Stockholm University

Molecular mechanism(s) underlying neurodegeneration in SCA7 disease

Role of NOX enzymes and oxidative stress

Abiodun Ajayi

Stockholm University

Molecular mechanism(s) underlying neurodegeneration in SCA7 disease

Role of NOX enzymes and oxidative stress

Abiodun Ajayi

ISBN: 978-91-7649-257-4

Printed in Sweden by Holmbergs, Malmö 2015

Distributor: Department of Neurochemistry, Stockholm University

Part of this thesis are based on a previous licentiate thesis

To my family

List of publications

The thesis is based on the following publications, which are referred to in the text by their indicated roman numerals:

I. Yu X, **Ajayi A**, Boga NR, Ström AL. (2012). Differential degradation of full-length and cleaved ataxin-7 fragments in a novel stable inducible SCA7 model. J Mol Neurosci. 47:219-323.

II. **Ajayi A**, Yu X, Lindberg S, Langel U, Ström AL. (2012). Expanded ataxin-7 cause toxicity by inducing ROS production from NADPH oxidase complexes in a stable inducible Spinocerebellar ataxia type 7 (SCA7) model. BMC Neurosci. 13:86.

III. **Ajayi A**, Yu X, Wahlo-Svedin C, Tsirigotaki G, Karlström V, Ström AL. (2015). Altered p53 and NOX1 activity cause bioenergetic defects in a SCA7 polyglutamine disease model. Biochim Biophys Acta. 1847:418-428.

Ις. **Ajayi A**, Karlström V and Ström AL. NOX1 and p53 cross-talk in SCA7 polyglutamine toxicity. Manuscript

Other publications

Ajayi A, Yu X, Ström AL. (2012). The role of NADPH oxidase (NOX) enzymes in neurodegenerative disease. Front. Biol. DOI 10.1007/s11515-012-1250-y

Yu X, Muñoz-Alarcón A, **Ajayi A**, Webling KE, Steinhof A, Langel Ü, Ström AL. (2013). Inhibition of autophagy via p53-mediated disruption of ULK1 in a SCA7 polyglutamine disease model. J Mol Neurosci. 50:586-599.

Abstract

Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide expansion in the SCA7 gene, resulting in progressive ataxia and retinal dystrophy. SCA7 belongs to a group of neurodegenerative disorders called polyglutamine (polyQ) diseases, that share the common feature of glutamine tract expansions within otherwise unrelated proteins. Common suggested mechanisms by which polyQ expanded proteins induce toxicity include aggregation and induction of oxidative stress.

In this work, we examined the connection between oxidative stress, aggregation and toxicity in SCA7 disease. We show that expression of the SCA7 disease protein, ataxin-7 (ATXN7), results in elevated levels of ROS and oxidative stress, which in turn lead to toxicity. Our results also revealed that the oxidative stress further contributes to mutant ATXN7 aggregation. Moreover, we show, for the first time, that the major source of the elevated ROS in mutant ATXN7 cells is the increase activation of NOX1 enzymes. Interestingly, our results further revealed that the increased level of NOX1 activity together with altered p53 function leads to a metabolic shift in mutant ATXN7 expressing cells. Treatments with antioxidants, a NOX1 specific inhibitor or NOX1 knock-down, all decreased the ROS level, restored the metabolic shift and ameliorated the mutant ATXN7 induced toxicity. Taken together, we conclude that mutant ATXN7 activate NOX1 enzymes which results in oxidative stress, increased mutant ATXN7 aggregation, metabolic dysfunction and toxicity. NOX1 specific inhibition could thus be a potential therapeutic strategy for SCA7.

Contents

Abbreviations

AD	Alzheimer’s disease
AIF	Apoptosis-inducing factor
ALS	Amyotrophic lateral sclerosis
ATP	Adenosine Triphosphate
ATXN	Ataxin
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CBP	CREB-binding protein
CDP	CCAAT displacement protein
Cdx	Caudal-related homeodomain proteins
CNS	Central nervous system
COX	Cyclooxygenase
CRX	Cone-rod homeobox protein
CTCF	CCCTC-binding factor
DNA	Deoxyribonucleic acid
DRPLA	Dentatorubral and pallidoluysian atrophy
DUB	Deubiquitination
DUOX	Dual oxidase
ETC	Electron transport chain
FAD	Flavin adenine dinucleotide
GATA	GATA-binding factor
GLUT	Glucose transporter
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GST	Glutathione-S.transferase
GTP	Guanosine-5'-triphosphate
HAT	Histone acetyltransferase
HD	Huntington’s disease
HNF-1α	Hepatocyte nuclear factor-1α
KDa	Kilodalton
LOX	Lipooxygenase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MHtt	Mutant huntingtin
NADPH	Nicotinamide adenine dinucleotide phosphate
NES	Nuclear export signal
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLS	Nuclear localization signal
NMDA	N-methyl-D-aspartate
NOX	NADPH oxidase

NOXA1	NOX activating protein 1
NOXO1	NOX organizing protein 1
OxPHOS	Oxidative phosphorylation
PC12	Pheochromocytoma
PD	Parkinson's disease
PGC-1α	Peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-alpha
Phox	Phagocyte oxidase
PKA	Protein kinase A
PKC	Protein kinase C
PolyQ	Polyglutamine
PPAR	Peroxisome proliferator-activated receptor
Prx	Peroxiredoxin
RORα	RAR-related orphan receptor alpha
ROS	Reactive oxygen species
SAGA	Spt/Ada/Gcn5 acetylase
SBMA	spinal and bulbar muscular atrophy
SCA	Spinocerebellar ataxia
SCA7	Spinocerebellar ataxia type 7
SH3	SRC Homology 3 Domain
SOD	Superoxide dismutase
SOR	Superoxide reductase
SP	Specificity protein
STAGA	SPT3-TAF(II)31-GCN5L acetylase
TBP	TATA-binding protein
TIGAR	TP53-inducible glycolysis and apoptosis regulator
Trx	Thioredoxin
TrxR	Thioredoxin reductase
UPS	Ubiquitin–proteasome system
USP	Ubiquitin-specific protease
Vit	Vitanine
ZBD	Zinc binding domain

1. Introduction

1.1. Spinocerebellar ataxia type 7 (SCA7)

SCA7 is a progressive autosomal dominant inherited neurodegenerative disorder (**Holmberg M. et al, 1998**). It is the most common dominant spinocerebellar ataxia (SCA) in Sweden (**Jonasson J. et al, 2000**). Common neurological symptoms among SCA7 patients are ataxia, loss of vision as well as swallowing and speech difficulties (**Johansson J. et al, 1998, David G. et al, 1997, 1998**). Non-neurological symptoms like muscle stiffness, renal failure and congenital heart defects have also been reported in some infantile SCA7 patients (**Benton C. et al, 1998, Johansson J. et al, 1998, Donis KC. et al, 2015**). Common neuro-pathology in SCA7 patients includes atrophy of the cerebellum, brainstem and retina (**Martin J. et al, 1994, Enevoldson T. et al, 1994, Gouw LG. et al, 1994**). In the cerebellum, loss of Purkinje cells, granule cell and dentate nucleus cells correlates with ataxia (**Martin J. et al, 1994, Rüb U. et al, 2013, Seidel K. et al, 2012**). In the retina, degeneration of pigment epithelial cells, bipolar cells, ganglion cells and both rod and cone photoreceptor cells result in the loss of vision (**Martin J. et al, 1994**). In the brain-stem, loss of the inferior olivary and pontine nuclei neurons results in swallowing and speech difficulties (**Martin J. et al, 1994, Berciano J. 1982**). The mean onset age of SCA7 is about thirty years and the loss of vision distinguishes the disease from other cerebellar ataxias (**David G. et al, 1998**).

1.2. SCA7 gene mutation

SCA7 is caused by mutation/expansion of a CAG repeat in the translated region of the SCA7 gene located on chromosome area 3p12-p21.1 (**Holmberg M. et al, 1995, Benomar A. et al, 1995, Gouw LG. et al, 1995, Johansson J. et al, 1998, David G. et al, 1997**). Since CAG codes for the amino acid glutamine, the expansion of the CAG repeat results in an expanded polyglutamine (polyQ) domain in the N-terminal part of the ataxin-7 (ATXN7) protein (**David G. et al, 1997**). The CAG/polyQ repeat can expand to above 300 in SCA7 patients, while healthy persons have about 10 to 17 CAG repeats (**Michalik A. et al, 2004)**. The number of glutamine repeats observed in the majority of SCA7 patients ranges between 45 and 75 repeats with an average number of 51 (**David G. et al, 1998**). The number of CAG repeats is inversely proportional to disease age of onset (the longer the repeat the earlier the age of disease onset) (**Johansson J. et al, 1998, David G. et al, 1998**). In situations where very long repeats are

inherited, SCA7 may appear with an early onset, rapidly progressive juvenile form, or even a very severe infantile death (**Benton C. et al, 1998, Johansson J. et al, 1998, David G. et al, 1997**). A correlation between the number of CAG repeats and initial disease symptom has also been reported (**Johansson J. et al, 1998, Hernandez-Castillo R. et al, 2013**). Visual impairment was the most common initial symptom observed in patients with 59 repeats or more, while ataxia predominated in patients with less than 59 repeats (**Johansson J. et al, 1998).** Since the CAG repeat is highly unstable, SCA7 families usually demonstrate a pattern of genetic anticipation, where subsequent generations inherit a longer repeat resulting in earlier onset. However, shorter repeats can also be inherited. The CAG instability occurs at the cellular level during genomic transmission or cell division and is greater in paternal than in maternal transmissions (**David G. et al, 1998, Lee JM. et al, 2010**). However, an infantile SCA7 case with a very long maternal CAG repeat expansion transmitted was recently reported (**Trang H. et al 2015**).

1.3. ATXN7 protein and function

As previously mentioned, the SCA7 gene encodes a protein called ATXN7 (**Garden G. and La Spada R. 2008, David G. et al, 1997**). There are two major isoforms of the ATXN7 protein, isoform a (892aa, 95 kDa) and b (945aa, 101 kDa) (sizes based on 10Qs) (**Einum DD. et al, 2003, Ström AL. et al, 2005)**. The difference between these two isoforms is a small sequence at the C terminal. Both isoforms are expressed in the central nervous system (CNS) (**Einum DD. et al, 2003, Ström AL. et al, 2005, Lindenberg S. et al, 2000, Cancel G. et al, 2000, Jonasson J. et al, 2002**). However, isoform a is predominantly located in the nucleus of the neurons, while isoform b is predominantly located in the cytoplasm (**Einum DD. et al, 2003, Ström AL. et al, 2005)**. The CCCTC-binding factor, also known as 11-zinc finger protein (CTCF), is a highly conserved multi-functional transcription regulator that strictly controls the ATXN7 expression (**Sopher BL. et al, 2011**).

Both isoforms contain a proline rich region (PRR) that is capable of binding to proteins containing a SRC Homology 3 (SH3) domain, a nuclear export signal (NES), two confirmed functional nuclear localization signals (NLS) (aa 378-393, 704-709) and a third putative NLS (aa 834-839) (**Taylor J. et al, 2006, Mushegian A. et al, 2000, Chen S. et al, 2004, Kaytor M. et al, 1999**). Recently, other ATXN7-like protein homologues namely, ATXN7L1, ATXN7L2 and ATXN7L3 as well as the yeast homologue, Sgf73 was identified

and their genes localized at distinct loci (**Helmlinger D. et al., 2004**). Sequence comparison of ATXN7-like protein homologues with ATXN7 in vertebrates revealed two conserved blocks, block I (aa 126–176) and block II (aa 341–400) of which block II corresponds to a folded zinc-binding domain (ZBD), present in all homologues **(Helmlinger D. et al, 2004, Bonnet J. et al, 2010).** A block III (aa 508–565) conserved in all homologues except ATXN7L3 and Sgf73 was also identified (**Helmlinger D. et al., 2004).** The polyQ domain, located N-terminal to block I is present in ATXN7, but is absent in sgf73, ATXN7L1, ATXN7L2 and ATXN7L3 (**Helmlinger D. et al. 2004).** Studies indicate that human ATXN7, as well as yeast Sgf73, are integral components of the SPT3-TAF (II) 31-GCN5L acetylase (STAGA) complex, known as Spt/Ada/Gcn5 acetylase (SAGA) in yeast (**Helmlinger D. et al., 2004, Sowa ME. et al, 2009, Lang G. et al, 2011**). STAGA/SAGA is a complex comprised of around 20 subunits and is a co-transcription activator complex with both histone deubiquitination (DUB) and acetylation (HAT) activity (**Weake VM. and Workman JL. 2008, Lee KK. et al, 2011, Lang G. et al, 2011**). STAGA/SAGA DUB activity is mediated via the ubiquitin specific protease 22 (USP22) and ubiquitin binding protein 8 (Ubp8) subunit in STAGA and SAGA respectively and has been shown to be required for replication and proliferation (**McCormick MA. et al, 2014, Lang G. et al, 2011, Zhao Y. et al, 2008)**. The DUB removes ubiquitin modification of histones H2B and H2A (**Zhang XY. et al, 2008 , Atanassov BS. et al, 2009, Atanassov BS. and Dent SY. 2011, Lang G. et al, 2011, Zhao Y. et al, 2008)**. STAGA/SAGA HAT activity is, however, mediated via the GCN5 subunit. GCN5 acetylates histones H3 or H4, as well as other non-histone substrates and has been shown to enhance transcriptional activity (**Kuo MH. et al, 1996**). The ATXN7 function in STAGA is not completely understood. However, ATXN7 has been shown to play essential role in the SAGA/STAGA complex activity and assembly (**Helmlinger D. et al., 2004, 2006, McMahon S. et al, 2005, Kohler A. et al, 2008, Lang G. et al, 2011**). ATXN7 is known to link the deubiquitination module and facilitate the integration of the module into the SAGA/STAGA complex **(Lee KK. et al, 2009, Samara NL. et al, 2010, Köhler A. et al, 2010)**. Depletion of ATXN7 was shown to cause dissociation of the deubiquitinase module from the SAGA complex, which resulted in alteration of the complex enzymatic activity (**Köhler A. et al, 2010, Samara NL. et al, 2010, Mohan RD. et al, 2014)**. As part of STAGA, ATXN7 is believed to regulate many genes linked to neurodegeneration and cancer (**Mohan RD. et al, 2014).** ATXN7 has also been

shown to be required for proper differentiation of photoreceptor and cerebellar Purkinje and granule neurons (**Yanicostas C. et al, 2012**). In addition, ATXN7 was linked to the regulation of cytoskeleton stability (**Nakamura Y. et al, 2012**). Could alteration of these functions due to the polyQ expansion in ATXN7 be the cause of SCA7?

1.4. SCA7 disease mechanisms

1.4.1. Loss of function

There are evidence suggesting that loss of normal ATXN7 function could contribute to SCA7 pathology. Studies have shown that ATXN7 deletion in Zebra fish and Drosophila leads to the loss of photoreceptor, cerebellar Purkinje and granule neurons (**Yanicostas C. et al, 2012, Mohan RD. et al, 2014**). Interestingly, the neurodegenerative symptoms displayed by the Drosophila ATXN7 knock-out are similar to those expressing expanded polyQ ATXN7 **(Mohan RD. et al, 2014).** The pathology in ATXN7 knock-out animals could be due to altered STAGA/SAGA functions (**Palhan VB. et al. 2005, McMahon SJ. et al., 2005, Chen Y. et al, 2012, Burke TL. et al, 2013)**. Mutant ATXN7 is incorporated into SAGA/STAGA and both STAGA/SAGA DUB and HAT activity are reported to be altered by the ATXN7 polyQ expansion (**Köhler A. et al, 2010, Samara NL. et al, 2010, Mohan RD. et al, 2014, Burke TL. et al, 2013).** However, other studies have reported that polyQ expansions in ATXN7 does not affect the activity of the STAGA/SAGA deubiquitination or acetylation module in SAGA/STAGA, but rather sequester these modules away or recruits the modules towards the wrong substrates (**Helmlinger D. et al, 2006, McCullough SD and Grant PA. 2010, Duncan CE. et al, 2013, Lan X. et al, 2015, Yang H. et al, 2015**). These results mean that loss of function of ATXN7 could explain the toxicity in SCA7.

1.4.2. Gain of function

Besides loss-of-function, gain-of-function mechanisms have also been suggested to cause SCA7. SCA7 belongs to the family of polyQ diseases, a group of neurodegenerative disorders that affect different brain region, but share the common feature of glutamine tract expansions within otherwise unrelated proteins (**Cummings J. and Zoghbi Y. 2000**). To date, nine polyQ diseases, including Huntington's disease (HD), dentatorubral and pallidoluysian atrophy (DRPLA), spinobulbar muscular atrophy (SBMA) and six forms of

Spinocerebellar ataxias (SCA1-3, 6, 7 and 17) have been identified (Table 1) (**Everett M. and Wood W. 2004, Orr T. and Zoghbi Y. 2007**).

Disease	**Protein**	**Pathogenic CAG repeat Length**	**Brain region most affected**
HD	Huntingtin	36-121	Striatum, cerebral cortex (**Graveland GA.. et al, 1985**)
SBMA	Androgen receptor	38-62	Motor neurons (**Banno H, et al, 2009**).
DRPLA	Atrophin 1	49.88	Cerebellum, basal ganglia, cerebral cortex (**Kumada S. et al, 2000**)
SCA1	Ataxin 1	40-82	Cerebellar Purkinje cells, dentate nucleus, brainstem (**Rüb U. et al, 2013)**
SCA2	Ataxin 2	32-200	Cerebellar Purkinje cells, brain stem, frontotemporal lobes (**Rüb U. et al, 2013)**
SCA3	Ataxin 3	61-84	Cerebellar dentate neurons, basal ganglia, brain stem, spinal cord (**Rüb U. et al, 2013)**
SCA6	Calcium channel α subunit	20-29	Cerebellar Purkinje cells, dentate nucleus, inferior olive (**Rüb U. et al, 2013)**
SCA7	Ataxin 7	37.306	Cerebellum, brain stem, visual cortex (**Rüb U. et al, 2013)**
SCA17	TATA box binding protein	20-29	Cerebellar Purkinje cells, inferior olive (**Rüb U. et al, 2013)**

Table 1. Family of polyglutamine disorders

These disorders are all except SBMA, autosomal dominant progressive neurodegenerative diseases and share a common CAG repeat threshold for

disease onset (**Orr T. and Zoghbi Y. 2007)**. Furthermore, all polyQ proteins, including ATXN7, share common features like misfolding, accumulation and aggregation (**Michalik A. and Van Broeckhoven C. 2003)**. These abnormal properties are believed to confer new (gain of function) toxic properties on the mutant proteins. Most compelling evidence for gain of function of polyQ proteins are the early reports obtained from generation of hypoxanthine phosphoribosyl transferase (HPRT) polyQ mice. Insertion of a 146 CAG/polyglutamine repeat into the gene/protein of HPRT, which does not normally contain a CAG/polyglutamine repeat, resulted in neurodegeneration, meaning that an expanded polyQ domain by itself is toxic **(Tallaksen-Greene SJ. et al, 2003, Ordway JM. et al, 1997, Marsh JL. et al, 2000).** In addition, mice lacking ataxin-1 (SCA1-null mice) was found to not show evidence of ataxia or neurodegeneration, while knock-in models with expanded polyQ ataxin-1 stretch developed ataxia and Purkinje cell degeneration (**Burright EN. et al, 1995, Matilla A. et al, 1998**). Moreover, early comparisons of knock-in and knock-out HD models and the fact that huntingtin homozygous knock-out embryos die early, was considered as another proof of the gain-of-function (**Wexler NS. et al, 1987, Duyao MP. et al, 1995, Nasir J. et al, 1995, Zeitlin S. et al, 1995).** During the last 20 years, several mechanisms through which expanded polyQ protein gain of function could contribute to toxicity have then been identified and will be discussed below, see section 1.5

Most polyQ proteins are ubiquitously expressed (**Ross CA et al, 1999**). However, each disease has a distinct, specific pattern of degeneration (Table 1). The vulnerability of specific neurons in each polyglutamine disease is thus difficult to reconcile with a common polyQ domain-induced toxicity. It is thus likely that the surrounding amino acids in each polyQ protein and specific interacting partner proteins may play a role. For example, inactivation/interaction of the tissue-specific transcription factor cone-rod homeobox protein (CRX) or Purkinje cells expressed protein R85/ponsin by the mutant ATXN7 protein contribute to the pathology in SCA7 disease (**La Spada R. et al, 2001, Chen S. et al, 2004, Jiang YJ. et al, 2013**). Taken together, it could be summarized that, polyQ disease pathology may result from loss-of-function and/or from a toxic gain-of-function.

1.5. PolyQ disease mechanisms

Many common polyQ disease mechanisms in neurons, as well as neighboring cells have been suggested. Evidence implicating involvement of multiple cell

types, including neighboring non-neuronal cells in polyQ toxicity is emerging **(Ilieva H. et al, 2009, Zoghbi HY. and Ort H. 2000).** For example, in HD models, it was shown that the disease mechanism is non-cell autonomous and it is based upon pathological cell–cell interactions **(Zoghbi HY. and Ort H. 2000).** The observation that Purkinje neurons die even when they do not express mutant ATXN7 or, even more provocatively, when mutant ATXN7 is expressed only within the Bergmann glia, demonstrate that non–cell autonomous disease mechanisms is also involved in SCA7 **(Custer SK. et al, 2006, Garden GA. et al., 2002).**

Ataxin-7 and many other polyQ proteins are subjected to proteolytic cleavage, mostly by caspases **(Young JE. et al, 2007, 2009, Weber JJ. et al., 2014, Guyenet SJ. et al, 2015, Garden GA. et al. 2002).** The proteolytically cleaved products are shorter and believed to form more aggregates and be more toxic. In fact, inhibition of cleavage is enough to ameliorate polyQ disease pathology in several models **(Graham RK. et al. 2006, Guyenet SJ. et al, 2015, Davies SW. et al, 1997, Sanchez I.. et al 2003, Skinner J. et al 1997).** Proteolytic fragments of mutant ATXN7 is predominantly detected in the nucleus and this may further enhance toxicity **(Young JE. et al, 2007).** Posttranslational modifications like phosphorylation, acetylation, ubiquitination, and sumoylation can influence polyQ protein cleavage (**Takahashi T. et al, 2010, Mookerjee S. et al 2009, Janer A. et al, 2010, Warby SC. et al, 2005).** Inhibition or enhancement of these modifications has been shown to ameliorate fragment formation and toxicity **(Warby SC. et al, 2005, Terashima T. et al, 2002, Janer A. et al, 2010).**

1.5.1. PolyQ protein aggregation

Expanded polyQ proteins are believed to fold into aberrant β-sheet conformations and then aggregate into oligomers, aggregates and inclusion bodies (IB), both in the cytoplasm and the nucleus (**Davies SW. et al, 1997, Sanchez I. et al 2003, Skinner J. et al, 1997, Yang W. et al, 2002, Holmberg M. et al, 1998**). The aggregates/IBs were early viewed as having a central role in polyQ-mediated pathology (**Sisodia S. 1998, Zoghbi Y. and Orr T. 2009, Taroni F. and DiDonato S. 2004, Furrer SA. et al, 2013, Holmberg M. et al, 1995**). Studies showed that aggregates or inclusion bodies could sequester transcription factors (see section 1.5.3), as well as disrupt axonal transport in neurons leading to toxicity (**Davies SW. et al, 1997, Morfini G. et al, 2005, Nucifora FC. Jr et al, 2001, Steffan JS. et al, 2000, 2001, Shirendeb UP. et**

al, 2012). However, the primary role of the aggregates is still unclear as more recent studies have argued that the aggregates might not be the main cause of polyQ toxicity and that the soluble misfolded monomers or smaller oligomers are likely the primary pathogenic agents (**Yoo SY. et al, 2003, Arrasate M. et al, 2004, Michalik A. and Van Broeckhoven C. 2003, Guyenet SJ. et al, 2015**). Some studies even suggest that aggregates or IBs could in fact be a protective measure by the cells (**Arrasate M. et al. 2004, Michalik A. and Van Broeckhoven C. 2003**).

1.5.2. Aberrant UPS and autophagy activity

The Ubiquitin–proteasome system (UPS) and macroautophagy are the two main protein quality control pathways used to degrade unwanted or damaged proteins by most cells (**Rubinsztein DC. 2006**). UPS or autophagy should degrade misfolded proteins, like expanded polyQ proteins, that otherwise hamper cell survival (**Smith SE. et al, 1996**, **Deter RL. et al, 1967**). UPS degrades small or short-lived proteins and occurs in both the cytoplasm and the nucleus, while macroautophagy (hereafter referred to as autophagy) degrades dysfunctional organelles, as well as proteins, but only occurs in the cytoplasm (**Smith SE. et al, 1996**, **Deter RL. et al, 1967.**

For a protein to be degraded by UPS, the protein is first tagged for degradation by attachment of a small protein called ubiquitin by ubiquitin ligases (**Rubinsztein DC. 2006**). Several additional ubiquitin proteins are then added, resulting in a polyubiquitin chain that is recognized by the proteasome, which contains proteases that degrades the substrate (**Rubinsztein DC. 2006**). PolyQ proteins, including ATXN7, have been reported to be resistant to proteasomal degradation and disrupt UPS activity (**Jana NR. et al, 2001, Bennett EJ. et al, 2007, Tydlacka S. et al, 2008, Wang HL. et al., 2007, Ortega Z. et al, 2010**). However, other studies have suggested that UPS is capable of degrading polyQ proteins (**Dantuma NP. and Bott LC. 2014**).

For a protein to be degraded by autophagy, the protein is isolated from the rest of the cell within a autophagosome, a double membrane vesicle. The autophagosome then fuses with a lysosome and the content is degraded by lysosomal hydrolases (**Majeski AE. and Dice JF. 2004**). Impairment of autophagy has been shown to result in accumulation of neuronal protein inclusions and neurodegeneration, meaning that autophagy protein quality control is crucial for neuronal cell survival (**Hara T. et al. 2006, Komatsu M.**

et al. 2007). Expanded polyQ proteins including ATXN7, have been shown to be degraded by autophagy as well as be resistant to autophagic degradation (**Qin Q. et al, 2006, Ravikumar B. et al, 2002, Yu X. et al 2012, 2013, Young JE. et al, 2007, Mookerjee S. et al 2009).** In fact, some polyQ expanded proteins have been shown to disrupt autophagy. For example, cells expressing mutant ATXN7 display decreased autophagy induction and autophagic capacity (**Yu X. et al, 2013, Young JE et al, 2007**). Treatments which enhance autophagy and promote degradation of mutant polyQ proteins has been shown to reduce mutant polyQ induced toxicity (**Yu X. et al, 2012, 2013, Wang HL. et al, 2013, Nisoli I. et al, 2010**). Taken together, mutant polyQ proteins tend to display disruption of normal cellular autophagy and/or UPS activity. This could lead to toxicity by disrupting the cellular homeostasis.

1.5.3. Transcriptional dys-regulation

Transcriptional dys-regulation is another common proposed mechanism through which polyQ proteins have been suggested to induce toxicity (**Cohen-Carmon D. and Meshorer E. 2012**). Gene expression could be altered when mutant polyQ proteins disrupt the function of transcription factors. Several transcription factors like CREB-binding protein (CBP) and steroid receptor coactivator1 (SRC1) are sequestered into expanded polyQ protein aggregates (**Steffan JS. et al., 2000, Nucifora FC. Jr et al, 2001, Stenoien DL. et al 1999**). Furthermore, mutant huntingtin, ataxin-3, (ATXN3), ATXN7 and atrophin-1 all interact or colocalize with specificity protein 1 (SP1), TAFII130 and TATA-binding protein (TBP), and thus represses transcription of some neuronal genes (**Yvert G. et al. 2001, Shimohata T. et al, 2000, Dunah AW. et al, 2002, Perez MK. et al, 1998**). Moreover, mutant ATXN7 was shown to suppress the activity of the transcription factor nuclear receptor RAR-related orphan receptor alpha (RORα) (**Ström AL. et al, 2005).** Other studies also reported that the normal function of ATXN7 to interact or activate the transcription factor CRX and other transcription machinery components was disturbed by mutant ATXN7 and this contribute to cone-rod dystrophy, cerebellar dysfunction and ataxia (**Yvert G. et al. 2001, La Spada R. et al, 2001, Chen S. et al, 2004, Chou AH et al, 2010**). Taken together, expanded polyQ protein seems to acquire additional function that allows them to interact with transcription factors and alter transcriptional regulation resulting in neurodegeneration (**McCullough SD. et al, 2012, Hong SE. et al, 2000, Miyata T. et al, 2010**).

1.5.4. PolyQ proteins and metabolic alterations

Energy in the form of ATP is generated in most cells through glycolysis and mitochondrial respiration. However, neurons are highly dependent on ATP generated from the mitochondria and do not appear to display increased glycolytic rate, like many other cells, upon mitochondrial inhibition (**Bolanos JP. et al., 2008, 2010**). The high reliance on mitochondria makes neurons vulnerable to metabolic dysfunction (**Kann O. and Kovács R. 2007, Nehlig A. and Coles JA. 2007**).

Mitochondria play a central role in cellular metabolism by generating ATP during oxidative phophorylation (OxPHOS), which in turn, is coupled to the electron transport chain (ETC) (**Warburg 1956, Weir EK. and Archer S. 2010**). The ETC contains five protein complexes (complex I, II, III, IV and V) sitting in the inner mitochondrial membrane. The transfer of electrons through ETC complexes I–IV provides the energy to drive protons against their concentration gradient across the inner mitochondrial membrane (out of the mitochondrial cytoplasm and into the mitochondria intramembrane space) (**Perry SW. et al, 2011**). This results in a net accumulation of H^+ in the intramembrane space and generation of a mitochondrial membrane potential ($\Delta\psi m$, a charge or electrical gradient). When protons then flow back into the mitochondria cytoplasm through the complex V, ATP is produced (**Perry SW. et al, 2011)**. Malfunctioning of the ETC complexes has been shown to result in mitochondrial dysfunction (**Davey GP. et al., 1998, Hroudová J. et at, 2011, Milakovic T. and Johnson GV. 2005**).

Several studies have linked altered glycolytic activity and/or mitochondrial complex dysfunction to polyQ, as well as other neurodegenerative diseases (**Damiano M. et al, 2010, Milakovic T. and Johnson GV. 2005, Power WJ. et al, 2007, Li X.J. et al, 2010, Gu M. et al, 1996, Bae BI. et al, 2005, Silva AC. et al, 2013**). For example, mitochondrial dysfunction was implicated in the pathogenesis of HD (**Gu M. et al, 1996),** SBMA (**Beauchemin AM. et al, 2001**), DRPLA (**Lodi R. t al., 2000**), SCA1 (**Kish SJ. et al., 1999**), SCA3 (**Laco et MN. et al., 2012**) and SCA7 **(Forsgren L .et al 1996**). In fact, mutant huntingtin (mHtt) and ataxin-3 has been shown to interact with the mitochondria (**Shirendeb UP. et al, 2012, Laco MN. et, al., 2012**). Recently, *in vivo* neurometabolic profiling in patients with SCA 1, 2, 3 and 7 revealed metabolic alterations (**Adanyeguh IM. et al, 2015**).

1.5.5. Oxidative stress and polyQ disease

Reactive oxygen species (ROS) are low-molecular-weight oxygen free radicals with an unpaired electron and in order to gain stability, they "steal" electrons from neighboring atoms or molecules (**Santos CX. et al, 2009, Jones DP. 2008**). Nucleic acids, proteins and lipids can be modified by ROS and hence become damaged (**Jones DP. 2008, Heales SJ. and Bolanos JP**. **2002).** A low level of ROS is involved in signal transduction and defense against harmful invasion by modification of specific proteins (**Heales SJ. and Bolanos JP**. **2002**). However, an elevated level of ROS, above the capacity of the anti-oxidant system, results in oxidative stress and excessive damage to cellular molecules (**Sies H. 1985, Sayre LM. et al, 2008**). Neurons are highly sensitive to oxidative stress due to an abundance of oxidative-sensitive lipids, low anti-oxidant defense capacity, a restricted renewal and regenerative capacity, as well as high usage of mitochondrial respiration (**Facecchia K. et al, 2011, Gandhi S. and Abramov A. 2012**). Oxidative stress has been linked to polyQ and other neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) (**Uttara B. et al, 2009, Greenamyre J. et al, 2004, Grimm S. et al, 2011, Tabrizi SJ. et al, 1999**). The oxidative stress in neurodegenerative disease could be caused by a decrease of antioxidant enzymes and/or an increased production of ROS (**Jones DP. 2006, Sayre LM. et al, 2008**).

Antioxidants are molecules that are capable of preventing the oxidation of other molecules (**Ionov M. et al, 2011, Coyle JT. and Puttfarcken P.** 1**993**). Cellular antioxidants include superoxide dismutases (SODs), superoxide reductases (SOR), catalases (CAT), peroxiredoxins (Prx), glutathione (GSH), glutathione peroxidases (GPx), glutathione reductase (GR), glutathione-S transferase (GST), thioredoxins (Trx) and thioredoxin reductase (TrxR). Low molecular weight antioxidants include vitamin C (Vit C) and alpha-tocopherol (also known as vitamin E (Vit E)) (**Fatokun A. et al, 2008**). Altered levels of various endogenous antioxidants have been reported in polyglutamine disorders. For example, increased levels of antioxidants like SODs, Gpx, CAT and Trx2 has been reported in several polyQ models and could be a response to the oxidative stress induced by polyQ proteins (**Reijonen S. et al, 2010, Mason P. et al, 2013)**. However, reduced levels of various antioxidants has also been reported in several polyQ disease models (**St-Pierre J. et al. 2006, Mason P. et al, 2013, Browne S. et al, 1997, Yu YC. et al, 2009**). The reduction of these molecules could contribute to the generation of oxidative stress and toxicity in polyQ

disease. The exact role of antioxidants and how their variations contribute to polyQ disease pathology is thus not completely understood. It is possible that mutant polyQ proteins could differentially trigger the induction or repression of the activity/expression levels of specific antioxidants.

ROS is normally the main product in many reactions, for instance by OxPHOS in mitochondria, xanthine and NADPH oxidase (XO and NOX, respectively), cyclooxygenase (COX) and lipooxygenase (LOX) (**Fatokun A. et al, 2008**). So far, four mechanisms by which polyQ proteins could result in increased generation of ROS have been reported. First, increased mitochondria ROS production due to mitochondria or mitochondria ETC dysfunction has been identified (**Browne S. et al, 1997, Maksimović I. et al, 2001, Ribeiro M. et al, 2013**). Studies have shown that mitochondria complex functions are altered by mutant polyQ proteins and this result in oxidative damage/stress and toxicity (**Browne S. et al, 1997, Maksimović I. et al, 2001, Ribeiro M. et al, 2013**). Second, aberrant transcriptional regulation of the peroxisome proliferator-activated receptor (PPAR) gamma co-activator 1-alpha (PGC-1α) has been shown to occur in HD models (**Cui L. et al, 2006**). PGC-1α is a transcriptional co-activator that regulates genes involved in energy metabolism and antioxidant defense (**St-Pierre et al. 2006)**. The study showed that mutant huntingtin mediated repression of PGC-1α transcriptional activity leads to the downregulation of ROS defense genes resulting in increased oxidative damage and neuronal death (**St-Pierre et al. 2006)**. Third, inclusion bodies formed by polyQ proteins are shown to act as centers for ROS generation (**Firdaus W. et al, 2006**). Fourth, polyQ proteins were shown to activate NADPH oxidase (NOX) enzymes leading to increased ROS generation **(Bertoni A. et al, 2011, Valencia A. et al, 2013**). NOX enzymes, which are the most relevant to this thesis, will be discussed further below.

1.6. The NOX family

The NOX family of enzymes transfers electrons across biological membranes to oxygen in the cell exterior or in intracellular compartments thereby generating ROS (**Katsuyama M. et al, 2012, Ago T. et al, 2010, Bedard K. and Krause K. 2007**). There are seven members in the NOX family namely: NOX1-5 and dual oxidase 1 and 2 (DUOX1 and DUOX2) (**Lambeth JD. and Neish AS. 2014, Katsuyama M. et al, 2012, Bedard K. and Krause K. 2007, Ago T. et al, 2010**). All NOX enzymes contain at least six transmembrane domains plus a flavin adenine dinucleotide (FAD) and a nicotinamide adenine dinucleotide

phosphate (NADPH) binding domain in the cytosolic C-terminal (**Katsuyama M. et al, 2012, Ago T et al, 2010, Bedard K. and Krause K. 2007**). The electron is transferred from the substrate NADPH, through FAD and two heme groups coordinated by the transmembrane helixes, to oxygen (**Bedard K. and Krause K. 2007)**.

1.6.1. NOX family expression in neurons

NOX enzyme activity and expression have been reported in the brain, including in neurons, astrocytes and microglia (**Hernandes MS. and Britto LR. 2012, Nayernia Z. et al, 2014**). In neurons, the most abundant isoforms reported are NOX1, NOX2, NOX3, and NOX4. NOX2 is expressed in all regions of the forebrain, midbrain and hindbrain, with particularly high levels of NOX2 in neurons in the hippocampus (CA1 and CA3 areas), cortex, amygdala, striatum and thalamus (**Noh KM. and Koh JY. 2000, Serrano F. et al. 2003, Tejada-Simon MV. et al., 2005**). NOX1, NOX2, NOX3, as well as NOX4 have been reported in cerebellum and photoreceptor cells, the most affected neurons in SCA7 (**Mizuki K, et al,1998, Coyoy A. et al., 2008, Bhatt L. et al., 2010, Nayernia Z. et al, 2014).** NOX5, DUOX1 and DUOX2 expression have also been reported in the brain **(Hernandes MS. and Britto LR. 2012).**

1.6.2. Regulation of NOX activity

The NOX members differ in their dependency on cytosolic components for their enzymatic activity (**Chuong Nguyen MV. et al, 2015**). NOX1 and NOX3 uses the NOX organizing protein 1 (NOXO1), the NOX activating protein 1 (NOXA1) and the Guanosine-5'-triphosphate (GTP) binding protein, Rac1, while NOX2 (also known as gp91^{phox}) uses p47^{phox} (NOXO1 homologue), p67^{phox} (NOXA1 homologue), p40^{phox} and Rac1 or 2 (**Katsuyama M. et al, 2012, Ago T. et al, 2010, Banfi B. et al, 2001**). NOX4, NOX5, DUOX1 and DUOX2 requires no additional cytosolic subunits (**Katsuyama M. et al, 2012, Ago T. et al, 2010, Banfi B. et al, 2001**).

NOX family enzyme activity is regulated by the protein expression of the NOX enzyme itself, as well as by the expression and the assembly of the regulatory subunits (**Lambeth J. et al, 2007**). The NOX2 enzyme has been most extensively studied and is believed to be regulated in a complex manner. Transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and CCAAT displacement protein (CDP) have been identified as a regulator of NOX2 expression (**Skalnik DG. et al, 1991, Anrather J. et al,**

2006). However, for full activity of NOX2, the assembly of a multi-subunit complex consisting of NOX2, $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and Rac1/2 must take place (**Katsuyama M. et al, 2012, Ago T. et al, 2010**). Upon cell stimulation, Rac and $p47^{phox}$ are independently redistributed from the cytosol to the plasma membrane and recruited to the NOX2 complex. Phosphorylation of $p47^{phox}$ on several serine residues induces a conformational change and triggers the translocation to the membrane allowing $p47^{phox}$ to interact with $p22^{phox}$. The movement of $p47^{phox}$ brings with it the other subunits, $p67^{phox}$ and $p40^{phox}$. Upon recruitment, the $p67^{phox}$ binds to NOX2 and induces the enzymatic activity. It is believed that $p40^{phox}$ modification is involved in the modulation of NOX2 activity by facilitating the membrane translocation of $p47^{phox}$ and $p67^{phox}$ (**Katsuyama M. et al, 2012, Ago T. et al, 2010, Lopes LR. et al, 2004**). NOX1 expression has been reported to be regulated by transcription factors GATA-binding factor 6 (GATA-6), hepatocyte nuclear factor-1α (HNF-1α), and caudal-related homeodomain proteins (Cdx) (**Valente A. et al, 2008, Brewer A. et al, 2006**). NOX1 can be modulated in different cells by different stimuli including growth factors and growth-related agonists like angiotensin II in vascular smooth muscle cells, lipopolysaccharide (LPS) in gastric mucosal cells , oxidative stress in vascular smooth muscle cells, hypoxia in pulmonary epithelial cells and Interferon-γ in colon cancer cells (**Geiszt M. et al, 2003, Kawahara T. et al, 2005, Goyal P. et al, 2004, Wingler K. et al, 2001, Stanic B. et al, 2010**). Full activation of NOX1 requires the assembly of its cytosolic sub-units, NOXO1, NOXA1 and Rac through mechanisms yet to be fully established (**Hernandes MS. and Britto LR. 2012, Geiszt M. et al 2003, Hordijk P. 2006**). However, the activity of NOX1 is positively and negatively controlled by NOXO1 and NOXA1 phosphorylation respectively (**Hordijk P. 2006, Kroviarski Y. et al, 2010, Yamamoto A. et al, 2013**). For example, phosphorylation of NOXO1 by protein kinase C (PKC) or protein kinase A (PKA) positively regulate NOX1-catalyzed ROS production (**Yamamoto A. et al, 2013**), while phosphorylation of NOXA1 by mitogen-activated protein kinase (MAPK), PKC or PKA negatively regulate NOX1-catalyzed ROS production (**Kroviarski Y. et al, 2010, Oh H. et al, 2010**). At present, no or few reports on the transcriptional regulation of NOX3-5 and DUOX1-2 are known. However, transcriptional regulation of NOX4 and NOX5 by NF-κB has been reported (**Manea A. et al, 2010, 2012**). It is believed that the NOX3 activation mechanism is similar to

NOX1, while NOX4 is constitutively active (**Hernandes MS. and Britto LR. 2012, Brown DI. and Griendling KK. 2009**). NOX5, DUOX1 and DUOX2 all have extended N-terminal Ca^{2+} binding EF-hand motifs and their activity is Ca^{2+} dependent (**Brown DI. and Griendling KK. 2009, Hernandes MS. and Britto LR. 2012**) However, reports that expression of DUOX1/2 enzymes are controlled by cyclic adenosine monophosphate (cAMP) remains debated (**Pachucki J. et al, 2004**).

1.6.3. NOX function in neurons

ROS produced by NOX has been implicated in the regulation of neuronal differentiation, regulation of angiotensin II effects, and N-methyl-D-aspartate (NMDA)-mediated synaptic plasticity (**Katsuyama M. et al, 2012, Gao HM. et al, 2012**). For example, it was shown that NOX2-mediated ROS is important to stimulate normal growth and proliferation of hippocampal neural stem cells (**Dickinson B. et al, 2011**). In addition, mice that lack functional NOX2 are deficient in synaptic plasticity, learning and memory (**Pao M. et al, 2004, Kishida K. et al, 2006**). NOX-dependent ROS was as well reported to stimulate retinal ganglion cell survival (**Mackey A. et al, 2008, Groeger G. et al, 2009**). In addition, the physiological level of superoxide produced from NOX2 was suggested to be essential for proper survival signalling in retinal cell lines (**Groeger G. et al, 2009**). However, much remains to be discovered about the roles of NOX-dependent ROS production in healthy neurons.

1.6.4. Role of NOX in neurodegenerative diseases

Involvement of NOX enzymes in neurodegeneration has been reported, although the mechanism is not completely understood **(Hernandes MS. and Britto LR. 2012, Angeloni C. et al, 2015, Cristóvão AC et al, 2012, Qin L. et al, 2013).** NOX1, NOX2 and NOX4 has been linked to neuronal oxidative stress, cerebrovascular dysfunctions and behavioral deficits in different neurodegenerative disease models (**Park L. et al., 2008, Wu D. et al., 2006, Marden JJ. et al. 2007, Choi DH. et al, 2012, Cristóvão AC et al, 2012**). Studies have also implicated NOX enzymes in polyQ diseases (**Maldonado PD. et al, 2010, Valencia A. et al, 2013**). Studies of the role of NOX in SCA7 was one main aim of this thesis, see result section.

1.7. p53 and polyQ disease

The p53 tumor suppressor protein regulates many cellular functions including cell cycle progression, apoptosis, neuronal differentiation, autophagy and

metabolism **(Bargonetti J. et al., 2002, Blum D. et al. 1997, de la Monte SM. et al, 1998, Duan W. et al, 2002, Kaya SS. et al., 1999**). For this thesis, the role of p53 in metabolic regulation is the most relevant. Many metabolic related genes are transcriptionally regulated by p53, including apoptosis-inducing factor (AIF), TP53-inducible glycolysis and apoptosis regulator (TIGAR) and glucose transporters (GLUTs) (**Liang Y. et al, 2013, Berkers CR. et al, 2013**). One way that p53 function is to slow down glycolysis and promote oxidative phosphorylation thus balancing the use of glycolysis and OxPHOS.

The p53 protein mainly functions as a transcription factor and is regulated by post-translational modifications. Examples of these modifications include phosphorylation, oxidation, ubiquitination and acetylation (**Gu W. et al., 1997, Ito A. et al, 2001, Vousden KH. 2002, Woods DB. et al, 2001, Wu HH. et al, 2000, Marchenko ND. et al, 2007**). These modifications are associated with changes in p53's activity and localization (**Ito A. et al, 2001, Liang SH. et al., 2001, Illuzzi J. et al, 2011**). Acetylation and/or phosphorylation promote p53 nuclear localization and transcriptional activity, while oxidation and mono-ubiquitination promotes cytoplasmic localization and impedes transcriptional activity (**Gu W. et al, 1997, Shieh SY. et al, 1997, Wu HH et al, 2000**). Other molecules have also been shown to modulate p53 DNA specificity and functions (**McLure KG. et al, 2004**). For example, NAD^+ binds to p53, induces a conformational change and inhibits p53's DNA binding function (**McLure KG. et al, 2004).** Interestingly, NAD^+ which is a known product of NOX enzymes is also known to induce the expression and activity of SIRT1, a deacetylase enzyme that control the level of p53 acetylation (**Hayashida S. et al, 2010, Puca R. et al, 2010**).

Change in subcellular localization of p53 has been reported in polyQ diseases (**Yu X. et al, 2013, Illuzzi J. et al, 2011, Guo X. et al, 2013**). A shift in p53 from the nucleus to the cytoplasm or the mitochondria have been observed in several polyQ disease models and supports the argument that p53 localization is altered in polyQ disease **(Guo X. et al, 2013, Yu X. et al., 2013, Tsoi H. et al, 2012**). Increased mitochondria and cytoplasmic p53 levels have been shown to promote apoptosis and inhibit autophagy, respectively (**Marchenko ND. et al, 2007, Yu X. et al, 2013**). Moreover, different polyQ aggregates were found to contain p53 (**Nucifora FC. Jr et al., 2001, Steffan JS. et al, 2000, 2001, Yu X. et al, 2013**). For instance, mutant Htt was shown to interact with p53 and sequester p53 into aggregates leading to toxicity. Sequestration of p53 into

aggregates in a SCA7 models was shown to result in reduced p53 transcription activity (**Yu X. et al, 2013**). However, other studies have reported increased levels of p53 regulated apoptotic genes, like Bcl-2-associated X protein (BAX), in SCA7 models (**Wang HL. et al 2005**). Moreover, deletion of p53 was shown to suppress mHtt and ataxin-1 (ATXN1)-induced degeneration in Drosophila and mice, respectively (**Steffan JS. et al, 2000, Bae BI. et al, 2005, Ryan AB. et al, 2006**). The role of p53 in polyQ disease is thus complex.

1.8. SCA7 disease diagnosis and therapeutics

Presently, there is no cure for SCA7 or the other polyQ disorders. Proper diagnosis and supportive therapy to manage the symptoms are the only available treatment. Diagnosis of SCA7 is done based on neurological examination e.g neuroimaging, assessment of visual acuity, visual fields and color vision and genetic testing. Supportive therapies that help to improve symptoms include the use of speech and communication therapy devices, which can help to manage cerebellar ataxia symptoms (**http://www.ncbi**). Use of sunglasses, limitation to UV exposure or optical aids for individuals with peripheral visual loss is also in use (**http://www.ncbi**). For patients with swallowing difficulties, video esophagrams is used to identify the food that is likely to trigger aspiration complication (**http://www.ncbi**).

Several potential therapeutic strategies are currently investigated for SCA7 and other polyQ diseases. First, efforts into testing allele-specific RNA silencing/interference strategies are ongoing (**Scholefield J. et al, 2009, Ramachandran PS. et al, 2014**). Non-allele specific silencing of ATXN7 has been shown to improve the disease phenotype in a mouse model of SCA7 (**Ramachandran PS. et al, 2014**). However, if the disease is caused by loss of function, a non-allele specific approach might not be that beneficial. Other strategies that aims at enhancing protein quality control systems, reducing oxidative stress through antioxidant stimulation, or increasing transcriptional activity have also been proposed based on the positive results obtained from various studies **(Cortes CJ. and La Spada AR. 2014, Kamat CD. et al, 2008, Katsuno M. et al, 2005, Ferrante RJ. et al, 2013**). Treatments aimed at increasing the level of brain-derived neurotrophic factor (BDNF) has been show to prevent the degeneration of mouse striatal neurons in HD models (**Pineda JR. et al. 2007**, **Silva A. et al, 2015**). At present, transplantation of stem cells, capable of secreting BDNF, is tested in 20 clinically diagnosed SCA1 patients in a phase II clinical study **(https://www.clinicaltrials**). In addition, over-

expression of hepatocyte growth factor (HGF), a pleiotrophic growth factor with highly potent neurotrophic activities on cerebellar neurons, was shown to attenuates the degeneration of Purkinje cells and Bergmann glia in a knock-in mouse model of spinocerebellar ataxia type 7 (**Noma S. et al, 2012**).

2. Aims of the thesis

The overall aim of this thesis was to investigate the molecular mechanisms by which polyQ expanded ATXN7 induce toxicity and whether modulating these mechanisms could ameliorate toxicity. Specific aims of each paper are listed below:

Paper I.

The aim of paper I was to establish and characterize a new stable inducible SCA7 model and to use this model to investigate how different ATXN7 species are degraded.

Paper II.

Paper II aimed to investigate the role and source of oxidative stress in mutant ATXN7 expressing cells. We also aimed to investigate whether ameliorating oxidative stress could constitute a potential therapeutic strategy in SCA7 disease.

Paper III.

The aim of paper III was to investigate the molecular mechanisms by which mutant ATXN7 cause metabolic dysfunction.

Paper IV.

The aim of paper IV was to further investigate the cross-talk between NOX1 and p53 in mutant ATXN7-induced metabolic dysfunction and toxicity.

3. Methodological consideration

The methods used in this thesis are described in detail in the contributing papers. This part will summarize the main methods utilized with some theoretical aspects. The statements below are valid for all papers when nothing else is stated.

3.1. Cell lines used

HEK 293T, HeLa and stable neuronal-like PC12 cells expressing wild-type or mutant ATXN7 were used.

3.1.1. HEK 293T cells

HEK 293T are transformed cancer cells originally derived from human embryonic kidney cells (**Graham FL. et al, 1977**). This cell line is good for research because it is of human origin and is easy to transfect. In addition, HEK cells express endogenous ATXN7.

3.1.2. HeLa cells

HeLa are transformed cancer cells originally derived from human cervical cells (**Scherer WF. et al, 1953**). Like HEK 293T cells, they are easy to transfect and express endogenous ATXN7 making them suitable for our research study.

3.1.3. Stable inducible Tet-off PC12 cell lines

PC12 is a neuronal-like cell line derived from a pheochromocytoma of the rat adrenal medulla (**Greene LA. and Tischler A. 1976**). Generation of stable inducible PC12 cell lines expressing N-terminal FLAG- and C-terminal GFP-tagged ATXN7 with 10 (FLQ10 line) or 65 (FLQ65 line) glutamines were made by transfecting a commercial PC12 Tet-off cell lines with FLQ10-pTRE-tight or FLQ65-pTRE-tight plasmid construct, respectively. In these cell lines, expression of the corresponding protein namely: ATXN7Q10-GFP or ATXN7Q65-GFP is induced upon removal of doxycline (Dox) from the media. Our choice of the Tet-off system over the Tet-on system was to avoid any possible interference by doxycycline on our readouts when ATXN7 is expressed (**Ermak G. et al, 2003**). Colonies with GFP tagged ATXN7 expression levels similar to that of endogenous ATXN7 were selected to avoid over-expression effects.

3.2. Cell treatments and transfection

To study the role of UPS and autophagy in paper I, inhibitors or activators of UPS and/or autophagy was used. Rapamycin and trehalose were used as

autophagy activators (**Guertin DA. and Sabatini DM. 2007, Sarkar S. et at, 2007**). Rapamycin inhibits the mammalian target of rapamycin (mTOR), a negative regulator of autophagy, while the exact mechanism of trehalose is not known, but is believed to be mTOR independent (**Guertin DA. and Sabatini DM. 2007, Sarkar S. et at, 2007)**. NH_4Cl or 3-MA was used to inhibit autophagy (**Hart PD. et al, 1983, Gordon AH. et al, 1980, Seglen PO. and Gordon PB. 1982**). NH_4Cl inhibit autophagosome-lysosome fussion, while 3-MA inhibit the formation of autophagosomes (**Gordon AH. et al, 1980, Seglen PO. and Gordon PB. 1982).** Epoxomycin was used as inhibitor of proteasome catalytic function (**Meng L. et al, 1999**).

To study the role of oxidative stress in paper II and III, antioxidants or pro-oxidants were used. N-acetyl cysteine (NAC) or Vitamin E (Vit E) was used as antioxidants (**Aruoma I. et al, 1989, Burton G. and Traber M. 1990**). NAC act as an antioxidant by regulating GSH metabolism, while vit E acts as a chain-breaking antioxidant that prevents the propagation of free radical reactions (**Aruoma I. et al, 1989, Burton G. and Traber M. 1990)**. Hydrogen peroxide (H_2O_2) or buthionine sulphoximine (BSO) was used as pro-oxidants (**Kato H. et al, 1997, Skapek S. et al, 1988**). H_2O_2 directly generate reactive oxygen species, while BSO deplete GSH (**Kato H. et al, 1997, Skapek S. et al, 1988**).

To study the activity and role of NOX complexes in oxidative stress, general or specific inhibitors were used in paper II, III and IV. Apocynin and gp91ds-TAT (NOX inhibitor peptide conjugated to a cell penetrating peptide named TAT) was used as more general NOX inhibitors (**Vejrazka M. et al, 2006, Rey F. et al, 2001**). Apocynin inhibits the $p47^{phox}$ subunit of the NADPH oxidase, while gp91ds-TAT inhibits the interaction of NOX2 with $p47^{phox}$ thereby inhibiting NOX enzyme assembly and activation. Based on sequence homology, apocynin and gp91ds-tat may also affect NOX1 assembly (**Tejada-Simon MV. et al., 2005**). However, an antioxidant effect of apocynin has also been reported (**Heumüller S. et al, 2010**). For more specific inhibition, NOX1 siRNA and ML171 was therefore also used (**Gianni D. et al, 2010**). ML171 inhibits the assembly of NOX1 complexes and the selectivity for NOX1 (IC50 of 0.25 μM) over other NADPH oxidases (IC50 > 3 μM) has been shown (**Gianni D. et al, 2010**).

To study mitochondrial activity/function in paper III, inhibitors of mitochondria complexes I-V were used. Rotenone, 3-nitropropionic acid (3-NP), antimycin A,

sodium azide (NaN_3) or oligomycin was used as inhibitors of mitochondria complex I, II, III, IV and V, respectively (**Forkink M. et al, 2014, Cillero-Pastor B. et al, 2013**).

To study the role of p53 proper in papers III and IV, a p53 inducer, Nutlin-3, was used (**Vassilev LT. et al, 2004**). Nutlin-3 binds to the binding site of p53 on MDM2 thereby inhibiting degradation of p53 (**Vassilev LT. et al, 2004)**. To study p53 oxidation in paper IV, a pro-oxidant to sulfhydryls groups, diamide was used (**Leichert LI. and Jakob U. 2006**). Diamide forms a reversible disulphide bond with sulfhydryls group on proteins (**Leichert LI. and Jakob U. 2006).**

To study protein half-lives in paper IV, a protein synthesis inhibitor, Cycloheximide (CHX), was used (**Poehlsgaard J. and Douthwaite S. 2005**).

Cells were transfected using polyethyleneimine (PEI), lipofectamine2000 (Invitrogen) or lipofectamine RNAiMAX.

3.3. Analysis of protein expression

Western blot was used to analyze the expression levels of specific proteins. Total or subcellular protein fractions were obtained from cells and boiled together with sodium dodecyl sulphate (SDS) to denature the proteins and gives them a negative net charge in proportion to their size (**Weber K and Osborn M. 1969**). This difference in charge was then used to separate proteins according to size on a polyacrylamide gel that is run in an electric field. The separated proteins were further transferred to a membrane with the help of an electric current. Specific proteins were then probed by using specific antibodies directed against the target protein of interest. A horseradish peroxidase (HRP) coupled secondary antibody, which binds to the primary antibody, was then added in order to visualize the protein by enhanced chemiluminescence (ECL) (**Veitch NC. 2004, Mruk DD. and Cheng CY, 2011**). The ECL contains luminol and hydrogen peroxide (H_2O_2) (**Mruk DD and Cheng CY, 2011**). On addition of the ECL, HRP will catalyze the oxidation of luminol generating a sensitized intermediate reagent near the molecule of interest. The intermediate product is further oxidized by H_2O_2 to produce a triplet-excited carbonyl, which emits light when it decays to the singlet carbonyl (**Mruk DD and Cheng CY, 2011**). This signal was captured on a charge-coupled device (CCD) camera. The relative abundance of the target protein was quantified by densiometric analysis using Image lab software. Both the specificity and sensitivity of this technique

depends on the antibody. If there is cross reactivity of the antibody, the result might be difficult to interpret and the specificity of the antibody should be confirmed using either over-expression or knock-down/knock-out system of the target protein.

3.4. Analysis of mutant ATXN7 aggregation

Analysis of mutant ATXN7 aggregation was done using filter trap assay and microscopy. For filter trap assay, cells were lysed, samples centrifuged and the pellet fractions containing aggregated materials filtered through a nitrocellulose membrane with a pore size of 0.2 μm in diameter using vacuum aspiration. Under these conditions, aggregated materials larger than 0.2 μm in diameter will be trapped on the membrane. Probing of the membrane was then performed using primary ATXN7 antibody and a corresponding horseradish peroxidase (HRP) coupled-secondary antibody. Visualization was performed using enhanced chemiluminescence (ECL). The benefit of this method is that quantification of the result is easy and objective.

For microscopy, cells grown on glass cover-slips were washed with phosphate-buffered saline (PBS) and fixed with paraformaldehyde to stop all life processes and to prevent disintegration of the cellular structure. Cells were then permeabilized with Triton X-100 to create holes in the plasma membrane and create access to intracellular or intraorganellar antigens. Cells were then incubated with primary antibodies and with fluorescent secondary antibodies. Slides were washed and mounted using Vectashield-mounting medium. Fluorescence microscopy was carried out using a Leica DM IRBE epifluorescence microscope with a ×100 objective. The advantage of microscopy analysis is that specific structures and location of aggregates can be studied.

3.5. Toxicity measurement

Toxicity was measured with WST-1 and membrane integrity assay. The WST-1 assay is based on the enzymatic cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases present in viable cells. This assay can be used to measure cell proliferation or toxicity (**https://www.caymanchem.com/pdfs**). To measure toxicity and not proliferation, we normalized the absorbance value with the protein concentration in each sample. By doing this, we can rule out readouts that correspond to changes in proliferation. Advantage of using the WST-1 salt is that the salt yields a water-soluble cleavage product, which can be measured directly without

any further solubilization. The assay requires no washing step making it easy and fast to perform. In addition, WST-1 is more stable in contrast to MTT, XTT or MTS.

Membrane integrity as a measure of toxicity was determined by analyzing leakage of lactate dehydrogenase (LDH) from the cytoplasm of damaged cells. Measurement of leaked LDH from the cytoplasm into the surrounding culture medium has been widely accepted as a valid method to estimate toxicity when normalized with the total LDH value or protein concentration (**Decker T. and Lohmann-Matthes ML, 1988**). The assay is based on LDH dependent enzymatic conversion of resazurin to resorufin whose fluorescens can be measured. The advantage of this assay is that it is easy to handle, require little time and is non-destructive (cells can be further analyzed by other method) since it can be performed on culture media.

3.6. GSH assay

The total level of reduced glutathione was measured using the Glutathione assay kit (GSH.Glo^{TM}). In cells, GSH is oxidized to glutathione disulphide (GSSH) by reactive oxygen species and GSSH can then be converted back to GSH by glutathione reductase (GSR). Measuring the total level of reduced glutathione (GSH) provides information about ROS mediated depletion of GSH. The assay is based on the conversion of a luciferin derivative into luciferin in the presence of glutathione, catalyzed by glutathione S-transferase (GST). The signal generated is coupled to firefly luciferase. Light from the luciferase is dependent on the amount of luciferin formed, which is in turn dependent on the amount of GSH present. The kit provides a stable signal with a half-life greater than 2 hours thereby eliminating the need for strictly timed luminescent detection. In addition, only a small sample is required because of the enhanced sensitivity.

3.7. ROS measurement

Total ROS was analyzed using the oxidative-sensitive dye, dichloro-fluorescein-diacetate (DCF-DA). DCF-DA is colorless and non-fluorescent and can passively diffuse into the cell (**Zulueta JJ. et al, 1997**). On entering the cell, the acetate groups is cleaved by intracellular esterases to yield a non-fluorescent compound which is later oxidised by ROS into 2', 7' –dichlorofluorescein (DCF) , a highly fluorescent compound whose fluorescens can be measured. Advantage of DCF-DA over other dyes is that it is easy to use and extremely sensitive to changes in the redox state of a cell.

Mitochondrial ROS levels were measured by the highly cationic sensitive probe, Mito- SOX™ Red. Mito- SOX™ Red passively diffuses into the cell and is rapidly and selectively targeted to the mitochondria. The cationic triphenylphosphonium substituent on the dye is responsible for the uptake of the probe in actively respiring mitochondria. Once in the mitochondria, MitoSOX™ Red is oxidized by superoxide, but not by other ROS or reactive nitrogen species (RNS) (**Mukhopadhyay P. et al, 2007**). The oxidized product becomes highly fluorescent and can easily be measured.

3.8. Mitochondria membrane potential

Mitochondria membrane potential ($\Delta\psi m$) was measured with the fluorescent probe tetramethylrhodamine ethyl ester (TMRE). TMRE is a cell-permeant, cationic, red-orange fluorescent dye that is readily sequestered by active mitochondria in proportion to the membrane potential ($\Delta\Psi$) thus allowing for assessment of mitochondrial polarization status (**Perry SW. et al, 2011**). Cells with polarized mitochondria will fluoresce red while cells with depolarized mitochondria will have diminished levels of red fluorescence. Advantage of this dye is that it does not form aggregates in membranes like JC-1 and does not interact with membrane proteins (**Perry SW. et al, 2011**).

3.9. Semi-quantitative RT-PCR

Extraction of mRNA from neuronal like PC12 cells was done using RNeasy kit from Qiagen. First, cells were lysed and homogenized using a lysis buffer supplement with beta-mercaptoethanol. The use of beta-mercaptoethanol prevents degradation of RNA by denaturing RNAses. Ethanol is then added to the lysate to precipitate nucleic acids from the lysate. The ethanol was removed by centrifugation and DNase1 digestion used to remove DNA resulting in a product containing only the RNA. After several washing steps to get rid of any leftover salt contaminant, the clean RNA was then eluted using RNase-free water. The quantity and quality was then determined by nanodrop and agarose gel analyses. The expression of specific genes was analyzed by semi-quantitative RT-PCR. For this, equal amouts of RNA was first reverse transcribed to cDNA using RevertAid H minus First strand cDNA kit from Fermentas. Validated rat primers from Qiagen or Biorad was then used to amplify the specific genes of interest and quantifications of the PCR reactions performed at a specific cycle number for each transcript using a ChemiDoc XRS + imaging system and the Image Lab software.

3.10. Biotin labeling of oxidized p53

Reaction of sulfhydryls groups in p53 with ROS results in disulphide bonds, which are reversible. To analyze the level of p53 with oxidized sulfhydryls groups, cells were lysed in biotin labeling lysis buffer (BLLB) containing maleimide to stably block all free, non-oxidized sulfhydryl groups, see Figure 1. Maleimide reacts only with the free sulfhydryl groups that have not been oxidized, forming a stable irreversible carbon-sulfur bond. After this step sulfhydryl groups oxidized by ROS was reduce by treatment with DTT. At this step, the amount of reduced sulfhydryl groups is proportional to the amount of ROS oxidized sulfhydryl groups that was present in the cell. In the next step, the reduced sulfhydryl groups are allowed to react with biotin-maleimide. The biotin-maleimide will react with all reduced sulfhydryl groups forming a sulfhydryls-maleimide-biotin linked complex. Streptavidin-sepharose beads are then incubated with the sample to specifically pull down the complexes. The immobilized streptavidin binds to all biotinylated proteins. The beads are then washed and resuspended in SDS-PAGE loading buffer to detach and denature all bead bound proteins. SDS-PAGE and western blot analysis was then performed to analyze the level of oxidized p53.

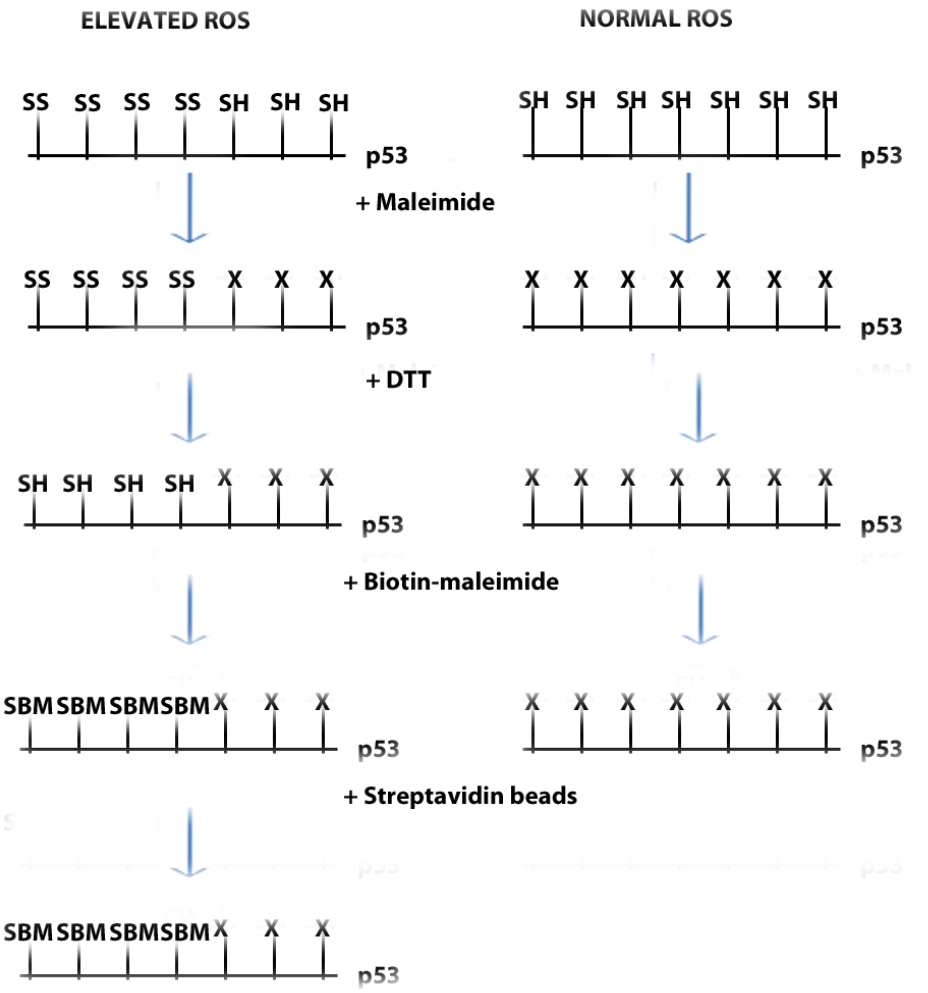

Figure 1. Schematic illustration of biotin labeling of oxidized p53. X= reacted with maleimide. SBM= reacted with biotin-maleimide.

3.11. Pulse Chase

Pulse chase was used to determine the half-life of proteins. Following different lengths of treatment with cycloheximide, which blocks protein translation, cells, were harvested and the level of the protein of interest analyzed by western blot as described above. The half-live, i.e., the time required to reduce target protein level by 50 %, was calculated using the formula: $M_t = M_0 \times (1 / 2)^{t/T}$, where T=the half-life, M_0=the amount at 0 h, and M_t=maximum time.

4. Results and discussion

4.1. Mutant ATXN7 expression, aggregation and toxicity in a stable inducible SCA7 model (Paper I & II)

To study the properties and toxic effects of the mutant ATXN7 protein, we generated a novel stable inducible SCA7 model. In this PC12 model called (FLQ65), full-length ATXN7 carrying 65Qs and a C-terminal GFP tag is expressed upon removal of Dox from media. Control cells, (FLQ10) expressing GFP tagged full-length ATXN7 with 10Qs were also generated. Upon induction of ATXN7Q65-GFP or ATXN7Q10-GFP, full-length ATXN7, as well as cleaved fragments of both the wild-type and mutant ATXN7 protein could be observed (Paper I, fig. 1A). In FLQ10 cells, high levels of one fragment between 40 and 50 kDa was observed, whereas two fragments between 40 and 45 kDa were detected in FLQ65 cells (Paper I, fig. 1A). Fragments with similar sizes, generated by caspase cleavage, have been reported in other SCA7 models (**Young JE et al, 2007**). Our results also revealed the presence of aggregated mutant ATXN7 material from day 3 onwards in FLQ65 cells, whereas no aggregates were detected in FLQ10 cells (Paper I, fig. 1C and Paper II, fig 1B). Moreover, we found that full-length mutant ATXN7 can aggregate, as inclusions could be detected with the C-terminal GFP tag (Paper 1, fig. 1E). However, compared to the number of inclusion detected with an N-terminal ATXN7 antibody, the GFP positive inclusions were much fewer, suggesting that inclusions mostly consist of ATXN7 fragments (paper I, fig 1E and data not shown). This is consistent with other studies showing that cleaved fragments are more prone to aggregate **(Sanchez I.. et al 2003, Skinner J. et al 1997).** In addition, we showed that induction of the mutant ATXN7 protein leads to cellular toxicity, whereas induction of wild-type ATXN7 did not cause cellular toxicity (Paper I, fig. 1D, Paper II fig 1C and D), similar to previous studies (**Furrer SA. et al, 2013, Mookerjee S. et al 2009, Helmlinger D. et al, 2004**). The mutant ATXN7-induced toxicity could arise from both the full-length and cleaved species (**Guyenet SJ. et al, 2015).** Interestingly, aggregates were visible from day 3, full-length mutant ATXN7 expression was detected from day 6, however toxicity was only displayed from day 9 onwards. Expression of mutant ATXN7 hence needs to be present for some time before toxicity develops in this model, mimicking the progressive nature of SCA7 in patients (**Johansson J. et al, 1998, David G. et al, 1997**).

4.2. Differential degradation of full-length and mutant ATXN7 fragments (Paper I)

Improving the clearance of expanded polyQ proteins from cells have been suggested as a potential therapy (**Wang HL. et al, 2013, Nisoli I. et al, 2010**). UPS and autophagy are the two main pathways used by most cells to degrade misfolded proteins (**Rubinsztein DC. 2006**). Using the newly constructed SCA7 model, we first investigated how the wild-type and mutant ATXN7 protein are cleared/degraded. Our result showed that full-length and cleaved fragments of mutant ATXN7 are differentially degraded. While UPS was essential for the clearance of full-length mutant ATXN7, as well as the wild-type ATXN7 full-length and Q10 ATXN7 fragment (Paper I, fig. 3A-C, 4A and B, 5A-B), autophagy essentially contributed to the degradation of mutant ATXN7 fragments and aggregates (Paper I, fig. 5B, 6A and B, and 8A-E). In contrast to our findings, another study found that neither UPS, nor autophagy, had any effect on mutant ATXN7 fragment clearance (**Mookerjee S. et al 2009**). This discrepancy could be due to the usage of a stable versus a transient transfection model or the formation of different sized fragments. Cleavage of ATXN7 is thought to occur in the nucleus and result in loss of the nuclear export signal (NES) **(Young JE et al, 2007)**. Fragments generated thus have to rely on diffusion through the nuclear pore to exit the nucleus. However, proteins larger than 40-60 kDa cannot diffuse through the nuclear pores (**Grünwald D. et al. 2011**). The fragments generated in the study by Mookerjee et al carrying 92Qs, would thus more likely be trapped in the nucleus, where autophagy is absent, compared to the fragments in our study with 65Qs.

Our data clearly show that mutant ATXN7 can be degraded (Paper I). However, to see if the clearance is slower than that of wild-type ATXN7, we next investigated the half-life of the wild-type and mutant ATXN7 protein. To do this, we took advantage of the ability to switch the ATXN7 expression on or off in the stable FLQ10/FLQ65 cell models, allowing us to study the ATXN7 protein turn-over without the use of protein synthesis inhibitors like cycloheximide. After allowing FLQ10 and FLQ65 cells to express ATXN7 for 12 days further expression was turn-off by the addition of doxycycline. The ATXN7 level was then analyzed at various time points by western blot and the half-lives calculated. The result revealed a half-life for full-length wild-type ATXN7 and the wild-type fragment to be 4.5 and 4.7 h, respectively (Paper I, fig. 2A and B). These estimated half-lives are consistent with previous studies indicating a relative short half-life for wild-type ATXN7 (**Yoo SY. et al. 2003,**

Yvert G. et al. 2001). In contrast, both full-length mutant and cleaved fragments of mutant ATXN7 displayed early accumulation after further expression was turned off (Paper I, fig. 2A and B). However, a rapid clearance, which we hypothesize could be due to aggregation rather than degradation, was then observed (Paper I, fig. 2A and B). Analysis of the aggregated ATXN7 material revealed a half-life of 34.3 h (Paper I, fig. 2B). This result show that the expanded polyglutamine domain enhances the stability of ATXN7 through rapid aggregation, a phenomenon also reported in another study (**Yvert G. et al. 2001**).

4.3. Mutant ATXN7 induced-oxidative stress enhances aggregation and toxicity (Paper I & II)

Oxidative stress has been implicated in the pathology of several neurodegenerative diseases, including some polyQ diseases. To investigate if oxidative stress plays a role in SCA7 toxicity, we measured the ROS and GSH levels in the inducible FLQ65 SCA7 model. The results showed progressively elevating levels of ROS and decreased GSH levels consistent with oxidative stress in induced FLQ65 cells expressing mutant ATXN7 (Paper II, fig. 1E and F). To establish if there is a link between the oxidative stress, aggregation and toxicity in SCA7, we treated the mutant ATXN7 expressing cells with drugs like H_2O_2 and BSO, which promote oxidative stress. The results showed that promoting oxidative stress increases mutant ATXN7 aggregation (Paper II, fig. 4B and D). Similarly, other studies have shown that H_2O_2 treatment enhances mutant huntingtin aggregation and cell death (**Goswami A. et al, 2006**). We also treated the mutant ATXN7 expressing cells with antioxidants (NAc or Vit E) or increased the expression of some antioxidants through transfection with SOD1 or RORa. These treatments all resulted in reduced levels of ROS and aggregation as well as restored cellular viability in mutant ATXN7 expressing cells (Paper II, fig. 2A, C, D, F and 3A and B). Consistent with this, other studies have demonstrated similar effects of antioxidant on aggregation and toxicity. For example, over-expression of SOD1 was shown to reduce mutant huntingtin aggregation and cell death (**Goswami A. et al, 2006**). This means that antioxidant might be used as a therapeutic treatment in SCA7 and possibly other polyQ diseases. In fact, an earlier study in a HD mouse model suggested a potential protective role of antioxidants (**Beal M. and Ferrante R. 2004, Huntington Study Group. 2001**). However, antioxidant treatment in clinical

has so far been reported to produce less positive effects (**Peyser CE. et al, 1995**).

4.4. Increased NOX1 activity underlies the oxidative stress-induced toxicity (Paper II, III and IV)

To determine the cause of the oxidative stress in the FLQ65 SCA7 model, we first evaluated the status of the antioxidant system. Our results showed increased levels of SOD1 and GSTA3 in mutant ATXN7 cells (Paper II, fig. 5A). Increased levels of SOD1 and GSTA3 most likely indicate that the mutant ATXN7 cells are trying to cope with the mutant ATXN7-induced oxidative stress by up regulating some antioxidants. However, we also detected reduced levels of CAT in the mutant ATXN7 expressing cells (Paper II, fig. 4A). Interestingly, reduced levels of CAT have also been reported in SCA3 and HD models (**Yu YC. et al, 2009**). The reduced CAT levels could compromise clearance of H_2O_2 and may contribute to the oxidative stress in polyQ disease. However, in the SCA7 model, the levels of ROS was increased before CAT enzyme down-regulation could be detected suggesting that the decreased CAT level is not the main source of ROS generation in mutant ATXN7 expressing cells.

In an attempt to further investigate the source of the ROS in the mutant ATXN7 cells, we therefore next measured ROS generation by mitochondria or NOX enzymes. Our results showed that the increased ROS is generated by NOX enzymes and not from mitochondria, as general NOX enzyme inhibitors reversed the elevated ROS levels in mutant ATXN7 cells (Paper II, fig. 6A, B and D). To identify which NOX enzyme is involved in the increased ROS production in FLQ65 cells, we first analyzed the expression level of NOX1 and 2, the two main NOX family members present in PC12 cells. Analysis of NOX family members revealed an increase in the expression level of NOX1, while the NOX2 expression level remained unchanged (Paper III, 1B-F). This suggests that NOX1 is the NOX enzyme activated by mutant ATXN7. To further verify this, we used a NOX1 specific inhibitor or NOX1 siRNA knock-down. NOX1 inhibition using ML171 or knock-down, both reduced the ROS generation and ameliorated cellular toxicity induced by mutant ATXN7 (Paper III, fig. 2A-F). To further investigate the mechanism behind the increased NOX1 activity, we also measured NOX1 stability in mutant ATXN7 cells. The results revealed a NOX1 half-life of 35.3 h in mutant ATXN7 expressing cells, whereas a half-life of 14.5 h was identified in non-induced control cells (Paper IV, fig. 1B). This

result suggests that the elevated level/activity of NOX1 in SCA7 cells could at least in part be due to an increased NOX1 protein stability.

Several other polyQ disease studies have also identified NOX activation as a cause of oxidative stress and toxicity (**Bertoni A. et al, 2011, Ajayi A. et al, 2015**). However, reduced NOX activity in striatal cells expressing the mutant huntingtin protein has also been reported (**Ribeiro M. et al, 2013**). In most studies linking NOX to polyQ toxicity, NOX2 has been implicated as the main target of expanded-polyQ proteins (**Bertoni A. et al, 2011, Valencia A. et al, 2013**). Bertoni et al showed that both mutant ataxin-2 and Huntingtin are selectively sequestered into the lipid raft membrane compartment and interact with NOX2/gp91^{phox}, resulting in increased level of ROS and subsequently toxicity (**Bertoni A. et al, 2011)**. However, whether polyQ proteins directly interact with NOX2 has been questioned by others (**Valencia A. et al, 2013**). Why NOX1, and not NOX2, is preferentially activated by mutant ATXN7 is not known. We could however speculate that mutant ATXN7 could target the cytosolic subunits of NOX1 (NOXO1 or NOXA1) and not NOX2 (p47^{phox} or p67^{phox}) thereby triggering the activation or assembly of the NOX1 complex. Another reason could be that mutant ATXN7 could preferentially affect transcription factors like GATA-6, HNF-1α or Cdx that are known to regulate NOX1 expression (**Valente A. et al, 2008, Brewer A. et al, 20069).**

4.5. Metabolic shift and energy reduction in mutant ATXN7 expressing cells (Paper III)

Metabolic dysfunction has been implicated in several polyQ diseases including SCA7. Moreover, activation and up-regulation of NOX1 has been linked to altered metabolism in cancer cells (**Lu W. et al, 2012, Rezvani HR. et al, 2011, Prata C. et al, 2008)**. We therefore hypothesized that mutant ATXN7-induced NOX1 activation could contribute to the metabolic alterations in SCA7. To investigate this, we first measured the mitochondrial respiratory activity by analyzing the oxygen consumption and the mitochondrial membrane potential in FLQ65 SCA7 cells. Our results showed reduced oxygen consumption and mitochondrial membrane potential in mutant ATXN7 expressing cells (Paper III fig. 4A, B, fig. 5F and G). In contrast, no change in oxygen consumption or the mitochondrial membrane potential could be observed in control cells (Paper III fig. 4B, fig. 5F and G). Next we measured the glycolytic activity in mutant ATXN7 expressing cells. An increased glycolytic activity evident by increased

glucose consumption, as well as increased lactate and LDH levels and activity was observed in mutant ATXN7 cells (Paper III, fig. 3A, B, D and E, fig. 5C). These data suggest that mutant ATXN7 results in a metabolic shift with reduced mitochondrial respiration and instead increased glycolysis. This shift could reduce the total ATP production and indeed, we could detect reduced ATP levels in mutant ATXN7 cells (Paper III, fig 4H). Consistent with our results, reduced oxygen consumption and ATP levels, as well as increased glycolysis, have been reported in other polyQ disease models (**Milakovic T. et al, 2005, Weydt P .et al, 2006, Ravikumar B. et al, 2003, Kita H, et al., 2002**).

Interestingly, treatment with antioxidants, NOX enzyme inhibitors, as well as NOX1 knock-down reversed the metabolic shift (Paper III, fig. 5A-G). These results suggest the involvement of NOX1 in the metabolic dysfunction. Put together, our results highlight the importance of mitochondrial dysfunction and metabolic alteration in SCA7 disease. We hypothesize that the increased glycolysis in FLQ65 SCA7 cells may be an attempt by the mutant ATXN7 expressing cells to source for more energy. However, since neurons may not be able to up-regulate glycolysis, this might not be possible in the patient brain and could contribute to the high vulnerability of neurons in SCA7 (**Bolanos JP. et al., 2008, 2010**).

4.6. Altered p53 properties in mutant ATXN7 expressing cells (Paper III and IV)

We recently showed that p53 co-aggregates with mutant ATXN7 and that the nuclear levels, as well as the general transcriptional activity of p53 is reduced in a SCA7 model (**Yu X. et al, 2013**). Furthermore, one important p53 function is to slow down glycolysis and promote mitochondrial respiration. To investigate if the reduce nuclear level of p53 contributes to the observed altered metabolism in SCA7 cells; we measured the mRNA levels of some key p53 regulated metabolic genes including AIF, GLUT1 and TIGAR. AIF is induced by p53 and is required for the maintenance of mitochondrial complex I and III integrity (**Stambolsky P. et al, 2006**), whereas negative regulation of glucose transporter 1 (GLUT1) expression and induction of TIGAR by p53 suppresses glycolysis (**Bensaad K. et al, 2006, Schwartzenberg-Bar-Yoseph F. et al 2004**). Our result showed decrease mRNA levels of TIGAR and AIF, as well as a small, but non-significant, increase in GLUT1 mRNA in mutant ATXN7 cells (Paper III, fig. 6B). In agreement with the mRNA results, we observe a gradual decrease in the protein levels of TIGAR and AIF, but a gradual increase in GLUT1 in cells

expressing mutant ATXN7 protein (Paper III, fig. 7A-E). These data suggest that dys-regulation of p53 target genes could contribute to the reduced mitochondrial respiration and increased glycolysis observed in FLQ65 cells. To further reassert this hypothesis, we next investigated whether increasing the p53 levels could reverse the mutant ATXN7 induced metabolic alterations. Our result shows that treatment with Nutlin-3, which increased the p53 level, restored the expression levels of TIGAR, AIF and GLUT1, as well as reversed the metabolic shift and the up regulation of NOX1 expression (Paper III, fig. 8A-D). These results indicate that p53 is involved in the metabolic alteration and could be involved in negative regulation of NOX1 transcription.

The ROS generated through NOX1 activation could potentially oxidize p53 and thereby promote cytoplasmic localization of p53 and reduce p53 transcriptional activity (**Wu HH. et al, 2000**). Next, we therefore analyzed the p53 sub-cellular localization and oxidation level in SCA7 cells in the presence or absence of the NOX1 inhibitor ML171. Our results showed that the increased NOX1 activity in SCA7 cells results in increased p53 oxidation and altered the cellular p53 localization (Paper IV, fig 2A-C, 3C). Taken together, our data suggests that a feed forward-loop between p53 and NOX1 could occur in SCA7 cells (Paper III, fig. 9). In this loop, the reduced p53 transcriptional activity induces NOX1 expression and activity resulting in more NOX1 produced ROS, which in turn leads to increased oxidation of p53, further reduction of p53 transcriptional activity, metabolic dysregulation, oxidative stress and toxicity.

5. Conclusions

We have gained new understanding of the molecular mechanisms by which the mutant ATXN7 protein induces toxicity in SCA7 disease. Our results show that oxidative stress, NOX1 complex activation, as well as p53 dys-regulation play a role in disease pathology. For each paper, we have obtained the following conclusions:

Paper I:

- Mutant ATXN7 expression causes cellular toxicity.
- UPS is the major degradation pathway for full-length wild-type and mutant ATXN7.
- However, autophagy significantly contributes to the degradation of mutant ATXN7 fragments and aggregates.

Paper II:

- Mutant ATXN7 induced oxidative stress contributes to aggregation and toxicity.
- Antioxidant treatment ameliorates the cellular toxicity induced by mutant ATXN7.
- Mutant ATXN7 expression leads to oxidative stress via over-activation of NOX enzymes.

Paper III:

- Mutant ATXN7 expression leads to specific NOX1 up-regulation and activation.
- NOX1 activation induces a metabolic shift and energy deficits in mutant ATXN7 expressing cells.
- Reduced nuclear p53 function leads to altered levels of metabolic genes and contributes to the metabolic dysfunction in SCA7 cells.

Paper IV:

- NOX1 activation promotes translocation of p53 from the nucleus to the cytoplasm in mutant ATXN7 expressing cells.
- NOX1 activation enhances p53 oxidation in mutant ATXN7 expressing cells
- Mutant ATXN7 stabilizes the NOX1 enzyme.

Our findings will help to further identify and investigate the factors that influence mutant ATXN7 toxicity and will hopefully help to develop a potential therapeutic approach for SCA7 and other polyQ diseases.

6. Populärvetenskaplig sammanfattning på svenska

Spinocerebellär ataxi typ 7 (SCA7) är en neurodegenerativ sjukdom som påverkar lillhjärnan, näthinnan och hjärnstammen. SCA7 orsakas av en mutation / förlängning av en CAG upprepning i den kodande delen av SCA7 genen. Eftersom CAG kodar för aminosyran glutamin, resulterar expansionen av CAG upprepningen i ett förlängt polyglutamin (polyQ) domän i den N-terminala delen av proteinet ataxin-7 (ATXN7). Trots att orsaken till SCA7 har varit känd i flera år saknas fortfarande effektiva behandlingsstrategier för att bota sjukdomen. För att kunna utveckla sådanna strategier är det viktigt att förstå de bakomliggande sjukdomsmekanismerna i detalj. Förrutom SCA7 orsakas 8 andra neurodegenerativa sjukdomar av förlängda glutamin domän i annars obesläktade proteiner. Dessa sjukdomar kallas polyglutamin (polyQ) sjukdomar och gemensamma sjukdomsmekanismer genom vilka de polyQ expanderade proteiner inducerar toxicitet har föreslagits. I denna studie har betydelsen av oxidativ stress och aggregering/ihopklumpning av det muterade ATXN7 proteinet undersökts i modeller av SCA7. Vi visar att uttrycket av muterat ATXN7 resulterar i förhöjda nivåer av reaktiva syraradikaler (ROS) och oxidativ stress, vilket i sin tur leder till toxicitet. Våra resultat visar också att den oxidativa stressen bidrar till ytterligare aggregering av muterat ATXN7. Dessutom visar vi för första gången att den största källan till den förhöjda ROS nivån i SCA7 celler är ökad aktivering av NOX1 enzymer. Intressant, visade våra resultat vidare att den ökade NOX1 aktivitet, tillsammans med förändringar i proteinet p53s funktioner leder till metaboliska förändringar och reducerad energi produktion i SCA7 celler. Behandling med antioxidanter såväl som inhibering av NOX1 minskade ROS nivån, återställde de metaboliska förändringarna och förbättrade SCA7 cellernas överlevnad. Sammantaget drar vi slutsatsen att muterat ATXN7 aktiverar NOX1 enzymer vilket resulterar i oxidativ stress, ökad ATXN7 aggregering, metaboliska förändringar och toxicitet. Specifik inhibering av NOX1 skulle därmed kunna vara en potentiell terapeutisk strategi för SCA7.

7. Acknowledgements

My biggest thanks go to God almighty for seeing me through the PhD program.

Special thanks to my supervisor, Anna Lena Ström, for her patient, love and willingness to guide me through my PhD study. Your contribution in my life will live forever.

I wish to thank both present and past members of my group: Xin, Narasimha, Galateia, Victor, Anne and Carolina for their contribution to this work and their support in general.

Big thanks to everyone at the department for their cooperation, understanding, love and positive discussion. I will always remember you all.

Special thanks to all my teachers for their academic support and advice.

8. References

Adanyeguh IM, Henry PG, Nguyen TM, Rinaldi D, Jauffret C, Valabregue R, Emir UE, Deelchand DK, Brice A, Eberly LE, Öz G, Durr A, Mochel F. (2015). In vivo neurometabolic profiling in patients with spinocerebellar ataxia types 1, 2, 3, and 7. Mov Disord. 30:662-670.

Ago T, Kuroda J, Kamouchi M, Sadoshima J, Kitazono T. (2010). Pathophysiological roles of NADPH oxidase/nox family proteins in the vascular system. -Review and perspective-. Circ J. 75:1791-1800.

Ajayi A, Yu X, Lindberg S, Langel U, Ström AL.(2012). Expanded ataxin-7 cause toxicity by inducing ROS production from NADPH oxidase complexes in a stable inducible Spinocerebellar ataxia type 7 (SCA7) model. BMC Neurosci. 13:86.

Ajayi A, Yu X, Wahlo-Svedin C, Tsirigotaki G, Karlström V, Ström AL. (2015). Altered p53 and NOX1 activity cause bioenergetic defects in a SCA7 polyglutamine disease model. Biochim Biophys Acta.;1847:418-428.

Angeloni C, Prata C, Dalla Sega FV, Piperno R, Hrelia S. (2015). Traumatic brain injury and NADPH oxidase: a deep relationship. Oxid Med Cell Longev. 2015:370312.

Anrather J, Racchumi G, Iadecola C. (2006). NF-kappaB regulates phagocytic NADPH oxidase by inducing the expression of gp91phox. J. Biol. Chem. 281:5657–5667.

Arrasate M, Mitra S, Schweitzer S, Segal M and Finkbeiner S. (2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431:805–810.

Aruoma I, Halliwell B, Hoey B, Butler J. (1989). The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med. 6:593-597.

Atanassov BS, Dent SY. (2011). USP22 regulates cell proliferation by deubiquitinating the transcriptional regulator FBP1. EMBO Rep. 12:924-930.

Atanassov BS, Evrard YA, Multani AS, Zhang Z, Tora L, Devys D, Chang S, Dent SY. (2009). Gcn5 and SAGA regulate shelterin protein turnover and telomere maintenance. Mol Cell. 35:352-364.

Bae BI, , Igarashi S, Fujimuro M, Agrawal N, Taya Y, Hayward SD, Moran TH, Montell C, Ross CA, Snyder SH,Xu H Sawa A. (2005). p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. Neuron. 47:29-41.

Banfi B, Molnár G, Maturana A, Steger K, Hegedûs B, Demaurex N, Krause K. (2001). A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes. J. Biol. Chem. 276:37594-37601.

Banno H, Katsuno M, Suzuki K, Tanaka F, Sobue G. (2009). Neuropathology and therapeutic intervention in spinal and bulbar muscular atrophy. Int J Mol Sci.10:1000-1012.

Bargonetti, J. and Manfredi, J. J. (2002). "Multiple roles of the tumor suppressor p53." Curr Opin Oncol 14: 86-91.

Beal M. F. & Ferrante R. J. (2004). Experimental therapeutics in transgenic mouse models of Huntington's disease. Nature Rev. Neurosci. 5:373–384.

Beauchemin AM, Gottlieb B, Beitel LK, Elhaji YA, Pinsky L, Trifiro MA. Cytochrome c. (2001). oxidase subunit Vb interacts with human androgen receptor: a potential mechanism for neurotoxicity in spinobulbar muscular atrophy. Brain Res Bull . 56: 285-297.

Bedard K , Krause K. (2007). The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev.1:245-313.

Bennett EJ, Shaler TA, Woodman B, Ryu KY, Zaitseva TS, Becker CH, Bates GP, Schulman H, Kopito RR. (2007). Global changes to the ubiquitin system in Huntington's disease. Nature. 448:704-708.

Benomar A, Krols L, Stevanin G, Cancel G, LeGuern E, David G, Ouhabi H, Martin JJ, Dürr A, Zaim A, et al. (1995). The gene for autosomal dominant cerebellar ataxia with pigmentary macular dystrophy maps to chromosome 3p12-p21.1. Nat Genet. 10:84-88.

Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH. (2006). TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell. 126:107-20.

Benton C, de Silva R, Rutledge S, Bohlega S, Ahisaw T, Zoghbi H. (1998). Molecular and clinical studies in SCA-7 define a broad clinical spectrum and the infatile phonotype. Neurology. 51. 1081-1086.

Berciano J. (1982). Olivopontocerebellar atrophy. A review of 117 cases. J Neurol Sci. 53:253-272.

Berkers CR, Maddocks OD, Cheung EC, Mor I, Vousden KH. (2013). Metabolic regulation by p53 family members. Cell Metab. 18:617-633.

Bertoni A, Giuliano P, Galgani M, Rotoli D, Ulianich L, Adornetto A, Santillo MR, Porcellini A, Avvedimento VE. (2011). Early and late events induced by polyQ-expanded proteins: identification of a common pathogenic property of polyQ-expanded proteins. J Biol Chem.286:4727-4741.

Bhatt L, Groeger G, McDermott K, Cotter T G. (2010). Rod and cone photoreceptor cells produce ROS in response to stress in a live retinal explant system. Mol Vis, 16: 283–293.

Blum, D., Wu, Y., Nissou, M. F., Arnaud, S., Alim Louis, B. and Verna, J. M. (1997). "p53 and Bax activation in 6-hydroxydopamine-induced apoptosis in PC12 cells." Brain Res 751:139-142.

Bolanos, J.P., Almeida, A., Moncada, S. (2010) . Glycolysis: a bioenergetic or a survival pathway? Trends in biochemical sciences, 35:145-149.

Bolanos, J.P., Delgado-Esteban, M., Herrero-Mendez, A., Fernandez-Fernandez, S., Almeida A. (2008). Regulation of glycolysis and pentose-phosphate pathway by nitric oxide: impact on neuronal survival. Biochimica et biophysica 1777: 789-793.

Bonnet J, Wang YH, Spedale G, Atkinson RA, Romier C, Hamiche A, Pijnappel WW, Timmers HT, Tora L, Devys D, Kieffer B. (2010). The structural plasticity of SCA7 domains defines their differential nucleosome-binding properties. EMBO Rep.11:612-618.

Brewer C, Sparks E, Shah A. (2006). Transcriptional regulation of the NADPH oxidase isoform, Nox1, in colon epithelial cells: role of GATA-binding factor(s). Free Radic. Biol. Med. 40:260–274.

Brown D.I. and Griendling K.K. (2009). Nox proteins in signal transduction. Free Radic. Biol. Med. 47:1239-1253.

Browne S.E, Bowling A.C, MacGarvey U, Baik M.J, Berger S.C, Muqit M.M, Bird E.D. and Beal M.F. (1997). Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann. Neurol., 41: 646–653.

Burke TL, Miller JL, Grant PA. (2013). Direct inhibition of Gcn5 catalytic activity by polyglutamine-expanded Ataxin-7. J Biol Chem. [Epub ahead of print].

Burright EN, Clark HB, Servadio A, Matilla T, Feddersen RM, Yunis WS, Duvick LA, Zoghbi HY, Orr HT. (1995). SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. Cell. 82:937-948.

Burton GW, Traber MG. (1990). Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr. 10:357-382.

Cancel G, Duyckaerts C, Holmberg M, Zander C, Yvert G, Lebre AS, Ruberg M, Faucheux B, Agid Y, Hirsch E, Brice A. (2000). Distribution of ataxin-7 in normal human brain and retina. Brain. 12:2519-2530.

Chen S, Peng GH, Wang X, Smith AC, Grote SK, Sopher BL, La Spada AR. (2004). Interference of Crx-dependent transcription by ataxin-7 involves interaction between the glutamine regions and requires the ataxin-7 carboxy-terminal region for nuclear localization. Hum Mol Genet. 13:53-67.

Chen YC, Gatchel JR, Lewis RW, Mao CA, Grant PA, Zoghbi HY, Dent SY. (2012). Gcn5 loss-of-function accelerates cerebellar and retinal degeneration in a SCA7 mouse model. Hum Mol Genet. 21:394-405.

Choi D.H, Cristóvão A.C, Guhathakurta S, Lee J, Joh T.H, Beal, M.F, Kim Y.S. (2012). NADPH oxidase 1-mediated oxidative stress leads to dopamine neuron death in Parkinson's disease. Antioxid. Redox Signal, 16:1033-1045.

Chou AH, Chen CY, Chen SY, Chen WJ, Chen YL, Weng YS, Wang HL. (2010). Polyglutamine-expanded ataxin-7 causes cerebellar dysfunction by inducing transcriptional dysregulation. Neurochem Int. 56:329-339.

Chuong Nguyen MV, Lardy B, Paclet MH, Rousset F, Berthier S, Baillet A, Grange L, Gaudin P, Morel F. (2015). [NADPH oxidases, Nox: new isoenzymes family]. Med Sci (Paris). 31:43-52.

Cillero-Pastor B, Rego-Pérez I, Oreiro N, Fernandez-Lopez C, Blanco FJ. (2013). Mitochondrial respiratory chain dysfunction modulates metalloproteases -1, -3 and -13 in human normal chondrocytes in culture.BMC Musculoskelet Disord. 14:235.

Cohen-Carmon D, Meshorer E. (2012). Polyglutamine (polyQ) disorders: the chromatin connection. Nucleus. 3:433-441.

Cortes CJ, La Spada AR. (2014). The many faces of autophagy dysfunction in Huntington's disease: from mechanism to therapy. Drug Discov Today. 19:963-971.

Coyle JT, Puttfarcken P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. Science. 1993 262:689-695.

Coyoy A, Valencia A, Guemez-Gamboa A, Morán J. (2008). Role of NADPH oxidase in the apoptotic death of cultured cerebellar granule neurons. Free Radic Biol Med, 45: 1056–1064.

Cristóvão AC, Guhathakurta S, Bok E, Je G, Yoo SD, Choi DH, Kim YS. (2012). NADPH oxidase 1 mediates α-synucleinopathy in Parkinson's disease. J Neurosci. 32:14465-14477.

Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. (2006). Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell. 127: 59-69.

Cummings J. and Zoghbi Y. (2000). TRINUCLEOTIDE REPEATS: Mechanisms and Pathophysiology. Annu. Rev. Neurosci. 23:217–247.

Custer SK, Garden GA, Gill N, Rueb U, Libby RT, Schultz C, Guyenet SJ, Deller T, Westrum LE, Sopher BL, La Spada AR. (2006). Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport. Nat. Neurosci. 9:1302–1311.

Damiano M1, Galvan L, Déglon N, Brouillet E. (2010). Mitochondria in Huntington's disease. Biochim Biophys Acta. 1802:52-61.

Dantuma NP, Bott LC. (2014). The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. Front Mol Neurosci. 7:70.

Davey, G.P., Peuchen, S., Clark, J.B. (1998). Energy thresholds in brain mitochondria. Potential involvement in neurodegeneration. The Journal of biological chemistry, 273: 12753-12757.

David G, Abbas N, Stevanin G, Dürr A, Yvert G, Cancel G, Weber C, Imbert G, Saudou F, Antoniou E, Drabkin H, Gemmill R, Giunti P, Benomar A, Wood N, Ruberg M, Agid Y, Mandel JL, Brice A. (1997). Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. Nat Genet. 17:65-70.

David G, Durr A, Stevanin G, Cancel G, Abbas N, Benomar A. (1998). Molecular and clinical correlations in autosomal dominant cerebellar ataxia with progressive macular dystrophy. Hum Mol Genet. 7. 167-170.

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP. (1997). Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 90:537-548.

de la Monte, S. M., Sohn, Y. K., Ganju, N. and Wands, J. R. (1998). "P53- and CD95-associated apoptosis in neurodegenerative diseases." Lab Invest 78:401-411.

Decker T, Lohmann-Matthes ML. (1988). A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. J Immunol Methods. 115:61-69.

Deter RL, Baudhuin P, De Duve C. (1967). Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. J Cell Biol. 35:C11-16.

Dickinson BC, Peltier J, Stone D, Schaffer DV, Chang CJ. (2011). Nox2 redox signaling maintains essential cell populations in the brain. Nat Chem Biol. 7:106-112.

Donis KC, Mattos EP, Silva AA, Furtado GV, Saraiva-Pereira ML, Jardim LB, Saute JA. (2015). Infantile spinocerebellar ataxia type 7: Case report and a review of the literature. J Neurol Sci. 118-121.

Duan, W., Zhu, X., Ladenheim, B., Yu, Q. S., Guo, Z., Oyler, J., Cutler, R. G., Cadet, J. L., Greig, N. H. and Mattson, M. P. (2002). "p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism." Ann Neurol 52:597-606.

Dunah A. W., Jeong H., Griffin A., Kim Y.-M., Standaert D. G., Hersch S. M. et al. (2002). Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science 296: 2238–2243.

Duncan CE, An MC, Papanikolaou T, Rugani C, Vitelli C, Ellerby LM. (2013). Histone deacetylase-3 interacts with ataxin-7 and is altered in a spinocerebellar ataxia type 7 mouse model.Mol Neurodegener.8:42. [Epub ahead of print].

Duyao MP, Auerbach AB, Ryan A, Persichetti F, Barnes GT, McNeil SM, Ge P, Vonsattel JP, Gusella JF, Joyner AL, et al. (1995). Inactivation of the mouse Huntington's disease gene homolog Hdh. Science. 269:407-410.

Einum DD, Clark AM, Townsend JJ, Ptacek LJ, Fu YH. (2003). A novel central nervous system-enriched spinocerebellar ataxia type 7 gene product. Arch Neurol. 60:97-103.

Enevoldson TP, Sanders MD, Harding AE. (1994). Autosomal dominant cerebellar ataxia with pigmentary macular dystrophy. A clinical and genetic study of eight families. Brain. 117:445-460.

Ermak G, Cancasci VJ, Davies KJ. (2003). Cytotoxic effect of doxycycline and its implications for tet-on gene expression systems. Anal Biochem. 318:152-154.

Everett M and Wood W. (2004). Trinucleotide repeats and neurodegenerative disease. Brain 127, 2385–2405.

Facecchia K, Fochesato LA, Ray SD, Stohs SJ, Pandey S. (2011). Oxidative toxicity in neurodegenerative diseases: role of mitochondrial dysfunction and therapeutic strategies. J Toxicol. 683728.

Fatokun AA, Stone TW, Smith RA. (2008). Oxidative stress in neurodegeneration and available means of protection. Front Biosci, 13:3288–3311.

Ferrante RJ, Kubilus JK, Lee J, Ryu H, Beesen A, Zucker B, Smith K, Kowall NW, Ratan RR, Luthi-Carter R, Hersch SM. (2003). Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice.. J Neurosci. 23:9418-9427.

Firdaus WJ, Wyttenbach A, Giuliano P, Kretz-Remy C, Currie RW, Arrigo AP. (2006). Huntingtin inclusion bodies are iron-dependent centers of oxidative events. FEBS J. 273:5428-5441.

Forkink M, Manjeri GR, Liemburg-Apers DC, Nibbeling E, Blanchard M, Wojtala A, Smeitink JA, Wieckowski MR, Willems PH, Koopman WJ. (2014). Mitochondrial hyperpolarization during chronic complex I inhibition is sustained by low activity of complex II, III, IV and V.Biochim Biophys Acta. 1837:1247-1256.

Forsgren L, Libelius R, Holmberg M, von Döbeln U, Wibom R, Heijbel J, Sandgren O, Holmgren G. (1996). Muscle morphology and mitochondrial investigations of a family with autosomal dominant cerebellar ataxia and retinal degeneration mapped to chromosome 3p12-p21.1. J Neurol Sciences. 144:91–98.

Furrer SA, Waldherr SM, Mohanachandran MS, Baughn TD, Nguyen KT, Sopher BL, Damian VA, Garden GA, La Spada AR. (2013). Reduction of mutant ataxin-7 expression restores motor function and prevents cerebellar synaptic reorganization in a conditional mouse model of SCA7. Hum Mol Genet. 22:890-903.

Gandhi S, Abramov AY.(2012). Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev. 428010.

Gao HM, Zhou H, Hong JS. (2012). NADPH oxidases: novel therapeutic targets for neurodegenerative diseases. Trends Pharmacol Sci. 33, 295-303.

Garden G.A, Libby R.T, Fu Y.H Y, et al. (2002). Polyglutamineexpanded ataxin-7 promotes non-cell-autonomous purkinje cell degeneration and displays proteolytic cleavage in ataxic transgenic mice. J. Neurosci. 22:4897–4905.

Garden GA. and La Spada AR. (2008). Molecular pathogenesis and cellular pathology of spinocerebellar ataxia type 7 neurodegeneration. Cerebellum 22, 138–149.

Geiszt M, Lekstrom K, Brenner S, Hewitt SM, Dana R, Malech HL, Leto TL. (2003). NAD(P)H oxidase 1, a product of differentiated colon epithelial cells, can partially replace glycoprotein 91phox in the regulated production of superoxide by phagocytes. J. Immunol. 171:299-306.

Gianni D, Taulet N, Zhang H, DerMardirossian C, Kister J, Martinez L, Roush WR, Brown SJ, Bokoch GM, Rosen H. (2010). A novel and specific NADPH oxidase-1 (Nox1) small-molecule inhibitor blocks the formation of functional invadopodia in human colon cancer cells. ACS Chem Biol. 5:981-993.

Gordon AH, Hart PD, Young MR. (1980). Ammonia inhibits phagosome-lysosome fusion in macrophages. Nature. 286:79-80.

Goswami A, Dikshit P, Mishra A, Mulherkar S, Nukina N, Jana NR. (2006). Oxidative stress promotes mutant huntingtin aggregation and mutant huntingtin-dependent cell

death by mimicking proteasomal malfunction. Biochem Biophys Res Commun. 342:184-190.

Gouw LG, Digre KB, Harris CP, Haines JH, Ptacek LJ. (1994). Autosomal dominant cerebellar ataxia with retinal degeneration: clinical, neuropathologic, and genetic analysis of a large kindred. Neurology. 44:1441-1447.

Gouw LG, Kaplan CD, Haines JH, Digre KB, Rutledge SL, Matilla A, Leppert M, Zoghbi HY, Ptácek LJ. (1995). Retinal degeneration characterizes a spinocerebellar ataxia mapping to chromosome 3p. Nat. Genet. 10:89-93.

Goyal P, Weissmann N, Grimminger F, Hegel C, Bader L, Rose F, Fink L, Ghofrani H., Schermuly R, Schmidt H, Seeger W, Hanze J. (2004). Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. Free Radic. Biol. Med. 36:1279–1288.

Graham FL, Smiley J, Russell WC, Nairn R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J Gen Virol. 36:59-574.

Graham, R.K., Deng, Y., Slow, E.J., Haigh, B., Bissada, N., Lu, G., Pearson, J., Shehadeh, J., Bertram, L., Murphy, Z., et al. (2006). Cleavage at the Caspase-6 Site Is Required for Neuronal Dysfunction and Degeneration Due to Mutant Huntingtin. Cell 125, 1179–1191.

Graveland GA, Williams RS, DiFiglia M. (1985). Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. Science. 227:770-773.

Greenamyre J.T. and Hastings T.G. (2004). Biomedicine. Parkinson's—divergent causes, convergent mechanisms. Science: 304: 1120-1122.

Greene LA, Tischler AS. (1976). Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci U S A. 73:2424-2428.

Grimm S, Hoehn A, Davies KJ, Grune T. (2011). Protein oxidative modifications in the ageing brain: consequence for the onset of neurodegenerative disease. Free Radic Res. 45:73-88.

Groeger G, Mackey A M, Pettigrew C A, Bhatt L, Cotter T G (2009). Stress-induced activation of Nox contributes to cell survival signalling via production of hydrogen peroxide. J Neurochem. 5: 1544–1554.

Grünwald D, Singer RH, Rout M. (2011). Nuclear export dynamics of RNA- protein complexes. Nature. 475:333-341.

Gu, M., Gash, M.T., Mann, V.M., Javoy-Agid, F., Cooper, J.M., Schapira, A.H. (1996). Mitochondrial defect in Huntington's disease caudate nucleus. Annals of neurology, 39: 385-389.

Gu, W. and Roeder, R. G. (1997). "Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain." Cell 90:595-606.

Guertin DA, Sabatini DM.(2007). Defining the role of mTOR in cancer.Cancer Cell. 12:9-22.

Guo X, Disatnik MH, Monbureau M, Shamloo M, Mochly-Rosen D, Qi X. (2013). Inhibition of mitochondrial fragmentation diminishes Huntington's disease-associated neurodegeneration. J Clin Invest. 123:5371-5388.

Guyenet SJ, Mookerjee SS, Lin A, Custer SK, Chen SF, Sopher BL, La Spada AR, Ellerby LM. (2015). Proteolytic Cleavage of Ataxin-7 Promotes SCA7 Retinal Degeneration and Neurological Dysfunction. Hum Mol Genet. [Epub ahead of print].

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. (2006). Suppression of basal

autophagy in neural cells causes neurodegenerative disease in mice. Nature. 441:885-889.

Hart PD, Young MR, Jordan MM, Perkins WJ, Geisow MJ.(1983). Chemical inhibitors of phagosome-lysosome fusion in cultured macrophages also inhibit saltatory lysosomal movements. A combined microscopic and computer study.J Exp Med. 158:477-492.

Hayashida S, Arimoto A, Kuramoto Y, Kozako T, Honda S, Shimeno H, Soeda S. (2010). Fasting promotes the expression of SIRT1, an NAD+ -dependent protein deacetylase, via activation of PPARalpha in mice. Mol Cell Biochem, 339:285-292.

Heales, S. J. and Bolanos, J. P. (2002). Impairment of brain mitochondrial function by reactive nitrogen species: the role of glutathione in dictating susceptibility. Neurochemistry International 406: 469-474.

Helmlinger D, Abou-Sleymane G, Yvert G, Rousseau S, Weber C, Trottier Y, Mandel L. and Devys D. (2004). Disease progression despite early loss of polyglutamine protein expression in SCA7 mouse model. J. Neurosci. 24: 1881–1887.

Helmlinger D, Hardy S, Abou-Sleymane G, Eberlin A, Bowman AB, Gansmüller A, Picaud S, Zoghbi HY, Trottier Y, Tora L, Devys D. (2006). Glutamine-expanded ataxin-7 alters TFTC/STAGA recruitment and chromatin structure leading to photoreceptor dysfunction. PLoS Biol. 4:e67.

Helmlinger D, Hardy S, Sasorith S, Klein F, Robert F, Weber C, Miguet L, Potier N, Van-Dorsselaer A, Wurtz JM, Mandel JL, Tora L, Devys D. (2004). Ataxin-7 is a subunit of GCN5 histone acetyltransferase-containing complexes. Hum Mol Genet 13: 1257–1265.

Helmlinger D, Tora L, Devys, D. (2006). Transcriptional alterations and chromatin remodeling in polyglutamine diseases. Trends Genet. 22: 562–570.

Hernandes MS, Britto LR. (2012). NADPH oxidase and neurodegeneration. Curr Neuropharmacol. 10:321-327.

Hernandez-Castillo CR, Alcauter S, Galvez V, Barrios FA, Yescas P, Ochoa A, Garcia L, Diaz R, Gao W, Fernandez-Ruiz J. (2013). Disruption of visual and motor connectivity in spinocerebellar ataxia type 7. Mov Disord. 28:1708-1716.

Heumüller S, Wind S, Barbosa-Sicard E, Schmidt HH, Busse R, Schröder K, Brandes RP. (2010). Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant. Hypertension. 51:211-217.

Holmberg M, Duyckaerts C, Dürr A, Cancel G, Gourfinkel-An I, Damier P, Faucheux B, Trottier Y, Hirsch EC, Agid Y, Brice A. (1998). Spinocerebellar ataxia type 7 (SCA7): a neurodegenerative disorder with neuronal intranuclear inclusions. Hum Mol Genet. 7:913-918.

Holmberg M, Johansson J, Forsgren I, Heijbel J, Sandgren O, Holmgren G. (1995). Localization of autosomal dominant cerebellar ataxia associated with retinal degeneration and anticipation to chromosomes 3p12-p21.1 Hum Mol Genet. 4:1441-1445.

Hong SE, Shugart YY, Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND, Walsh CA.(2000). Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat Genet. 26:93-96.

Hordijk PL. (2006). Regulation of NADPH oxidases: the role of Rac proteins. Circ Res. 98:453-462.

Hroudová J, Fisar Z, Korábečný J, Kuča K. (2011). In vitro effects of acetylcholinesterase inhibitors and reactivators on Complex I of electron transport chain. Neuro Endocrinol Lett.32:259-263.

http://www.ncbi.nlm.nih.gov/books/NBK1256/
https://www.caymanchem.com/pdfs/10008883.pdf
https://www.clinicaltrials.gov/ct2/results?term=sca3&Search=Search
Huntington Study Group. (2001). A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. Neurology 57, 397–404.
Ilieva H, Polymenidou M, Cleveland DW. (2009). Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J Cell Biol. 187:761-772.
Illuzzi JL, Vickers CA, Kmiec EB. (2011). Modifications of p53 and the DNA damage response in cells expressing mutant form of the protein huntingtin. J Mol Neurosci. 45:256-268.
Ionov M, Burchell V, Klajnert B, Bryszewska M, Abramov AY. (2011). "Mechanism of neuroprotection of melatonin against beta-amyloid neurotoxicity," Neuroscience. 180:229-237.
Ito A., Lai C. H, Zhao X., Saito S., Hamilton M. H., Appella E. and Yao T. P. (2001). "p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2." Embo J 20:1331-1340.
Jana NR, Zemskov EA, Wang Gh, Nukina N. (2001). Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. Hum Mol Genet. 10:1049-1059.
Janer A, Werner A, Takahashi-Fujigasaki J, Daret A, Fujigasaki H, Takada K, Duyckaerts C, Brice A, Dejean A, Sittler A. (2010). SUMOylation attenuates the aggregation propensity and cellular toxicity of the polyglutamine expanded ataxin-7.Hum Mol Genet. 19:181-195.
Jiang YJ, Zhou CJ, Zhou ZR, Wu M, Hu HY. (2013). Structural basis for recognition of the third SH3 domain of full-length R85 (R85FL)/ponsin by ataxin-7. FEBS Lett. 5793:550-554.
Johansson J, Forsgren L, Sandgren O, Brice A, Holmgren G. and Holmberg, M. (1998) . Expanded CAG repeats in Swedish spinocerebellar ataxia type 7 (SCA7) patients: effect of CAG repeat length on the clinical manifestation. Hum Mol Genet.7:171-176.
Jonasson J, Juvonen V, Sistonen P, Ignatius J, Johansson D, Björck EJ, Wahlström J, Melberg A, Holmgren G, Forsgren L, Holmberg M. (2000). Evidence for a common Spinocerebellar ataxia type 7 (SCA7) founder mutation in Scandinavia. Eur J Hum Genet. 8:918-922.
Jonasson J., Ström AL, Hart P, Brännström T, Forsgren L, Holmberg M. (2002). Expression of ataxin-7 in CNS and non-CNS tissue of normal and SCA7 individuals. Acta Neuropathol. 104:29-37.
Jones D.P. (2006). Redefining oxidative stress. Antioxid. Redox Signal. 8: 1865-1879.
Jones D.P. (2008). Radical-free biology of oxidative stress. Am. J. Physiol. Cell Physiol. 295: C849-868.
Kamat CD, Gadal S, Mhatre M, Williamson KS, Pye QN, Hensley K. (2008). Antioxidants in central nervous system diseases: preclinical promise and translational challenges. J Alzheimers Dis. 15:473-493.
Kann O, Kovács R. (2007). Mitochondria and neuronal activity. Am J Physiol Cell Physiol. 292:C641-657.
Kato H, Sugino N, Takiguchi S, Kashida S, Nakamura Y. (1997). Roles of reactive oxygen species in the regulation of luteal function. Rev Reprod. 2:81-83.
Katsuno M, Sang C, Adachi H, Minamiyama M, Waza M, Tanaka F, Doyu M, Sobue G. (2005). Pharmacological induction of heat-shock proteins alleviates

polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci U S A. 102:16801-16806.

Katsuyama M, Matsuno K, Yabe-Nishimura C. (2012). "Physiological roles of NOX/NADPH oxidase, the superoxide-generating enzyme." J Clin Biochem Nutr 50: 9-22.

Kawahara T, Kohjima M, Kuwano Y, Mino H, Teshima-Kondo S, Takeya R, Tsunawaki S, Wada A, Sumimoto H, Rokutan K. (2005). Helicobacter pylori lipopolysaccharide activates Rac1 and transcription of NADPH oxidase Nox1 and its organizer NOXO1 in guinea pig gastric mucosal cells. Am. J. Physiol. Cell Physiol. 288:C450–C457.

Kaya, S. S., Mahmood, A., Li, Y., Yavuz, E., Goksel, M. and Chopp, M. (1999). "Apoptosis and expression of p53 response proteins and cyclin D1 after cortical impact in rat brain." Brain Research 818:23-33.

Kaytor M.D, Duvick L.A, Skinner P.J, Koob M.D, Ranum L.P. and Orr H.T. (1999). Nuclear localization of the spinocerebellar ataxia type 7 protein, ataxin-7. Hum. Mol. Genet.8:1657–1664.

Kish SJ, Mastrogiacomo F, Guttman M, Furukawa Y, Taanman JW, Dozic S, et al. (1997). Decreased brain protein levels of cytochrome oxidase subunits in Alzheimer's disease and in hereditary spinocerebellar ataxia disorders: a nonspecific change? J Neurochem. 72: 700-707.

Kishida KT, Hoeffer CA, Hu D, Pao M, Holland SM, Klann E. (2006). Synaptic plasticity deficits and mild memory impairments in mouse models of chronic granulomatous disease. Mol Cell Biol. 26:5908-5920.

Kita H, Carmichael J, Swartz J, Muro S, Wyttenbach A, Matsubara K, Rubinsztein DC, Kato K. (2002). Modulation of polyglutamine-induced cell death by genes identified by expression profiling. Hum Mol Genet.11:2279-2287.

Kohler A, Schneider M, Cabal GG, Nehrbass U, Hurt E. (2008) Yeast Ataxin-7 links histone deubiquitination with gene gating and mRNA export. Nat Cell Biol 10: 707–715.

Komatsu M, Wang QJ, Holstein GR, Friedrich VL Jr, Iwata J, Kominami E, Chait BT, Tanaka K, Yue Z. (2007). Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc Natl Acad Sci U S A. 104:14489-14494.

Kroviarski Y, Debbabi M, Bachoual R, Périanin A, Gougerot-Pocidalo MA, El-Benna J, Dang PM. (2010). Phosphorylation of NADPH oxidase activator 1 (NOXA1) on serine 282 by MAP kinases and on serine 172 by protein kinase C and protein kinase A prevents NOX1 hyperactivation. FASEB J. 24:2077-2092.

Kumada S, Hayashi M, Mizuguchi M, Nakano I, Morimatsu Y, Oda M. (2000). Cerebellar degeneration in hereditary dentatorubral-pallidoluysian atrophy and Machado-Joseph disease. Acta Neuropathol. 99:48-54.

Kuo M.H., Brownell J.E., Sobel, R.E., Ranalli T.A., Cook R.G., Edmondson D.G., Roth S.Y., Allis C.D. (1996). Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature 383: 269–272.

Köhler A, Zimmerman E, Schneider M, Hurt E, Zheng N.(2010). Structural basis for assembly and activation of the heterotetrameric SAGA histone H2B deubiquitinase module.Cell. 141:606-617.

La Spada R, Fu Y, Sopher BL, Libby RT, Wang X, Li LY. (2001) Polyglutamine-expanded ataxin-7 antagonizes crx function and induces cone-rod dystrophy in a mouse model of sca7. Neuron 31:913–927.

Laco MN, Oliveira CR, Paulson HL, Rego AC. (2012). Compromised mitochondrial complex II in models of Machado-Joseph disease. Biochim Biophys Acta. 1822: 139-149.

Lambeth JD, Kawahara T, Diebold B. (2007). Regulation of Nox and Duox enzymatic activity and expression. Free Radic Biol Med. 43:319-331.

Lambeth JD, Neish AS. (2014). Nox enzymes and new thinking on reactive oxygen: a double-edged sword revisited. Annu Rev Pathol. 9:119-145.

Lan X, Koutelou E, Schibler AC, Chen YC, Grant PA, Dent SY. (2015). Poly(Q) Expansions in ATXN7 Affect Solubility but Not Activity of the SAGA Deubiquitinating Module. Mol Cell Biol. 35:1777-1787.

Lang G, Bonnet J, Umlauf D, Karmodiya K, Koffler J, Stierle M, Devys D, Tora L. (2011). The tightly controlled deubiquitination activity of the human SAGA complex differentially modifies distinct gene regulatory elements. Mol Cell Biol. 31:3734-37344.

Lee JM, Zhang J, Su AI, Walker JR, Wiltshire T, Kang K, Dragileva E, Gillis T, Lopez ET, Boily MJ, Cyr M, Kohane I, Gusella JF, MacDonald ME, Wheeler VC.(2010). A novel approach to investigate tissue-specific trinucleotide repeat instability. BMC Syst Biol.4:29.

Lee KK, Sardiu ME, Swanson SK, Gilmore JM, Torok M, Grant PA, Florens L, Workman JL, Washburn MP. (2011). Combinatorial depletion analysis to assemble the network architecture of the SAGA and ADA chromatin remodeling complexes. Mol Syst Biol. 7:503.

Lee KK, Swanson SK, Florens L, Washburn MP, Workman JL. (2009). Yeast Sgf73/Ataxin-7 serves to anchor the deubiquitination module into both SAGA and Slik(SALSA) HAT complexes. Epigenetics Chromatin 18;2-2.

Leichert LI1, Jakob U. (2006). Global methods to monitor the thiol-disulfide state of proteins in vivo. Antioxid Redox Signal. 8:763-772.

Li, X.J., Orr, A.L., Li, S. (2010). Impaired mitochondrial trafficking in Huntington's disease. Biochimica et biophysica acta, 1802: 62-65.

Liang Y, Liu J, Feng Z. (2013). The regulation of cellular metabolism by tumor suppressor p53. Cell Biosci. 3:9.

Liang, S. H. and Clarke, M. F. (2001). "Regulation of p53 localization." Eur J Biochem 268:2779-2783.

Lindenberg S, Yvert G, Mu¨ ller K, Landwehrmeyer G.B. (2000). Expression analysis of ataxin-7 mRNA and protein in human brain: evidence for a widespread distribution and focal protein accumulation. Brain Pathol. 10: 385–394.

Lodi R, Schapira AH, Manners D, Styles P, Wood NW, Taylor DJ, et al. (2000). Abnormal in vivo skeletal muscle energy metabolism in Huntington's disease and dentatorubropallidoluysian atrophy. Ann Neurol. 48: 72-76.

Lopes L.R, Dagher M.C, Gutierrez A, Young B, Bouin A.P, Fuchs A, Babior B.M. (2004). Phosphorylated p40PHOX as a negative regulator of NADPH oxidase. Biochemistry.43:3723-3730.

Lu W, Hu Y, Chen G, Chen Z, Zhang H, Wang F, Feng L, Pelicano H, Wang H, Keating MJ, Liu J, McKeehan W, Luo Y, Huang P. (2012). Novel role of NOX in supporting aerobic glycolysis in cancer cells with mitochondrial dysfunction and as a potential target for cancer therapy. PLoS Biol.10:e1001326.

Mackey A M, Sanvicens N, Groeger G, Doonan F, Wallace D, Cotter T G. (2008). Redox survival signalling in retina-derived 661W cells. Cell Death Differ, 8: 1291–1303.

Majeski AE, Dice JF.(2004). Mechanisms of chaperone-mediated autophagy.Int J Biochem Cell Biol. 36:2435-2444.

Maksimović ID, Jovanović MD, Colić M, Mihajlović R, Mićić D, Selaković V, Ninković M, Malicević Z, Rusić-Stojiljković M, Jovicić A. (2001). Oxidative damage and

metabolic dysfunction in experimental Huntington's disease: selective vulnerability of the striatum and hippocampus. Vojnosanit Pregl.3:237-242.

Maldonado PD, Molina-Jijón E, Villeda-Hernández J, Galván-Arzate S, Santamaría A, Pedraza-Chaverrí J. (2010). NAD(P)H oxidase contributes to neurotoxicity in an excitotoxic/prooxidant model of Huntington's disease in rats: protective role of apocynin. J Neurosci Res. 88:620-629.

Manea A, Manea SA, Florea IC, Luca CM, Raicu M. (2012). Positive regulation of NADPH oxidase 5 by proinflammatory-related mechanisms in human aortic smooth muscle cells. Free Radic Biol Med. 52:1497-1507.

Manea A, Tanase LI, Raicu M, Simionescu M. (2010). Transcriptional regulation of NADPH oxidase isoforms, Nox1 and Nox4, by nuclear factor-kappaB in human aortic smooth muscle cells. Biochem Biophys Res Commun. 396:901–907.

Marchenko ND, Wolff S, Erster S, Becker K, Moll UM. (2007). Monoubiquitylation promotes mitochondrial p53 translocation. EMBO J. 26:923-934.

Marden J J, Harraz M M, Williams A J, Nelson K, Luo M, Paulson H, Engelhardt J F. (2007). Redox modifier genes in amyotrophic lateral sclerosis in mice. J Clin Invest. 117: 2913–2919.

Marsh, J.L., Walker, H., Theisen, H., Zhu, Y.Z., Fielder, T., Purcell, J. and Thompson, L.M. (2000). Expanded polyglutamine peptides alone are intrinsically cytotoxic and cause neurodegeneration in Drosophila. Hum. Mol. Genet., 9, 13-25.

Martin JJ, Van Regemorter N, Krols L, Brucher JM, de Barsy T, Szliwowski H, Evrard P, Ceuterick C, Tassignon MJ, Smet-Dieleman H, et al. (1994). On an autosomal dominant form of retinal-cerebellar degeneration: an autopsy study of five patients in one family. Acta Neuropathol. 88:277-286.

Mason RP, Casu M, Butler N, Breda C, Campesan S, Clapp J, Green EW, Dhulkhed D, Kyriacou CP, Giorgini F. (2013). Glutathione peroxidase activity is neuroprotective in models of Huntington's disease. Nat Genet. 45:1249-1254.

Matilla A, Roberson ED, Banfi S, Morales J, Armstrong DL, Burright EN, Orr HT, Sweatt JD, Zoghbi HY, Matzuk MM. (1998). Mice lacking ataxin-1 display learning deficits and decreased hippocampal paired-pulse facilitation.J Neurosci. 18:5508-5516.

McCormick MA, Mason AG, Guyenet S, Dang W, Garza RM, Ting MK, Moller RM, Berger SL, Kaeberlein M, Pillus L, La Spada AR, Kennedy BK. (2014). The SAGA histone deubiquitinase module controls yeast replicative lifespan via Sir2 interaction. Cell Rep. 8:477-486.

McCullough SD, Grant PA . (2010). Histone acetylation, acetyltransferases, and ataxia-alteration of histone acetylation and chromatin dynamics is implicated in the pathogenesis of polyglutamin eexpansion disorders. Adv Protein Chem Struct Biol 79:165–203.

McCullough SD, Xu X, Dent SY, Bekiranov S, Roeder RG, Grant PA. (2012). Reelin is a target of polyglutamine expanded ataxin-7 in human spinocerebellar ataxia type 7 (SCA7) astrocytes. Proc Natl Acad Sci U S A.109:21319-21324.

McLure KG, Takagi M, Kastan MB. (2004). NAD+ modulates p53 DNA binding specificity and function. Mol Cell Biol. 24:9958-9967.

McMahon S.J, Pray-Grant M.G, Schieltz D, Yates 3rd J.R, Grant, P.A. (2005). Polyglutamine-expanded spinocerebellar ataxia-7 protein disrupts normal SAGA and SLIK histone acetyltransferase activity. Proc. Natl. Acad. Sci. U.S.A. 102. 8478–8482.

Meng L, Mohan R, Kwok BH, Elofsson M, Sin N, Crews CM. (1999). Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo antiinflammatory activity. Proc Natl Acad Sci U S A. 96:10403-10408.

Michalik A, Martin JJ, Van Broeckhoven C. (2004). Spinocerebellar ataxia type 7 associated with pigmentary retinal dystrophy. Eur J Hum Genet. 12:2-15.

Michalik A, Van Broeckhoven C. (2003). Pathogenesis of polyglutamine disorders: aggregation revisited. Hum Mol Genet. 2:R173-186.

Milakovic, T., Johnson, G.V. (2005). Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin. The Journal of biological chemistry, 280: 30773-30782.

Miyata T, Ono Y, Okamoto M, Masaoka M, Sakakibara A, Kawaguchi A, Hashimoto M, Ogawa M. (2010). Migration, early axonogenesis, and Reelin-dependent layer-forming behavior of early/posterior-born Purkinje cells in the developing mouse lateral cerebellum. Neural Dev.5:23.

Mizuki K, Kadomatsu K, Hata K, Ito T, Fan Q W, Kage Y, Fukumaki Y, Sakaki Y, Takeshige K, Sumimoto H. (1998). Functional modules and expression of mouse p40(phox) and p67(phox), SH3-domaincontaining proteins involved in the phagocyte NADPH oxidase complex. Eur J Biochem, 251: 573–582.

Mohan RD, Abmayr SM, Workman JL. (2014). Pulling complexes out of complex diseases: Spinocerebellar Ataxia 7. Rare Dis. 14;2:e28859.

Mohan RD, Dialynas G, Weake VM, Liu J, Martin-Brown S, Florens L, Washburn MP, Workman JL, Abmayr SM. (2014). Loss of Drosophila Ataxin-7, a SAGA subunit, reduces H2B ubiquitination and leads to neural and retinal degeneration. Genes Dev. 28:259-272.

Mohan RD, Workman JL, Abmayr SM. (2014). Drosophila models reveal novel insights into mechanisms underlying neurodegeneration. Fly (Austin). 83:148-152.

Mookerjee S, Papanikolaou T, Guyenet SJ, Sampath V, Lin A, Vitelli C, DeGiacomo F, Sopher BL, Chen SF, La Spada AR, Ellerby LM. (2009). Posttranslational modification of ataxin-7 at lysine 257 prevents autophagy-mediated turnover of an N-terminal caspase-7 cleavage fragment. J Neurosci. 29:15134-15144.

Morfini G, Pigino G, Brady ST. (2005). Polyglutamine expansion diseases: failing to deliver. Trends Mol Med. 11:64-70.

Mruk DD, Cheng CY. (2011). Enhanced chemiluminescence (ECL) for routine immunoblotting: An inexpensive alternative to commercially available kits. Spermatogenesis. 1:121-122.

Mukhopadhyay P, Rajesh M, Yoshihiro K, Haskó G, Pacher P. (2007). Simple quantitative detection of mitochondrial superoxide production in live cells. Biochem Biophys Res Commun. 358:203-208.

Mushegian AR, Vishnivetskiy SA, Gurevich VV. (2000). Conserved phosphoprotein interaction motif is functionally interchangeable between ataxin-7 and arrestins. Biochemistry. 39:6809-6813.

Nakamura Y, Tagawa K, Oka T, Sasabe T, Ito H, Shiwaku H, La Spada AR, Okazawa H. (2012). Ataxin-7 associates with microtubules and stabilizes the cytoskeletal network. Hum Mol Genet.5:1099-1010.

Nasir J, Floresco SB, O'Kusky JR, Diewert VM, Richman JM, Zeisler J, Borowski A, Marth JD, Phillips AG, Hayden MR. (1995). Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell. 81:811-823.

Nayernia Z, Jaquet V, Krause KH. (2014). New insights on NOX enzymes in the central nervous system. Antioxid Redox Signal.20:2815-2837.

Nehlig, A., Coles, J.A. (2007). Cellular pathways of energy metabolism in the brain: is glucose used by neurons or astrocytes? Glia, 55: 1238-1250.

Nisoli I, Chauvin JP, Napoletano F, Calamita P, Zanin V, Fanto M, Charroux B. (2010). Neurodegeneration by polyglutamine Atrophin is not rescued by induction of autophagy. Cell Death Differ. 17:1577-1587.

Noh K M, Koh J Y . (2000). Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes. J Neurosci, 23: RC111.

Noma S, Ohya-Shimada W, Kanai M, Ueda K, Nakamura T, Funakoshi H. (2012). Overexpression of HGF attenuates the degeneration of Purkinje cells and Bergmann glia in a knockin mouse model of spinocerebellar ataxia type 7. Neurosci Res. 73:115-121.

Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, Dawson TM, Ross CA. (2001). Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science. 291:2423-2428.

Oh H, Jung HY, Kim J, Bae YS. (2010). Phosphorylation of serine282 in NADPH oxidase activator 1 by Erk desensitizes EGF-induced ROS generation. Biochem Biophys Res Commun. 394:691-696.

Ordway JM, Tallaksen-Greene S, Gutekunst CA, Bernstein EM, Cearley JA, Wiener HW, Dure LS, Lindsey R, Hersch SM, Jope RS, Albin RL, Detloff PJ. (1997). Ectopically expressed CAG repeats cause intranu- clear inclusions and a progressive late onset neurological phenotype in the mouse. Cell 91:753–763.

Orr T and Zoghbi, Y. (2007). Trinucleotides repeat disorders. Annu. Rev. Neurosci. 30:576–621.

Ortega Z, Díaz-Hernández M, Maynard CJ, Hernández F, Dantuma NP, Lucas JJ. (2010). Acute polyglutamine expression in inducible mouse model unravels ubiquitin/proteasome system impairment and permanent recovery attributable to aggregate formation. J Neurosci. 30:3675-3688.

Pachucki J, Wang D, Christophe D, Miot F. (2004). Structural and functional characterization of the two human ThOX/Duox genes and their 5'-flanking regions. Mol. Cell. Endocrinol. 214:53–62.

Palhan VB, Chen S, Peng GH, Tjernberg A, Gamper AM, Fan Y, Chait BT, La Spada AR, Roeder RG. (2005). Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration.Proc Natl Acad Sci U S A 102:8472– 8477.

Pao M, Wiggs EA, Anastacio MM, Hyun J, DeCarlo ES, Miller JT, Anderson VL, Malech HL, Gallin JI, Holland SM. (2004). Cognitive function in patients with chronic granulomatous disease: a preliminary report. Psychosomatics. 45:230-234.

Park L, Zhou P, Pitstick R, Capone C, Anrather J, Norris E H, Younkin L, Younkin S, Carlson G, McEwen B S, Iadecola C. (2008). Nox2- derived radicals contribute to neurovascular and behavioral dysfunction in mice overexpressing the amyloid precursor protein. Proc Natl Acad Sci USA, 105: 1347–1352.

Perez M. K., Paulson H. L., Pendse S. J., Saionz S. J., Bonini N. M. and Pittman R. N. (1998) . Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. J. Cell Biol. 143: 1457–1470.

Peyser CE, Folstein M, Chase GA, Starkstein S, Brandt J, Cockrell JR, Bylsma F, Coyle JT, McHugh PR, Folstein SE. (1995). Trial of d-alpha-tocopherol in Huntington's disease. Am J Psychiatry. 152:1771-1775.

Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA. (2011). Mitochondrial membrane potential probes and the proton gradient: a practical usage guide. Biotechniques. 50:98-115.

Pineda JR, Rubio N, Akerud P, Urbán N, Badimon L, Arenas E, Alberch J, Blanco J, Canals JM. (2007). Neuroprotection by GDNF-secreting stem cells in a Huntington's disease model: optical neuroimage tracking of brain-grafted cells. Gene Ther. 14:118-128.

Poehlsgaard J, Douthwaite S. (2005). The bacterial ribosome as a target for antibiotics. Nat Rev Microbiol.3:870-881.

Powers, W.J., Videen, T.O., Markham, J., McGee-Minnich, L., Antenor-Dorsey, J.V., Hershey, T., Perlmutter, J.S. (2007). Selective defect of in vivo glycolysis in early Huntington's disease striatum. Proceedings of the National Academy of Sciences of the United States of America, 104: 2945-2949.

Prata C, Maraldi T, Fiorentini D, Zambonin L, Hakim G, Landi L. (2008). Nox-generated ROS modulate glucose uptake in a leukaemic cell line. Free Radic Res. 42:405-414.

Puca R, Nardinocchi L, Starace G, Rechavi G, Sacchi A, Givol D, D'Orazi G. (2010). Nox1 is involved in p53 deacetylation and suppression of its transcriptional activity and apoptosis. Free Radic Biol Med, 48:1338-1346.

Qin L, Liu Y, Hong JS, Crews FT. (2013). NADPH oxidase and aging drive microglial activation, oxidative stress, and dopaminergic neurodegeneration following systemic LPS administration. Glia. ;61:855-868.

Qin Q, Inatome R, Hotta A, Kojima M, Yamamura H, Hirai H, Yoshizawa T, Tanaka H, Fukami K, Yanagi S. (2006). A novel GTPase, CRAG, mediates promyelocytic leukemia protein-associated nuclear body formation and degradation of expanded polyglutamine protein. J Cell Biol. 172:497-504.

Ramachandran PS, Bhattarai S, Singh P, Boudreau RL, Thompson S, Laspada AR, Drack AV, Davidson BL. (2014). RNA interference-based therapy for spinocerebellar ataxia type 7 retinal degeneration. PLoS One. 9:e95362.

Ramachandran PS, Boudreau RL, Schaefer KA, La Spada AR & Davidson BL. (2014). Non-allele specific silencing of ataxin-7 improves phenotypes in a mouse model of Spinocerebellar ataxia type 7. Molecular Therapy 22: 1635-1642.

Ravikumar B, Duden R, Rubinsztein DC. (2002). Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum Mol Genet. 11:1107-1117.

Ravikumar B, Stewart A, Kita H, Kato K, Duden R, Rubinsztein DC. (2003). Raised intracellular glucose concentrations reduce aggregation and cell death caused by mutant huntingtin exon 1 by decreasing mTOR phosphorylation and inducing autophagy. Hum Mol Genet. 12:985-994.

Reijonen S, Kukkonen JP, Hyrskyluoto A, Kivinen J, Kairisalo M, Takei N, Lindholm D, Korhonen L. (2010). Downregulation of NF-kappaB signaling by mutant huntingtin proteins induces oxidative stress and cell death. Cell Mol Life Sci 67:1929–1941.

Rey FE, Cifuentes ME, Kiarash A, Quinn MT, Pagano PJ. (2001). Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O(2)(-) and systolic blood pressure in mice. Circ Res. 89:408-414.

Rezvani HR, Rossignol R, Ali N, Benard G, Tang X, Yang HS, Jouary T, de Verneuil H, Taïeb A, Kim AL, Mazurier F. (2011). XPC silencing in normal human keratinocytes triggers metabolic alterations through NOX-1 activation-mediated reactive oxygen species. Biochim Biophys Acta. 1807:609-619.

Ribeiro M, Silva AC, Rodrigues J, Naia L, Rego AC. (2013). Oxidizing effects of exogenous stressors in Huntington's disease knock-in striatal cells--protective effect of cystamine and creatine. Toxicol Sci. 136:487-499.

Ross CA, Wood JD, Schilling G, Peters MF, Nucifora FC Jr, Cooper JK, Sharp AH, Margolis RL, Borchelt DR. (1999). Polyglutamine pathogenesis. Philos Trans R Soc Lond B Biol. 354:1005-1011.

Rubinsztein DC. (2006). The roles of intracellular protein-degradation pathways in neurodegeneration. Nature. 443:780-786.

Ryan AB, Zeitlin SO, Scrable H. (2006). Genetic interaction between expanded murine Hdh alleles and p53 reveal deleterious effects of p53 on Huntington's disease pathogenesis. Neurobiol Dis. 24:419-427.

Rüb U, Schöls L, Paulson H, Auburger G, Kermer P, Jen JC, Seidel K, Korf HW, Deller T. (2013). Clinical features, neurogenetics and neuropathology of the polyglutamine spinocerebellar ataxias type 1, 2, 3, 6 and 7. Prog Neurobiol. 104:38-66.

Samara NL, Datta AB, Berndsen CE, Zhang X, Yao T, Cohen RE, Wolberger C. (2010). Structural insights into the assembly and function of the SAGA deubiquitinating module.Science. 328:1025-1029.

Sanchez I, Mahlke C, Yuan J. (2003). Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. Nature 421:373-379.

Santos C.X, Anaka L.Y, Wosniak J, Laurindo F.R. (2009). Mechanisms and Implications of Reactive Oxygen Species Generation During the Unfolded Protein Response: Roles of Endoplasmic Reticulum Oxidoreductases, Mitochondrial Electron Transport and NADPH Oxidase. Antioxid. Redox Signal. 10:2409-2427.

Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. (2007). Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. J Biol Chem. 282:5641-5652.

Sayre LM, Perry G, Smith MA. (2008). Oxidative stress and neurotoxicity. Chem Res Toxicol. 21:172-188.

SCHERER WF, SYVERTON JT, GEY GO. (1953). Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. J Exp Med. 97:695-710.

Scholefield J, Greenberg LJ, Weinberg MS, Arbuthnot PB, Abdelgany A, Wood MJ. (2009). Design of RNAi hairpins for mutation-specific silencing of ataxin-7 and correction of a SCA7 phenotype. PLoS One. 4:e7232.

Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. (2004). The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Res. 64:2627-2633.

Seglen PO, Gordon PB. (1982). 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. Proc Natl Acad Sci U S A. 79:1889-1892.

Seidel K, Siswanto S, Brunt ER, den Dunnen W, Korf HW, Rüb U. (2012). Brain pathology of spinocerebellar ataxias. Acta Neuropathol. 124:1-21.

Serrano F, Kolluri N S,Wientjes F B, Card J P, Klann E. (2003). NADPH oxidase immunoreactivity in the mouse brain. Brain Res, 988:193–198.

Shieh, S. Y., Ikeda, M., Taya, Y. and Prives, C. (1997). "DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2." Cell 91:325-334.

Shimohata T., Nakajima T., Yamada M., Uchida C., Onodera O., Naruse S. et al. (2000). Expanded polyglutamine streches interact with TAFII130, interfering with CREB-dependent transcription. Nat. Genet. 26: 29–36.

Shirendeb UP, Calkins MJ, Manczak M, Anekonda V, Dufour B, McBride JL, Mao P, Reddy PH. (2012). Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. Hum Mol Genet. 21:406-420.

Sies, H.(1985). Oxidative Stress: Introductory Remarks. London: Academic Press.

Silva A, Naia L, Dominguez A, Ribeiro M, Rodrigues J, Vieira OV, Lessmann V, Rego AC. (2015). Overexpression of BDNF and Full-Length TrkB Receptor Ameliorate Striatal Neural Survival in Huntington's Disease. Neurodegener Dis. [Epub ahead of print].

Silva AC, Almeida S, Laço M, Duarte AI, Domingues J, Oliveira CR, Januário C, Rego AC. (2013). Mitochondrial respiratory chain complex activity and bioenergetic alterations in human platelets derived from pre-symptomatic and symptomatic Huntington's disease carriers. Mitochondrion. 13:801-809.

Sisodia S. (1998). Nuclear inclusions in glutamine repeat disorders: are they pernicious, coincidental, or beneficial? Cell 95:1-4.

Skalnik D. G, Strauss E, Orkin S. H. (1991). CCAAT displacement protein as a repressor of the myelomonocytic-specific gp91-phox gene promoter. J. Biol. Chem. 266:16736–16744.

Skapek SX, Colvin OM, Griffith OW, Elion GB, Bigner DD, Friedman HS.. (1988). Enhanced melphalan cytotoxicity following buthionine sulfoximine-mediated glutathione depletion in a human medulloblastoma xenograft in athymic mice. Cancer Res 48: 2764–2767.

Skinner J, Koshy T, Cummings J, Klement A, Helin K, Servadio A, Zoghbi Y, Orr T. (1997). Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. Nature 389:971-974.

Smith SE, Koegl M, Jentsch S. (1996). Role of the ubiquitin/proteasome system in regulated protein degradation in Saccharomyces cerevisiae. Biol Chem. 377:437-446.

Sopher BL, Ladd PD, Pineda VV, Libby RT, Sunkin SM, Hurley JB, Thienes CP, Gaasterland T, Filippova GN, La Spada AR. (2011). CTCF regulates ataxin-7 expression through promotion of a convergently transcribed, antisense noncoding RNA. Neuron. 70:1071-1084.

Sowa ME, Bennett EJ, Gygi SP, Harper JW. (2009). Defining the human deubiquitinating enzyme interaction landscape. Cell. 138:389-403.

St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, Spiegelman BM. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell.127:397-408.

Stambolsky P, Weisz L, Shats I, Klein Y, Goldfinger N, Oren M, Rotter V. (2006). Regulation of AIF expression by p53. Cell Death Differ.13:2140-2149.

Stanic B, Katsuyama M, Miller FJ Jr. (2010). An oxidized extracellular oxidation-reduction state increases Nox1 expression and proliferation in vascular smooth muscle cells via epidermal growth factor receptor activation. Arterioscler Thromb Vasc Biol. 30:2234-2241.

Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM. (2000). The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci U S A. 97:6763-6788.

Steffan, J. S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B. L., Kazantsev, A., Schmidt, E., Zhu, Y. Z., Greenwald, M., Kurokawa, R., Housman, D. E., Jackson, G. R., Marsh, J. L. and Thompson, L. M. (2001). "Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila." Nature 413:739-743.

Stenoien D .L., Cummings C. J., Adams H. P., Mancini M. G., Patel K., DeMartino G. N. et al. (1999). Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by HDJ-2 chaperone. Hum. Mol. Genet. 8: 731–741.

Ström AL, Forsgren L, Holmberg M. (2005). A role for both wild-type and expanded ataxin-7 in transcriptional regulation. Neurobiol Dis. 20:646-655.

Ström AL, Forsgren L, Holmberg M. (2005). Identification and characterization of Spinocerebellar Ataxia Type 7 (SCA7) isoform SCA7b in mice. Biochim Biophys Acta. 1731:149-153.

Tabrizi, S.J., Cleeter, M.W., Xuereb, J., Taanman, J.W., Cooper, J.M., Schapira, A.H. (1999). Biochemical abnormalities and excitotoxicity in Huntington's disease brain. Annals of neurology, 45: 25-32.

Takahashi T, Katada S, Onodera O. (2010). Polyglutamine diseases: where does toxicity come from? what is toxicity? where are we going? J Mol Cell Biol. 2:180-91.

Tallaksen-Greene SJ, Ordway JM, Crouse AB, Jackson WS, Detloff PJ, Albin RL. (2003). Hprt(CAG)146 mice: age of onset of behavioral abnormalities, time course of neuronal intranuclear inclusion accumulation, neurotransmitter marker alterations, mitochondrial function markers, and susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J Comp Neurol. 465:205-219.

Taroni F, DiDonato S. (2004). Pathways to motor incoordination: the inherited ataxias. Nat. Rev. Neurosci.5: 641-655.

Taylor J, Grote SK, Xia J, Vandelft M, Graczyk J, Ellerby LM, La Spada AR, Truant R. (2006). Ataxin-7 can export from the nucleus via a conserved exportin-dependent signal. J Biol Chem. 281:2730-2739.

Tejada-Simon M V, Serrano F, Villasana L E, Kanterewicz B I,Wu G Y,Quinn M T, Klann E. (2005). Synaptic localization of a functional NADPH oxidase in the mouse hippocampus. Mol Cell Neurosci. 29: 97–106.

Terashima T, Kawai H, Fujitani M, Maeda K, Yasuda H. (2002). SUMO-1 co-localized with mutant atrophin-1 with expanded polyglutamines accelerates intranuclear aggregation and cell death. Neuroreport. 13:2359-2364.

Trang H, Stanley SY, Thorner P, Faghfoury H, Schulze A, Hawkins C, Pearson CE, Yoon G6. (2015). Massive CAG repeat expansion and somatic instability in maternally transmitted infantile spinocerebellar ataxia type 7. JAMA Neurol. 72:219-223.

Tsoi H, Lau TC, Tsang SY, Lau KF, Chan HY. (2012). CAG expansion induces nucleolar stress in polyglutamine diseases. Proc Natl Acad Sci U S A. 109:13428-13433.

Tydlacka S, Wang CE, Wang X, Li S, Li XJ. (2008). Differential activities of the ubiquitin-proteasome system in neurons versus glia may account for the preferential accumulation of misfolded proteins in neurons. J Neurosci. 28:13285-13295.

Uttara B, Singh A.V, Zamboni P, Mahajan R.T. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol. 7: 65-74.

Valencia A, Sapp E, Kimm JS, McClory H, Reeves PB, Alexander J, Ansong KA, Masso N, Frosch MP, Kegel KB, Li X, DiFiglia M. (2013). Elevated NADPH oxidase activity contributes to oxidative stress and cell death in Huntington's disease. Hum Mol Genet. 22:1112-1131.

Valente AJ, Zhou Q, Lu Z, He W, Qiang M, Ma W, Li G, Wang L, Banfi B, Steger K, Krause KH, Clark RA, Li S. (2008). Regulation of NOX1 expression by GATA, HNF-1alpha, and Cdx transcription factors. Free Radic Biol Med. 44:430-443.

Veitch NC. (2004). Horseradish peroxidase: a modern view of a classic enzyme. Phytochemistry. 65:249-259.

Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA (2004). "In vivo activation of the p53 pathway by small-molecule antagonists of MDM2". Science 303:844–848.

Vejrazka M, Mícek R, Stípek S. (2006). Apocynin inhibits NADPH oxidase in phagocytes but stimulates ROS production in non-phagocytic cells. Biochim Biophys Acta.1722:143-147.

Vousden, K. H. (2002). "Activation of the p53 tumor suppressor protein." Biochim Biophys Acta 1602:47-59.

Wang HL, He CY, Chou AH, Yeh TH, Chen YL, Li AH. (2007). Polyglutamine-expanded ataxin-7 decreases nuclear translocation of NF-kappaB p65 and impairs NF-kappaB activity by inhibiting proteasome activity of cerebellar neurons. Cell Signal. 19:573-581.

Wang HL, Hu SH, Chou AH, Wang SS, Weng YH, Yeh TH. (2013). H1152 promotes the degradation of polyglutamine-expanded ataxin-3 or ataxin-7 independently of its ROCK-inhibiting effect and ameliorates mutant ataxin-3-induced neurodegeneration in the SCA3 transgenic mouse. Neuropharmacology. 70:1-11.

Wang HL, Yeh TH, Chou AH, Kuo YL, Luo LJ, He CY, Huang PC, Li AH. (2005). Polyglutamine-expanded ataxin-7 activates mitochondrial apoptotic pathway of cerebellar neurons by upregulating Bax and downregulating Bcl-x(L). Cell Signal. 18:541-552.

WARBURG O. (1956). On the origin of cancer cells. Science. 123:309-314.

Warby SC, Chan EY, Metzler M, Gan L, Singaraja RR, Crocker SF, Robertson HA, Hayden MR. (2005). Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo. Hum Mol Genet. 14:1569-1577.

Weake VM, Workman JL. (2008). Histone ubiquitination: triggering gene activity. Mol Cell. 29:653-663.

Weber JJ, Sowa AS, Binder T, Hübener J. (2014). From pathways to targets: understanding the mechanisms behind polyglutamine disease. Biomed Res Int. 701758.

Weber K, Osborn M. (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J Biol Chem. 244:4406-4412.

Weir EK, Archer SL. (2010). The role of redox changes in oxygen sensing. Respir Physiol Neurobiol. 174:182-191.

Wexler NS, Young AB, Tanzi RE, Travers H, Starosta-Rubinstein S, Penney JB, Snodgrass SR, Shoulson I, Gomez F, Ramos Arroyo MA, et al. (1989). Homozygotes for Huntington's disease. Nature. 326:194-197.

Weydt P, Pineda VV, Torrence AE, Libby RT, Satterfield TF, Lazarowski ER, Gilbert ML, Morton GJ, Bammler TK, Strand AD, Cui L, Beyer RP, Easley CN, Smith AC, Krainc D, Luquet S, Sweet IR, Schwartz MW, La Spada AR. (2006). Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. Cell Metab. 4:349-362.

Wingler K, Wunsch S, Kreutz R, Rothermund L, Paul M Schmidt H. (2001). Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo. Free Radic. Biol. Med. 31:1456–1464.

Woods, D. B. and Vousden, K. H. (2001). "Regulation of p53 function." Exp Cell Res 264:56-66.

Wu D C, Ré D B, Nagai M, Ischiropoulos H, Przedborski S. (2006). The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. Proc Natl Acad Sci USA, 103: 12132–12137.

Wu H.H.,Thomas J.A, and Momand J, (2000). p53 protein oxidation in cultured cells in response to pyrrolidine dithiocarbamate: a novel method for relating the amount of p53 oxidation in vivo to the regulation of p53-responsive genes. Biochem J, 351:87-93.

Yamamoto A, Takeya R, Matsumoto M, Nakayama KI, Sumimoto H. (2013). Phosphorylation of Noxo1 at threonine-341 regulates its interaction with Noxa1 and the superoxide-producing activity of Nox1. FEBS J. Doi: 10.1111.

Yang H, Liu S, He WT, Zhao J, Jiang LL, Hu HY. (2015). Aggregation of Polyglutamine-Expanded Ataxin-7 Specifically Sequesters Ubiquitin-Specific Protease 22 and Deteriorates Its Deubiquitinating Function in SAGA Complex. J Biol Chem. jbc.M114.631663. [Epub ahead of print]

Yang W, Dunlap R, Andrews B, Wetzel R. (2002). Aggregated polyglutamine peptides delivered to nuclei are toxic to mammalian cells. Hum. Mol. Genet. 11: 2905-2917.

Yanicostas C, Barbieri E, Hibi M, Brice A, Stevanin G, Soussi-Yanicostas N. (2012). Requirement for zebrafish ataxin-7 in differentiation of photoreceptors and cerebellar neurons. PLoS One. 7:e50705.

Yoo SY, Pennesi ME,Weeber EJ, Xu B, Atkinson R, Chen S, Armstrong DL, Wu SM, Sweatt JD, Zoghbi HY (2003). SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant ataxin-7 in neurons and abnormalities in short-term plasticity. Neuron 37:383–401.

Young, J.E., Garden, G.A., Martinez, R.A., Tanaka, F., Sandoval, C.M., Smith, A.C., Sopher, B.L., Lin, A., Fischbeck, K.H., Ellerby, L.M., et al. (2009). Polyglutamine-Expanded Androgen Receptor Truncation Fragments Activate a Bax-Dependent Apoptotic Cascade Mediated by DP5/Hrk. J. Neurosci. 29, 1987–1997.

Young, JE., Gouw, L., Propp, S., Sopher, B.L., Taylor, J., Lin, A., Hermel, E., Logvinova, A., Chen, S.F., Chen, S., et al. (2007). Proteolytic Cleavage of Ataxin-7 by Caspase-7 Modulates Cellular Toxicity and Transcriptional Dysregulation. J. Biol. Chem. 282, 30150–30160.

Yu X, Ajayi A, Boga NR, Ström AL. (2012). Differential Degradation of Full-length and Cleaved Ataxin-7 Fragments in a Novel Stable Inducible SCA7 Model. J. Mol. Neurosci. 2:219-233.

Yu X, Muñoz-Alarcón A, Ajayi A, Webling KE, Steinhof A, Langel U, Ström AL. (2013). Inhibition of Autophagy via p53-Mediated Disruption of ULK1 in a SCA7 Polyglutamine Disease Model. J Mol Neurosci. 50:586-599.

Yu YC, Kuo CL, Cheng WL, Liu CS, Hsieh M. (2009). Decreased antioxidant enzyme activity and increased mitochondrial DNA damage in cellular models of Machado-Joseph disease. Neurosci Res. 87:1884-1891.

Yvert G, Lindenberg K.S, Picaud S, Landwehrmeyer G.B, Sahel J.A, Mandel J.L. (2001). Expanded polyglutamines induce neurodegeneration and trans-neuronal alterations in cerebellum and retina of SCA7 transgenic mice. Hum. Mol. Genet. 10:2569–2579.

Yvert G, Lindenberg KS, Devys D, Helmlinger D, Landwehrmeyer GB, Mandel JL (2001). SCA7 mouse models show selective stabilization of mutant ataxin-7 and similar cellular responses in different neuronal cell types. Hum Mol Genet 10:1679–1692.

Zeitlin S, Liu JP, Chapman DL, Papaioannou VE, Efstratiadis A. (1995). Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. Nat Genet. 11:155-163.

Zhang XY, Pfeiffer HK, Thorne AW, McMahon SB. (2008). USP22, an hSAGA subunit and potential cancer stem cell marker, reverses the polycomb-catalyzed ubiquitylation of histone H2A. Cell Cycle. 7:1522-1154.

Zhao Y, Lang G, Ito S, Bonnet J, Metzger E, Sawatsubashi S, Suzuki E, Le Guezennec X, Stunnenberg HG, Krasnov A, Georgieva SG, Schüle R, Takeyama K, Kato S, Tora L, Devys D. (2008). A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Mol Cell. 29:92-101.

Zoghbi H.Y and Ort H.T.. (2000). Glutamine repeats and neurodegeneration. Annu. Rev. Neurosci. 23:217–247.

ZoghbiH Y, Orr HT. (2009). Pathogenic mechanisms of a polyglutamine-mediated neurodegenerative disease, Spinocerebellar Ataxia Type 1. J. Biol. Chem 284: 7425-7429.

Zulueta JJ, Sawhney R, Yu FS, Cote CC, Hassoun PM. (1997). Intracellular generation of reactive oxygen species in endothelial cells exposed to anoxia-reoxygenation. Am J Physiol. 272:L897-902.