49 Allele-specific AAV-based silencing of mutant ataxin-3 alleviates neuropathology and motor deficits in spinocerebellar ataxia type 3

Rui Jorge Nobre[1,2,3], Joana Saraiva[1], Magda Santana[1], Clelia Fusco[1], Susana Paixão[1], Catarina Miranda[1,3], Lorena Petrella[4], José Sereno[4], João Castelhano[4], Miguel Castelo-Branco[4], Miguel Sena-Esteves[5], Luis Pereira de Almeida[1,2,6].

[1]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal,[2]ViraVector - Viral Vectors for Gene Transfer Core Facility, Coimbra, Portugal,[3]Institute for Interdisciplinary Research (III), Coimbra, Portugal,[4]ICNAS - Institute of Nuclear Sciences Applied to Health, Coimbra, Portugal,[5]Neurology Department, Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA,[6]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Presenting author: Luis Pereira de Almeida

Contact: luispa@cnc.uc.pt

Introduction: Spinocerebellar ataxia type 3 or Machado-Joseph disease (MJD) is the most common dominantly inherited ataxia worldwide. It is associated with the expansion of a (CAG)n tract in the coding region of the causative gene MJD1/ATXN3, which translates into an expanded polyglutamine tract conferring toxic properties to the ataxin-3 protein, and inducing severe clinical features. Gene silencing targeting both mutant and non-mutant ataxin-3 alleles holds great promise for its treatment. Nevertheless, it is unknown whether neuronal cells in the human brain will tolerate long-term silencing of wild-type ataxin-3. Therefore, we aimed at developing an AAV-based miRNA gene therapy that would promote allele-specific silencing of mutant ataxin-3 and alleviation of MJD upon intracranial or intravenous injection.

Methods: Specific gene silencing miRNAs targeting SNPs in linkage disequilibrium with the disease-causing expansion were firstly designed and tested in modified neuronal cell lines. An AAV9 vector encoding the most effective artificial microRNA (AAV9-mirATAX3) was then generated and firstly validated in a lentiviral-based mouse model of MJD upon intracranial injection. Next, severely-impaired transgenic mice were intravenously-injected with AAV9-mirATAX3 at postnatal day one (PN1), submitted to behavioral tests at three different time points, to magnetic resonance imaging/spectroscopy (MRI/MRS) at PN75, and sacrificed at PN95.

Results: The silencing potential of the mirATX3 sequence demonstrated superior specificity in vitro compared to the silencing sequence previously reported. AAV9-mirATAX3's treatment reduced the number of protein aggregates and neuropathology in both animal models and led to significant improvements in behavioral tests in the transgenic model. Moreover, MRI/MRS data indicated that mirATXN3 treatment ameliorates the levels of a specific set of neurometabolites, which can be used as therapeutic biomarkers. The intravenous injection of adult animals further demonstrated that AAV9-mirATAX3 has also the ability to transduce the CNS of adult MJD transgenic mice.

Conclusion: This study provides compelling evidence that AAV9-mirATAX3 is able to silence mutant ataxin-3 in different SCA3 animal models, through different routes of administration, which may have an important impact on the treatment of MJD.

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