Chapter 16
Towards the Identification of Molecular Biomarkers of Spinocerebellar Ataxia Type 3 (SCA3)/Machado-Joseph Disease (MJD)

Manuela Lima and Mafalda Raposo

Abstract Whereas spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD) remains an untreatable disorder, disease-modifying compounds have begun being tested in the context of clinical trials; their success is dependent on the sensitivity of the methods used to measure subtle therapeutic benefits. Thus, efforts are being made to propose a battery of potential outcome measures, including molecular biomarkers (MBs), which remain to be identified; MBs are particularly pertinent if SCA3 trials are expected to enroll preataxic subjects. Recently, promising candidate MBs of SCA3 have emerged from gene expression studies. In this chapter we provide a synthesis of the cross-sectional and pilot longitudinal studies of blood-based transcriptional biomarkers conducted so far. Other alterations with potential to track the progression of SCA3, such as those involving mitochondrial DNA (mtDNA) are also referred. It is expected that a set of molecular biomarkers can be identified; these will be used in complementarity with clinical and imaging markers to fully track SCA3, from its preataxic phase to the disease stage.

Keywords Polyglutamine disorders · Biochemical markers · Transcriptional dysregulation · RNA · Trait biomarkers · State biomarkers

M. Lima (✉) · M. Raposo
Departamento de Biologia, Faculdade de Ciências e Tecnologia, Universidade dos Açores, Ponta Delgada, Portugal
e-mail: maria.mm.lima@uac.pt

M. Lima · M. Raposo
Instituto de Investigação e Inovação em Saúde (I3S), Universidade do Porto, Porto, Portugal

M. Lima · M. Raposo
Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal

C. Nóbrega and L. Pereira de Almeida (eds.), *Polyglutamine Disorders*, Advances in Experimental Medicine and Biology 1049,
https://doi.org/10.1007/978-3-319-71779-1_16

16.1 Spinocerebellar Ataxia Type 3 (SCA3): Overview

Spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD) is the most frequent of the autosomal dominant ataxias, displaying a worldwide prevalence of 1.5 cases per 100,000 inhabitants [1]. In the Portuguese Azores, to where the first descriptions of SCA3 trace (revised in [2]), an homogeneous cohort of patients has been described [3–5], whose importance for the understanding of several aspects of this disease has been reinforced by Raposo and collaborators [6]. A recent longitudinal epidemiological study of SCA3 in the Azores Islands reported an overall prevalence of 1 in each 2544 for the total archipelago, and of 1 in each 158 for the island of Flores [7].

SCA3 involves predominantly the cerebellar, pyramidal, extrapyramidal, motor neuron and oculomotor systems. Although the average age for the appearance of first symptoms is around 40 years, extremes from 4 to 70 years have been reported (revised in [8]). As a clinical entity, SCA3 is recognized as highly pleomorphic; pleomorphism is reflected on the marked variation of age at onset, the heterogeneity of the clinical features displayed by the different patients, as well as on the existence of particular/atypical clinical presentations (see, for example, [9]). Clinical heterogeneity is further evidenced by inter-patient differences in the rate of disease progression and magnitude of the associated disability.

SCA3 causative gene displays almost complete penetrance ($\sim$98%, in accordance with [10]), following an age-dependent pattern. The probability of being a mutation carrier and consequently a posteriori risk diminishes with age of asymptomatic at risk individuals, reaching approximately zero in 70 years old subjects [11].

ATXN3, SCA3 causative gene, was described as containing a polymorphic expanded and unstable CAG tract at exon 10 [12], consensually ranging from 14 to 42 repeats in normal chromosomes and from 52 to 91 repeats in chromosomes harboring its mutated form [13, 14]. *ATXN3* encodes for the ubiquously expressed ataxin-3, a cysteine protease whose main native role is that of a deubiquitinating enzyme (DUB) in the ubiquitin-proteasome pathway. Expansion of the polyglutamine (polyQ) tract above the pathological threshold initiates a cascade of pathogenic events that have been extensively studied (revised in [15, 16]). Aggregation of mutant ataxin-3, proteolytic cleavage, transcription dysregulation, mitochondrial dysfunction, axonal transport impairment, dysregulation of intracellular Ca^{2+} homeostasis and impairment of protein degradation are considered the main mechanisms implicated in SCA3 pathogenesis [16].

Despite the progresses made in the understanding of the underlying molecular mechanisms, disease-modifying therapies for SCA3 are still lacking, and only symptomatic approaches are currently available. So far interventional studies undertaken have failed to demonstrate an impact of compounds tested on disease progression. Therefore, despite corresponding to the SCA for which the highest number of interventional studies has been conducted, the large majority has been clearly power-limited; the lack of randomization, the absence of a placebo group

and the short duration of the trials are some of the limitations pinpointed (revised in [17]). Based on their own previous experience with an interventional study, Saute and collaborators [18] have provided insights on potential pitfalls in SCA3 clinical trials, drawing attention to the importance of improving their efficacy [18].

Empowering clinical trials emerges as a major goal of current SCA3 research, given the previously described scenario, in which anticipated trials should be lengthy and require large numbers of patients. Part of such empowerment is related with the increase in sensitivity of the instruments used to measure disease progression and detect subtle therapeutic benefits; it is therefore expected that molecular biomarkers (MBs) will have the potential to provide a crucial contribution to the quality of SCA3 interventional studies.

16.2 Molecular Biomarkers of SCA3

A biomarker is understood as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [19]. Biomarkers are frequently subdivided into "trait biomarkers" if they provide indications as to the presence/absence of disease and "state biomarkers" if they can inform about the severity of a certain pathology [20]. Biomarkers are expected to be useful in diagnostic, staging and prognosis of disease, as well as to aid in the prediction and monitoring of intervention [19].

As it would be expectable, clinical biomarkers correspond to the most investigated markers so far in SCA3. Several standardized clinical tests, including rating scales, have been developed to measure different aspects of the SCA3 phenotype. Such measures have been validated in studies of the natural history of the disease, targeting different cohorts of patients. Advantages pinpointed for the widespread use of clinical markers are the relatively low requirement in time and their reduced cost, as well as the fact that they can be obtained without the need for any sophisticated equipment [20]. Regardless of the progresses made with the development of more objective clinical scales, it is assumed that clinical measures are to a certain extent subjective (the complexity of the SCA3 phenotype further aggravates this limitation), insensitive to subtle changes in small periods of time, as well as potentially subjected to observational bias [20]. Also, current clinical measures are limited as to their usefulness in the preataxic stage of the disease, a phase that should be extremely important on what concerns the development of therapeutics.

Neuroimaging information holds the promise of sensitivity and informativity; neuroimaging indicators, such as specific volumetric alterations, are already being used as primary endpoint in clinical trials of neurodegenerative diseases similar to SCA3 (www.clinicaltrials.gov—clinical trial NCT02336633). Notwithstanding, it has to be acknowledged that although neuroimaging alterations in SCA3 are well documented in cross-sectional studies, longitudinal data is still scarce, a limitation

that will be necessary to overcome, if such type of biomarker is to be considered as a primary endpoint in future interventional trials of SCA3.

Because in slow progressing disorders, such as SCA3, the capture of small effects remains a challenge, the development of MBs is urgently needed. MBs should be crucial to improve sensitivity, when used in complement to clinical endpoints; they are also crucial because they can be applied to the presymptomatic stage, thus resolving one of the limitations of clinical markers. Furthermore, when ameliorating drugs will be available MBs being able to precociously detect pathogenic alterations will be of use to optimize therapeutics efficiency, since such compounds are expected to be more efficient if administrated to mutation carriers before disease.

16.2.1 Molecular Trait Biomarkers of SCA3

The CAG repeat at the *ATXN3* locus constitutes the primary trait biomarker of SCA3. In fact, the identification of the *ATXN3* gene opened prospects for the direct detection of the mutation, providing the grounds for molecular diagnosis and allowing presymptomatic (predictive) testing. The identification of carriers of the mutation before onset offers the possibility of understanding the full process of progression of the disease (Fig. 16.1), which in turn will be of major importance at

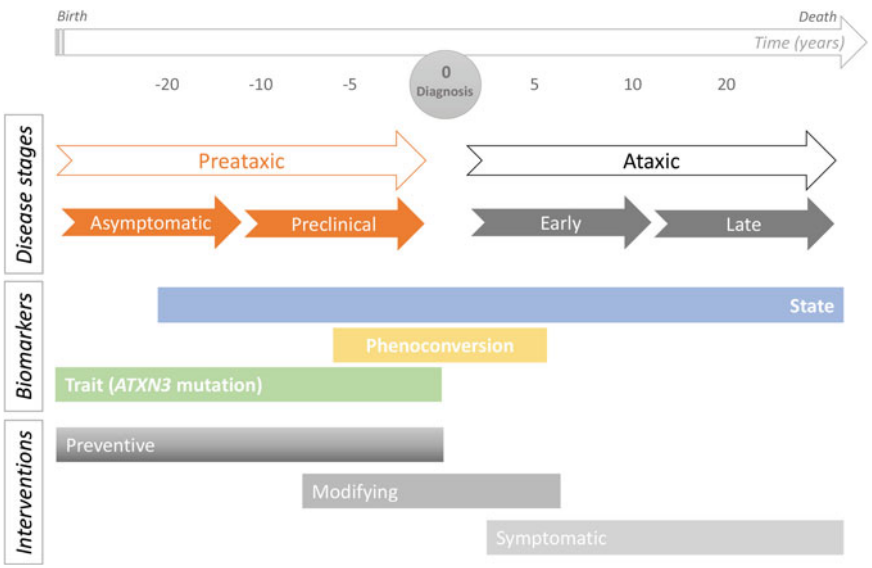

Fig. 16.1 Stages, type of biomarkers and interventional studies which can be performed during the natural course of SCA3

a stage where therapeutic offers will be available, as previously referred. In SCA3 patients, a negative correlation between the number of CAG repeats in the expanded allele and age at onset has been widely reported, the number of CAGs accounting for nearly 50–75% of the variation in the age of appearance of first symptoms (revised in [8]); in the Azorean SCA3 cohort, for example, the number of CAG repeats in the expanded allele explains 68% of the onset variance [21]. In addition to the size of the CAG tract, age at onset, specific symptoms, rate of progression as well as degree of severity are notably modifiable by other factors. Such factors could theoretically be environmental or genetic; probably both contribute to the specific final phenotype. Although research on genetic modifiers of SCA3 is still at an incipient stage, several genetic factors have already been investigated as modifiers of SCA3: the number of CAG repeats at several expansion loci [21–23]; allelic variants at the interleukine 6 (*IL6*; [24]), apolipoprotein E (*APOE*) [25, 26] and glucosylceramidase beta (*GBA*) [27] genes; variation in the 3'UTR at the *ATXN3* gene [28] and size of the normal SCA3 allele [29]. Moreover, for some of these genes/variants a possible cumulative effect has also been tested; in fact, Azorean SCA3 patients carrying the APOE*e2 and the IL6*C allele presented an onset which was anticipated by an average of 10 years [24]. More recently, exome sequencing has been proposed to study genetic modifiers of SCA3 (Manuela Lima, personal communication). The identification of genetic modifiers would allow: (1) targeting particular pathways/mechanisms for development of therapeutic interventions; (2) stratifying patients based on their genotype and incorporating this knowledge into the design of clinical trials. This type of stratification will empower clinical trials by controlling for the effect of the genetic background of participants [30]; and (3) improving the prediction of age at onset, aiming to provide better genetic counseling.

16.2.2 Molecular State Biomarkers of SCA3

16.2.2.1 Transcriptional biomarkers

As previously referred, abnormal conformation of mutated ataxin-3 promotes a gain of a toxic function compromising several cellular mechanisms, namely transcription. Transcriptional regulation seems to be affected by mutated ataxin-3 via two processes: (1) recruitment of transcription factors into polyQ-rich inclusions [31–33]; and (2) abnormal interactions with transcription factors and co-activators [34–38]. Transcriptional dysregulation was initially studied in cellular and animal models of SCA3 [35, 39, 40]. In such models, transcriptional alterations of genes involved in inflammatory processes, cell signaling and encoding cell-surface associated proteins has been described [35, 39, 40]. Based on this previous evidence Raposo and colleagues [6] hypothesized that in SCA3 patients the analysis of disease-specific

transcriptional changes in blood, a peripheral tissue, had the potential to allow the identification of novel biomarkers. A cross-sectional study with SCA3 patients and controls, using the Illumina Human V4-HT12 array, confirmed the presence of differences in expression between the two groups [6]. Twenty six genes, found to be up-regulated in patients, were selected for a first step of validation by quantitative real-time PCR (technical validation). From these 21 genes, fourteen were subsequently selected for validation by qPCR in a new set of SCA3 patients and controls. In this second validation step, the expression levels of *FCGR3B* (Fc fragment of IgG, low-affinity IIIb, receptor (CD16b)), *CSF2RA* (Colony-stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage), *CLC* (Charcot-Leyden crystal protein), *FPR2* (Formyl peptide receptor 2), *SLA* (Src-like-adaptor), *GPR97* (G protein-coupled receptor 97), *P2RY13* (Purinergic receptor P2Y, G-protein coupled, 13), *TNFSF14* (Tumor necrosis factor (ligand) superfamily, member 14), *SELPLG* (Selectin P ligand) and *YIPF6* (Yip1 domain family, member 6) were found to be 1.11–2.60-fold higher in patients when compared to controls. Noteworthy, *FCGR3B, P2RY13* and *SELPLG* genes were significantly up-regulated. Raposo and colleagues [6] further shown that, particularly for *FCGR3B* and *CLC*, patients with shorter disease duration tended to have higher expression levels when compared with patients with longer disease duration [6].

In a second approach Raposo and colleagues [41] listed molecules whose levels had been previously described in the literature as altered in SCA3 patients. The goal was to verify if expression levels of the selected genes *HSPB1* (heat shock 27 kDa protein 1), *DNAJB1* (DnaJ (Hsp40) homolog, subfamily B, member 1), *DNAJB12* (DnaJ (Hsp40) homolog, subfamily B, member 12), *DNAJB14* (DnaJ (Hsp40) homolog, subfamily B, member 14), *BAX* (BCL2-associated X protein), *BCL2* (B-cell CLL/lymphoma 2), *SOD2* (superoxide dismutase 2, mitochondrial), *IL1B* (interleukin 1, beta) and *IL6* (interleukin 6) correlated with disease. The authors concluded that *HSPB1* and *BCL2* were significantly down-regulated in patients compared to controls. Given the previously highlighted importance of the preclinical stage of SCA3 Raposo and colleagues [41] expanded this analysis to include samples from preataxic SCA3 subjects. mRNA levels adjusted for age at blood collection were obtained for a set of premanifest SCA3 subjects, patients and controls. *BCL2* levels were distinct in SCA3 subjects as compared to controls, although not being able to differentiate between premanifest carriers and patients. Moreover, lower levels of *IL6* mRNA were also found in preataxic carriers.

In the quest for SCA3 biomarkers, longitudinal studies should provide the best quality data; such studies, however, represent an important effort for researchers and patients and therefore are lacking for SCA3. Due to the availability of a homogeneous subset of SCA3 patients with several blood collection

points, Raposo and colleagues [41] investigated the behavior of *HSPB1* and *BCL2* mRNA levels in a longitudinal setup. Blood samples from SCA3 patients were collected at the baseline of the study and at a second moment. *BCL2* and *HSPB1* mRNA adjusted levels were found to be significantly different between the baseline and the second observational moment; during disease progression, the mRNA levels of *BCL2* and *HSPB1* increased.

16.2.2.2 Biomarkers of mitochondrial (mtDNA) depletion and damage

Amongst the several cellular pathways shown to be altered in the presence of mutated ataxin-3 are those associated with mitochondrial integrity and function. Because different compounds which improve energy metabolism defects or reduce oxidative stress (such as creatine and coenzyme Q10, amongst others) have been proposed as having the potential to ameliorate polyQ diseases, the investigation of the potential of mitochondrial alterations as biomarkers is pertinent. Metabolic alterations linked with mitochondrial function in SCA3 have been widely reported [42–44]. Furthermore, data on mtDNA depletion and increased damage (higher frequency of mutations, namely deletions) has also been produced [44–48]. Some of these previous reports indicated that alterations in the leukocytes mtDNA content had the potential to be used as biomarkers of SCA3; results from the several studies, however, were not consensual. Aiming to confirm previous findings of increased mtDNA depletion and damage, analysing a larger and independent set of patients, Raposo and colleagues [49] measured the levels of mitochondrial encoded NADH dehydrogenase 1 *(MT-ND1)*, NADH dehydrogenase 4 *(MT-ND4)* and ribonuclease P RNA component H1 *(RPPH1)* genes, which enabled the determination of the mtDNA content and the evaluation of the common deletion (m.8470_13446del4977 deletion, which is considered a marker of age). In this study, along with blood samples collected from SCA3 patients and community controls, samples from preataxic carriers were also analysed. Although differences in mtDNA content were not evidenced in carriers of the mutation, as compared to controls, the common deletion was significantly more frequent in patients and preataxic subjects than in controls, after adjusting for age at collection. In preataxic subjects, moreover, a significant correlation between the number of CAG repeats in expanded allele and the frequency of common deletion was obtained; the frequency of common deletion was lower in preataxic subjects carrying a higher number of CAGs [49].

Alterations in several molecules related with other SCA3-associated mechanisms, quantified either at the mRNA or protein level have been reported and are synthetized in Table 16.1.

Table 16.1 Candidate biomarkers linked to several SCA3-associated mechanisms, either identified using cross-sectional and longitudinal analysis

Mechanism	Type of study	Gene/ protein	Subjects	Main results	Reference
Autophagy	Cross-sectional	*BECN1*	Controls Patients	Levels of *BECN1* mRNA were 1.4 times higher in SCA3 patients compared to controls	[50]
	Cross-sectional	*SIRT1*/ SIRT1	Controls Patients	mRNA levels of *SIRT1* as well as protein levels were severely decreased in human fibroblasts of SCA3 patients in comparison with controls	[51]
Inflammation	Cross-sectional	Eotaxin	Controls Preataxic subjects patients	Eotaxin levels was found to be higher in SCA3 asymptomatic carriers and in patients	[52]
	Longitudinal		Patients	Eotaxin levels decreased after 360 days	
Insulin system	Cross-sectional	IGFBP3 IGF1 IGFBP1	Controls Patients	Low levels of insulin and IGFBP3 and high levels of insulin sensitivity (HOMA2), free IGFI, and IGFBP1 where obtained in SCA3 patients	[53]

16.3 Conclusions

The quest for MBs of SCA3 has started; although studies are presently in an exploratory phase, longitudinal studies have begun to aid in the validation of results from cross-sectional analysis. Candidate transcriptional biomarkers evaluated so far will need to be further tested to warrant their reliability; this means that their behavior should be independent from inter-individual variation and comorbidities, for example. Similar to what is expected in similar neurodegenerative diseases, it is likely that for SCA3 not only a single biomarker, but a set of different molecules, will have to be identified. This set of MBs will be use, in complementarity with clinical and imaging markers, to fully track SCA3, from its presymptomatic phase to the disease stage.

References

1. Ruano L, Melo C, Silva MC, Coutinho P (2014) The global epidemiology of hereditary ataxia and spastic paraplegia: a systematic review of prevalence studies. Neuroepidemiology 42 (3):174–183
2. Lima M, Bruges-Armas J, Bettencourt C (2012) Non-mendelian genetic aspects in spinocerebellar ataxias (scas): the case of Machado-Joseph disease (MJD). Spinocerebellar Ataxia, InTech
3. Coutinho P (1992) Doença de Machado-Joseph: Tentativa de definição. PhD dissertation, Universidade do Porto
4. Lima M, Mayer F, Coutinho P, Abade A (1997) Prevalence, geographic distribution, and genealogical investigation of Machado-Joseph disease in the Azores (Portugal). Hum Biol 69 (3):383–391
5. Lima M, Mayer FM, Coutinho P, Abade A (1998) Origins of a mutation: population genetics of Machado-Joseph disease in the Azores (Portugal). Hum Biol Int Rec Res 70(6):1011–1023
6. Raposo M, Bettencourt C, Maciel P, Gao F, Ramos A et al (2015) Novel candidate blood-based transcriptional biomarkers of Machado-Joseph disease. Mov Disord 30(7):968–975
7. de Araújo M, Raposo M, Kazachkova N, Vasconcelos J, Kay T, Lima M (2016) Trends in the epidemiology of spinocerebellar ataxia type 3/Machado-Joseph disease in the Azores Islands, Portugal. JSM Brain Sci 1(1):1001
8. Bettencourt C, Lima M (2011) Machado-Joseph disease: from first descriptions to new perspectives. Orphanet J Rare Dis 6:35
9. Bettencourt C, Raposo M, Kazachkova N, Cymbron T, Santos C et al (2011) The APOE ε2 allele increases the risk of earlier age at onset in Machado-Joseph disease. Arch Neurol 68 (12):1580–1583
10. Sequeiros J, Coutinho P (1993) Epidemiology and clinical aspects of Machado-Joseph disease. Adv Neurol 61:139–153
11. Bettencourt C, Santos C, Kay T, Vasconcelos J, Lima M (2008) Analysis of segregation patterns in Machado-Joseph disease pedigrees. J Hum Genet 53(10):920–923
12. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M et al (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8(3):221–228
13. Maciel P, Costa MC, Ferro A, Rousseau M, Santos CS et al (2001) Improvement in the molecular diagnosis of Machado-Joseph disease. Arch Neurol 58(11):1821–1827
14. Souza GN, Kersting N, Krum-Santos AC, Santos ASP, Furtado GV et al (2016) Spinocerebellar ataxia type 3/Machado-Joseph disease: segregation patterns and factors influencing instability of expanded CAG transmissions. Clin Genet 90(2):134–140
15. Evers MM, Toonen LJA, van Roon-Mom WMC (2014) Ataxin-3 protein and RNA toxicity in spinocerebellar ataxia type 3: current insights and emerging therapeutic strategies. Mol Neurobiol 49(3):1513–1531
16. do Costa MC, Paulson HL (2012) Toward understanding Machado-Joseph disease. Prog Neurobiol 97(2):239–257
17. Kazachkova N, Lima M (2015) Towards a therapeutic intervention in polyglutamine ataxias: from models to clinical trials. In: Atta-ur-Rahman (ed) Frontiers in clinical drug research - CNS and neurological disorders, pp. 77–130
18. Saute JAM, Rieder CRM, Castilhos RM, Monte TL, Schumacher-Schuh AF et al (2015) Planning future clinical trials in Machado Joseph disease: lessons from a phase 2 trial. J Neurol Sci 358(1–2):72–76
19. Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95
20. Weir DW, Sturrock A, Leavitt BR (2011) Development of biomarkers for Huntington's disease. Lancet Neurol 10(6):573–590

21. Raposo M, Ramos A, Bettencourt C, Lima M (2015) Replicating studies of genetic modifiers in spinocerebellar ataxia type 3: can homogeneous cohorts aid? Brain 138(Pt 12):e398
22. Jardim L, Silveira I, do Pereira ML, Céu Moreira M, Mendonça P et al (2003) Searching for modulating effects of SCA2, SCA6 and DRPLA CAG tracts on the Machado-Joseph disease (SCA3) phenotype. Acta Neurol Scand 107(3):211–214
23. Tezenas du Montcel S, Durr A, Bauer P, Figueroa K, Ichikawa Y et al (2014) Modulation of the age at onset in spinocerebellar ataxia by CAG tracts in various genes. Brain 137(Pt 9):2444–2455
24. Raposo M, Bettencourt C, Ramos A, Kazachkova N, Vasconcelos J et al (2016) Promoter variation and expression levels of inflammatory genes IL1A, IL1B, IL6 and TNF in blood of spinocerebellar ataxia type 3 (SCA3) patients. Neuromolecular Med 19(1):41–45
25. Bettencourt C, Santos C, Coutinho P, Rizzu P, Vasconcelos J et al (2011) Parkinsonian phenotype in Machado-Joseph disease (MJD/SCA3): a two-case report. BMC Neurol 11:131
26. Peng H, Wang C, Chen Z, Sun Z, Jiao B et al (2014) APOE ε2 allele may decrease the age at onset in patients with spinocerebellar ataxia type 3 or Machado-Joseph disease from the Chinese Han population. Neurobiol Aging 35(9):2179.e15–2179.e18
27. Siebert M, Donis KC, Socal M, Rieder C, Emmel VE et al (2012) Glucocerebrosidase gene variants in parkinsonian patients with Machado Joseph/spinocerebellar ataxia 3. Park Relat Disord 18(2):185–190
28. Long Z, Chen Z, Wang C, Huang F, Peng H et al (2015) Two novel SNPs in ATXN3 3' UTR may decrease age at onset of SCA3/MJD in Chinese patients. PLoS One 10(2):e0117488
29. França MC, Emmel VE, D'Abreu A, Maurer-Morelli CV, Secolin R et al (2012) Normal ATXN3 allele but not CHIP polymorphisms modulates age at onset in Machado-Joseph disease. Front Neurol 3:164
30. Gusella JF, MacDonald ME (2009) Huntington's disease: the case for genetic modifiers. Genome Med 1(8):80
31. Chai Y, Wu L, Griffin JD, Paulson HL (2001) The role of protein composition in specifying nuclear inclusion formation in polyglutamine disease. J Biol Chem 276(48):44889–44897
32. Perez MK, Paulson HL, Pendse SJ, Saionz SJ, Bonini NM, Pittman RN (1998) Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. J Cell Biol 143 (6):1457–1470
33. Takahashi J, Tanaka J, Arai K, Funata N, Hattori T et al (2001) Recruitment of nonexpanded polyglutamine proteins to intranuclear aggregates in neuronal intranuclear hyaline inclusion disease. J Neuropathol Exp Neurol 60(4):369–376
34. Evert BO, Araujo J, Vieira-Saecker AM, de Vos RAI, Harendza S et al (2006) Ataxin-3 represses transcription via chromatin binding, interaction with histone deacetylase 3, and histone deacetylation. J Neurosci 26(44):11474–11486
35. Evert BO, Vogt IR, Vieira-Saecker AM, Ozimek L, de Vos RAI et al (2003) Gene expression profiling in ataxin-3 expressing cell lines reveals distinct effects of normal and mutant ataxin-3. J Neuropathol Exp Neurol 62(10):1006–1018
36. Li F, Macfarlan T, Pittman RN, Chakravarti D (2002) Ataxin-3 is a histone-binding protein with two independent transcriptional corepressor activities. J Biol Chem 277(47):45004–45012
37. Nicastro G, Menon RP, Masino L, Knowles PP, McDonald NQ, Pastore A (2005) The solution structure of the Josephin domain of ataxin-3: structural determinants for molecular recognition. Proc Natl Acad Sci USA 102(30):10493–10498
38. Rodrigues A-J, Coppola G, Santos C, do Costa MC, Ailion M et al (2007) Functional genomics and biochemical characterization of the *C. elegans* orthologue of the Machado-Joseph disease protein ataxin-3. FASEB J. 21(4):1126–1136
39. Chou A-H, Yeh T-H, Ouyang P, Chen Y-L, Chen S-Y, Wang H-L (2008) Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. Neurobiol Dis 31(1):89–101

40. Evert BO, Vogt IR, Kindermann C, Ozimek L, de Vos RA et al (2001) Inflammatory genes are upregulated in expanded ataxin-3-expressing cell lines and spinocerebellar ataxia type 3 brains. J Neurosci 21(15):5389–5396
41. Raposo M (2017) Predicting and tracking Machado-Joseph disease: biomarkers of diagnosis and prognosis. PhD dissertation, Universidade dos Açores
42. Matsuishi T, Sakai T, Nagamitsu S, Shoji H, Ueda N et al (1996) Decreased cerebrospinal fluid levels of substance P in Machado-Joseph disease. J Neurol Sci 142(1–2):107–110
43. Pacheco LS, da Silveira AF, Trott A, Houenou LJ, Algarve TD et al (2013) Association between Machado-Joseph disease and oxidative stress biomarkers. Mutat Res 757(2):99–103
44. Yu Y-C, Kuo C-L, Cheng W-L, Liu C-S, Hsieh M (2009) Decreased antioxidant enzyme activity and increased mitochondrial DNA damage in cellular models of Machado-Joseph disease. J Neurosci Res 87(8):1884–1891
45. Kazachkova N, Raposo M, Montiel R, Cymbron T, Bettencourt C et al (2013) Patterns of mitochondrial DNA damage in blood and brain tissues of a transgenic mouse model of Machado-Joseph disease. Neurodegener Dis 11(4):206–214
46. Liu C-S, Cheng W-L, Kuo S-J, Li J-Y, Soong B-W, Wei Y-H (2008) Depletion of mitochondrial DNA in leukocytes of patients with poly-Q diseases. J Neurol Sci 264 (1–2):18–21
47. Ramos A, Kazachkova N, Silva F, Maciel P, Silva-Fernandes A et al (2015) Differential mtDNA damage patterns in a transgenic mouse model of Machado-Joseph disease (MJD/SCA3). J Mol Neurosci 55(2):449–453
48. Zeng A, Liu X, Shen L, Li W, Ding Z et al (2012) Analysis of mitochondrial DNA variations in a Chinese family with spinocerebellar ataxia. J Clin Neurosci 19(1):60–64
49. Raposo M, Ramos A, Kazachkova N, Teixeira B, Bettencourt C, Lima M (submitted) Accumulation of mitochondrial DNA common deletion since the preataxic stage of Machado-Joseph disease
50. Kazachkova N, Raposo M, Ramos A, Montiel R, Lima M (2017) Promoter variant alters expression of the Autophagic BECN1 gene: implications for clinical manifestations of Machado-Joseph disease. The Cerebellum
51. Cunha-Santos J, Duarte-Neves J, Carmona V, Guarente L, Pereira de Almeida L, Cavadas C (2016) Caloric restriction blocks neuropathology and motor deficits in Machado-Joseph disease mouse models through SIRT1 pathway. Nat Commun 11(7):11445
52. da Silva Carvalho G, Saute JAM, Haas CB, Torrez VR, Brochier AW, Souza GN, Furtado GV, Gheno T, Russo A, Monte TL, Schumacher-Schuh A, D'Avila R, Donis KC, Castilhos RM, Souza DO, Saraiva-Pereira ML, Torman VL, Camey S, Portela LV, Jardim LB (2016) Cytokines in Machado Joseph disease/Spinocerebellar Ataxia 3. The Cerebellum 15 (4):518–525
53. Saute JAM, da Silva ACF, Muller AP, Hansel G, de Mello AS, Maeda F, Vedolin L, Saraiva-Pereira ML, Souza DO, Arpa J, Torres-Aleman I, Portela LVC, Jardim LB (2011) Serum insulin-like system alterations in patients with Spinocerebellar Ataxia type 3. Mov Disord 26(4):731–735