med. 2003 Jun;9(6):768-73. doi: 10.1038/nm878.
49. Katsuno M, Adachi H, Inukai A, et al. Transgenic mouse models of spinal and bulbar muscular atrophy (SBMA). Cytogenet Genome Res. 2003;100(1-4):243-51. doi: 10.1159/000072860.
50. Chevalier-Larsen ES, O'Brien CJ, Wang H, et al. Castration restores function and neurofilament alterations of aged symptomatic males in a transgenic mouse model of spinal and bulbar muscular atrophy. J Neurosci. 2004;24:4778-4786.
51. Montie HL, Cho MS, Holder L, et al. Cytoplasmic retention of polyglutamine-expanded androgen receptor ameliorates disease via autophagy in a mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet. 2009 Jun 1;18(11):1937-50. doi: 10.1093/hmg/ddp115.
52. Chevalier-Larsen ES, Merry DE. Testosterone treatment fails to accelerate disease in a transgenic mouse model of spinal and bulbar muscular atrophy. Dis Models Mech. 2012 Jan;5(1):141-5. doi: 10.1242/dmm.007849.
53. Chua JP, Reddy SL, Merry DE, et al. Transcriptional activation of TFEB/ZKSCAN3 target genes underlies enhanced autophagy in spinobulbar muscular atrophy. Hum Mol Genet. 2014 Mar 1;23(5):1376-86. doi: 10.1093/hmg/ddt527.
54. Lieberman AP, Yu Z, Murray S, et al. Peripheral androgen receptor gene suppression rescues disease in mouse models of spinal and bulbar muscular atrophy. Cell Rep. 2014 May 8;7(3):774-84. doi: 10.1016/j.celrep.2014.02.008.
55. Takeyama K, Ito S, Yamamoto A, et al. Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. Neuron. 2002 Aug 29;35(5):855-64.
56. Scaramuzzino C, Casci I, Parodi S, et al. Protein arginine methyltransferase 6 enhances polyglutamine-expanded androgen receptor function and toxicity in spinal and bulbar muscular atrophy. Neuron. 2015 Jan 7;85(1):88-100. doi: 10.1016/j.neuron.2014.12.031.
57. Piccioni F, Pinton P, Simeoni S, et al. Androgen receptor with elongated polyglutamine tract forms aggregates that alter axonal trafficking and mitochondrial distribution in motor neuronal processes. FASEB J. 2002 Sep;16(11):1418-20. doi: 10.1096/fj.01-1035fje.
58. Simeoni S, Mancini MA, Stenoien DL, et al. Motoneuronal cell death is not correlated with aggregate formation of androgen receptors containing an elongated polyglutamine tract. Hum Mol Genet. 2000 Jan 1;9(1):133-44.
59. Stenoien DL, Cummings CJ, Adams HP, et al. Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. Hum Mol Genet. 1999;8(5):731-741.
60. Montie HL, Pestell RG, Merry DE. SIRT1 Modulates Aggregation and Toxicity through Deacetylation of the Androgen Receptor in Cell Models of SBMA. J Neurosci. 2011 Nov 30;31(48):17425-36. doi: 10.1523/JNEUROSCI.3958-11.2011.
61. Rusmini P, Crippa V, Giorgetti E, et al. Clearance of the mutant androgen receptor in motoneuronal models of spinal and bulbar muscular atrophy. Neurobiol Aging. 2013 Nov;34(11):2585-603. doi: 10.1016/j.neurobiolaging.2013.05.026.

62. Rusmini P, Sau D, Crippa V, et al. Aggregation and proteasome: the case of elongated polyglutamine aggregation in spinal and bulbar muscular atrophy. Neurobiol Aging. 2007 Jul;28(7):1099-111. doi: 10.1016/j.neurobiolaging.2006.05.015.
63. Darrington RS, Butler R, Leigh PN, et al. Ligand-dependent aggregation of polyglutamine-expanded androgen receptor in neuronal cells. Neuroreport. 2002 Nov 15;13(16):2117-20.
64. Renier KJ, Troxell-Smith SM, Johansen JA, et al. Anti-androgen flutamide protects male mice from androgen-dependent toxicity in three models of spinal bulbar muscular atrophy. Endocrinology. 2014 Apr 17:en20131756. doi: 10.1210/en.2013-1756.
65. Giorgetti E, Rusmini P, Crippa V, et al. Synergic prodegradative activity of Bicalutamide and trehalose on the mutant androgen receptor responsible for spinal and bulbar muscular atrophy. Hum Mol Genet. 2015 Jan 1;24(1):64-75. doi: 10.1093/hmg/ddu419.
66. Rusmini P, Polanco MJ, Cristofani R, et al. Aberrant Autophagic Response in The Muscle of A Knock-in Mouse Model of Spinal and Bulbar Muscular Atrophy. Sci Rep. 2015;5:15174. doi: 10.1038/srep15174.
67. Morimoto RI. Stress, aging, and neurodegenerative disease. New Engl J Med. 2006 Nov 23;355(21):2254-5. doi: 10.1056/NEJMcibr065573.
68. Kampinga HH, Craig EA. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nature reviews Mol Cell Biol. 2010 Aug;11(8):579-92. doi:10.1038/nrm2941.
69. Takayama S, Reed JC. Molecular chaperone targeting and regulation by BAG family proteins. Nature Cell Biol. 2001 Oct;3(10):E237-41. doi: 10.1038/ncb 1001-e237
70. Smith HL, Li W, Cheetham ME. Molecular chaperones and neuronal proteostasis. Semin Cell Dev Biol. 2015 Apr;40:142-52. doi: 10.1016/j.semcdb.2015.03.003.
71. Lilienbaum A. Relationship between the proteasomal system and autophagy. Int J Biochem Mol Biol. 2013;4(1):1-26.
72. Behl C. Breaking BAG: The Co-Chaperone BAG3 in Health and Disease. Trends Pharmacol Sci. 2016 Aug;37(8):672-88. doi: 10.1016/j.tips.2016.04.007.
73. Minoia M, Boncoraglio A, Vinet J, et al. BAG3 induces the sequestration of proteasomal clients into cytoplasmic puncta: Implications for a proteasome-to-autophagy switch. Autophagy. 2014 Jul 10;10(9):1603-21. doi: 10.4161/auto. 29409.
74. Gamerdinger M, Carra S, Behl C. Emerging roles of molecular chaperones and co-chaperones in selective autophagy: focus on BAG proteins. J Mol Med. 2011 Dec;89(12):1175-82. doi: 10.1007/s00109-011-0795-6.
75. Cristofani R, Crippa V, Rusmini P, et al. Inhibition of retrograde transport modulates misfolded protein accumulation and clearance in motoneuron diseases. Autophagy. 2017 Aug 3;13(8):1280-1303. doi: 10.1080/15548627.2017.1308985.
76. Cicardi ME, Cristofani R, Rusmini P, et al. Tdp-25 Routing to Autophagy and Proteasome Ameliorates its Aggregation in Amyotrophic Lateral Sclerosis Target Cells. Sci Rep. 2018 Aug 17;8(1):12390. doi: 10.1038/s41598-018-29658-2.
77. Klionsky DJ, Abdelmohsen K, Abe A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016;12(1):1-222. doi: 10.1080/15548627.2015.1100356.

78. Carra S, Sivilotti M, Chavez Zobel AT, et al. HspB8, a small heat shock protein mutated in human neuromuscular disorders, has in vivo chaperone activity in cultured cells. Hum Mol Genet. 2005 Jun 15;14(12):1659-69.
79. Carra S, Rusmini P, Crippa V, et al. Different anti-aggregation and pro-degradative functions of the members of the mammalian sHSP family in neurological disorders. Philos Trans Royal Soc London B. 2013 May 5;368(1617):20110409. doi: 10.1098/rstb.2011.0409.
80. Crippa V, Sau D, Rusmini P, et al. The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). Hum Mol Genet. 2010 Sep 1;19(17):3440-56. doi: 10.1093/hmg/ddq257.
81. Fontaine JM, Sun X, Hoppe AD, et al. Abnormal small heat shock protein interactions involving neuropathy-associated HSP22 (HSPB8) mutants. FASEB J. 2006 Oct;20(12):2168-70.
82. Irobi J, Almeida-Souza L, Asselbergh B, et al. Mutant HSPB8 causes motor neuron-specific neurite degeneration. Hum Mol Genet. 2010 Aug 15;19(16):3254-65. doi:10.1093/hmg/ddq234.
83. Ghaoui R, Palmio J, Brewer J, et al. Mutations in HSPB8 causing a new phenotype of distal myopathy and motor neuropathy. Neurology. 2016 Jan 26;86(4):391-8. doi:10.1212/WNL.0000000000002324.
84. Rusmini P, Crippa V, Cristofani R, et al. The Role of the Protein Quality Control System in SBMA. J Mol Neurosci 2016 Mar;58(3):348-64. doi: 10.1007/s12031-015-0675-6.
85. Chavez Zobel AT, Loranger A, Marceau N, et al. Distinct chaperone mechanisms can delay the formation of aggresomes by the myopathy-causing R120G alphaB-crystallin mutant. Hum Mol Genet. 2003 Jul 1;12(13):1609-20.
86. Wilhelmus MM, Boelens WC, Otte-Holler I, et al. Small heat shock protein HspB8: its distribution in Alzheimer's disease brains and its inhibition of amyloid-beta protein aggregation and cerebrovascular amyloid-beta toxicity. Acta Neuropathol. 2006 Feb;111(2):139-49. doi: 10.1007/s00401-005-0030-z.
87. Carra S, Seguin SJ, Lambert H, et al. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. J Biol Chem. 2008 Jan 18;283(3):1437-44. doi: 10.1074/jbc.M706304200.
88. Carra S, Seguin SJ, Landry J. HspB8 and Bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. Autophagy. 2008 Feb 16;4(2):237-9.
89. Bruinsma IB, Bruggink KA, Kinast K, et al. Inhibition of alpha-synuclein aggregation by small heat shock proteins. Proteins. 2011 Oct;79(10):2956-67. doi: 10.1002/prot.23152.
90. Seidel K, Vinet J, den Dunnen WF, et al. The HSPB8-BAG3 chaperone complex is upregulated in astrocytes in the human brain affected by protein aggregation diseases. Neuropathol Appl Neurobiol. 2011 Jun 23;38(1):39-53. doi: 10.1111/j.1365-2990.2011.01198.x.
91. Crippa V, D'Agostino VG, Cristofani R, et al. Transcriptional induction of the heat shock protein B8 mediates the clearance of misfolded proteins responsible for motor neuron diseases. Sci Rep. 2016 Mar 10;6:22827. doi: 10.1038/srep22827.