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**Partial loss of *Tip60* slows mid-stage neurodegeneration in a spinocerebellar ataxia type 1 (SCA1) mouse model**

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Objectives

Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disease caused by a polyglutamine tract expansion in the ATXN1 protein. This neurodegeneration is limited to cerebellar Purkinje cells, the brainstem and the spinal cord. This article will focus on the effects of ATXN1 on Purkinje cells. The normal function of ATXN1 is to regulate gene expression by interacting with a variety of transcription factors. The two proteins of interest in this paper are Tip60, an acetyltransferase tat-interactive protein 60 kDa, and Rora, a retinoid acid receptor-related orphan receptor alpha. Tip60 is thought to interact with ATXN1 through the ATXN1 and HMG-box protein 1 (AXH) domain, and ATXN1 and Tip60 are thought to complex with Rora to aid in Rora mediated gene expression.

This study is important because it investigates a possible pathway resulting in neuro-degeneration by AXTN1. This study looked at the biological importance of the ATXN1/Tip60 interaction, and the effect of Tip60 haploinsufficiency on cerebellar Purkinje cell degeneration and Rora gene expression. This study also looks to confirm the AXTN1/Tip60 interaction by the AXH domain of AXTN1 and look at the effect of phosphorylation on this interaction.

Experimental Approach and Results

Deletion constructs were designed to test various domains of the AXTN1 protein to determine which domains were important for Tip60 binding. These constructs were expressed in *E. coli* cells and run on a GST-pull-down assay with cloned human Tip60. Gel results showed that the only domain necessary for Tip60 binding to ATXN1 is the AXN domain. All constructs that contained the AXN domain were able to bind Tip60, and none of the constructs without the AXN domain were able to bind Tip60.

Next, they tested to see whether or not phosphorylation of serine at position S776 had an effect on ATXN1/Tip60 interaction. To do this, they created two mutants: an S776A substitution and an S776D substitution. The serine to alanine substitution represented a phospho-resistant substitution while the serine to aspartic acid substitution represented a phospho-mimicking substitution. The S776D phospho-mimicking substitution resulted in an increase of ATXN1/Tip60 binding, showing that phosphorylation of serine at position 776 enhances the ATXN1/Tip60 interaction.

Further experiments used mouse models on a mixed background of FVB;SV-129;C57BL/6. The mice used had phenotypes of wild type (WT), ATXN1[82Q], or ATXN1[82Q]:Tip60+/-. These mice were used to test for neurodegeneration by measuring Purkinje cell molecular thickness in WT, ATXN1[82Q] mice, and ATXN1[82Q]:Tip60+/- mice showing Tip60 haploinsufficiency. Immunofluorescence was used to measure the thickness of cerebellar Purkinje cells in these different phenotypes at nine, 12, 16, and 20 weeks of age. These ages represented early, mid-stage, and late-stage of disease, respectively. Quantitative analysis showed that WT mice showed the least degeneration, and that ATXN1[82Q]:Tip60+/- mice showed a decrease in neurodegeneration at 12-16 weeks during the mid-stage of disease. Degeneration at the early-stage and late-stage was the same for both ATXN1[82Q] and ATXN1[82Q]:Tip60+/- mice.

The final investigation involved the effect of Tip60 haploinsufficiency on Rora protein levels and Rora mediated gene expression. Western blot analysis showed that ATXN1[82Q]:Tip60+/- mice had an increase in Rora protein expression when compared to ATXN1[82Q] mice. Quantitative PCR was then used to determine expression of genes under the control of Rora. The genes tested had previously been shown to be decreased in SCA1 mice. There was no significant difference in gene expression between WT, ATXN1[82Q] mice, and ATXN1[82Q]:Tip60+/- mice at eight weeks of age, representing the early stage of disease. At 12 weeks, during the mid-stage of disease where a decrease in neurodegeneration had been previously shown in Purkinje cells, there was a significant increase in *Slc1a6* and *Pcp4* in ATXN1[82Q]:Tip60+/- mice compared to ATXN1[82Q] mice. At 20 weeks of age, there was also a significant increase in *Itpr,* and *Slc1a6* had increased significantly over the WT.

Conclusion

This article shows that Tip60 interacts with ATXN1 by the AXH domain based on a pull-down assay of various deletion constructs. It also shows that this interaction is strengthened by phosphorylation of the serine at position 776 based on the substitution of phospho-mimicking aspartic acid for serine. With the use of transgenic mice, they were able to show the effect of partial loss of Tip60 on the degeneration of cerebellar Purkinje cells. Tip60 haploinsufficiency showed a protective window during the mid-stage of disease for 12 to 16 week old mice. This suggests that Tip60 contributes to SCA1 disease pathogenesis, and that lower amounts of Tip60 result in less neurodegeneration. However, the protective effects of Tip60 haploinsufficiency are not present in the early stages of disease and do not extend into the late stages of disease. This would suggest that Tip60 does not play a role in the initiation of SCA1, but is involved only in the progression of the disease.

Tip60 haploinsufficiency also had an effect on Rora protein expression. Protein levels of Rora were increased in ATXN1[82Q]:Tip60+/- mice as compared to ATXN1[82Q] mice. Rora mediated gene expression of genes previously shown to be decreased in ATXN1[82Q] mice was also increased in those mice with partial loss of Tip60. These genes are known to bind Rora at the promoter, and some of them showed a significant increase in mice at both 12 weeks of age and 20 weeks of age. Therefore, the greater the expression of these genes, the greater the expression of Rora, although the effects of this increase in Rora are unclear. It is also unclear why *Slc1a6* expression in ATXN1[82Q]:Tip60+/- mice was able to increase so significantly over WT type mice.

Future Research

Continuing research in this area would include identifying other pathways and genetic markers involved in SCA1 pathogenesis. With respect to the protective effect of the partial loss of Tip60, a further step would be to determine what kind of effect a complete loss of Tip60 would have on disease pathogenesis. Tip60 knockout mice are lethal near blastocyst stage, so an alternative method of preventing Tip60 interaction would be required to test complete loss of Tip60. Possible options would include the removal of the AXH domain of ATXN1, or blocking the interaction of Tip60 and ATXN1 by some other competitive inhibitor. Complete loss of Tip60 may be able to further delay the progression of SCA1, or could possibly reveal an alternative role in disease initiation and progression.

Another area of study could be the effect of the up-regulation of Rora and Rora mediated genes. These genes were still significantly up-regulated during the late stages of disease, when the protective window of Tip60 haploinsufficiency was gone. It is possible that the continued up-regulation of Rora could have toxic effects on SCA1 pathogenesis. Perhaps this toxicity negates the protective effects of Tip60 haploinsufficiency.

A final question yet to be answered is the initiation of SCA1 pathogenesis. If Tip60 does not play a role in this initiation, then what does? At some point, the mutant ATXN1 will cause neurodegeneration, but it is unclear what this interaction, or possibly threshold is.

A critique of this paper would be that the role of both Tip60 and Rora are unclear. The paper says nothing about what the interaction of ATXN1 and Tip60 actually does; only that with a reduction in Tip60, SCA1 pathogenesis is slowed during mid-stage disease. The role of Rora in this disease is also unclear, along with what the up-regulation of Rora results in. Does Rora up-regulation increase disease pathogenesis, decrease pathogenesis, or have no effect on it at all? This would have been helpful information for this study, and given a better perspective of the entire disease process of SCA1.