

SUMMARY OF FINAL REPORT

RNAi therapy for Spinocerebellar ataxia type 7

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Background and aims:

Like many of the dominantly inherited ataxias, spinocerebellar ataxia 7 (SCA7) is caused by a polyglutamine expansion. The prevailing theory suggests that mutant ATAXIN-7 protein leads to a toxic gain-of-function mechanism of pathogenesis leading to neurodegeneration in the cerebellum and the retina. However, there is evidence to suggest that the function of the wild-type gene is required for cellular function. Although there are currently no treatments available, an ideal therapeutic strategy would be to silence/eliminate the ataxia-causing gene in a sequence specific manner. Exploiting RNA interference (RNAi) methods, we have demonstrated: identification of effective RNAi hairpins, one in the form of a miRNA, which show selective silencing of the mutant transcript and phenotypic correction; lentiviral expression of the RNAi hairpin in patient cells and identification of effective siRNAs that silence mouse *ataxin-7* in neurones.

In this project we planned to continue this work by; investigating the activity of the mutation-specific RNAi methods we have developed in patient-derived cells, using lymphoblastoid cells (LCLs) and induced pluripotent stem cell (iPSC)-derived neuronal cells; and by studying the efficacy of *ataxin-7* knockdown in mouse retina using identified siRNAs, and to what extent silencing can be tolerated *in vivo*.

Results:

We have established 17 iPSC-derived cell lines. These include lines from two SCA7 patients (a mother and son) which have yielded 15 iPSC lines. The repeat sizes of the son include an expansion of 51 repeats. His clinical report indicates an age of onset at 20 years of age. This 'early' onset may translate to an increased chance of identifying phenotypic changes in the neurones generated.

In addition we have identified transcriptional differences between patient and control human dermal fibroblast lines. These include Bex1 (Brain-expressed protein 1), DNAJA1 (a heat shock protein shown to be elevated in SCA7 brains) and UCHL1 (expressed in the brain and involved in ubiquitination of unwanted proteins, including polyglutamine proteins. These changes may be useful when assessing differences

between iPSC-derived neurones between patients and controls. Neurones from control lines have been generated (12 month progress report); we are currently growing patient iPSCs in order to complete neuronal differentiation.

We have demonstrated silencing of human endogenous ataxin-7 using both expressed hairpins and siRNAs. Thus we have established multiple effectors capable of silencing ataxin-7 in our patient and control cell lines, which will be used for silencing ataxin-7 in patient neurones. We have also started assessing the ability to transfect neurones using two novel transfection reagents so not as to stress the cells.

We have identified two to three effective siRNAs and an shRNA for silencing endogenous mouse ataxin-7.

We have identified endogenous mouse ataxin-7 in MEFs (mouse embryonic fibroblasts), BV-2s (microglia) and N2As (neuroblastoma cell line). Note the presence of a double band which may represent the second mouse isoform. We have collaborated with colleagues in Sweden who have kindly shared their antibody in order to detect endogenous ataxin-7 protein with the aim of demonstrating protein knockdown, however the stability of the protein (even wild-type) requires we optimise silencing to endure for >48 hours.

We have detected endogenous ataxin-7 in the retina of a wild-type mouse. Notably the protein is present in the nuclear and not the cytoplasmic protein fractions. We believe this is the first publication of retinal ataxin-7.

Lay summary of the results:

Spinocerebellar ataxia type 7 (SCA7) is a hereditary neurodegenerative condition for which no therapy exists at present. One of the ways to treat SCA7 would be to silence the mutant gene which causes the ataxia, using RNA interference (RNAi).

However, before we can test this in patients it is essential to make sure that we have followed all the necessary steps to evaluate the potential therapy. This can be done in two ways. Firstly, we want to show that this therapy works in cells from ataxia patients. Secondly, we have to show that silencing in a mouse is safe. In the last 18 months we have made a great deal of progress in this regard.

Firstly, it is important that we show that the small RNAi molecules we use to silence the mutant gene work in cells from patients with ataxia. We took blood cells from a number of patients and were able to show that our molecules silenced the gene to less than 50%. We also tested the molecules in skin cells and were able to get nearly 90% silencing using additional molecules. However, we also want to test these molecules in brain cells, to assess whether the molecules can prevent the pathologic process in these important cells. Since brain cells are difficult to get from people, we have established a wonderful technique whereby, we can take skin cells from patients, turn them into stem cells, and then turn them into brain cells. At this stage we have made over 16 stem cell lines from ataxia patients, and have shown we can make brain cells from these lines. We are currently working on adding our molecules to the brain cells. Importantly, the technique of making stem cells would allow us to apply this method to any other ataxia.

We have also made progress towards testing RNAi silencing therapy in mice. One of the crucial steps here is making sure we have identified the best molecules to silence the gene. In this regard we have developed several different types of molecules that can silence the mouse gene by 75%. We have also worked hard on making sure that we can *measure* the silencing of mouse gene which has been difficult in the past.

Before we can test these molecules in patients, we need to do a few more things. We have to investigate the condition in the patient brain cells we make and see if our RNAi molecules make them better. We also have to start putting the RNAi molecules we've designed into the mouse to test whether they are safe and work properly. These are the experiments we are continuing with at the moment. We hope that all of this work will bring us closer to a treatment in the future.

Benefits to people with ataxia arisen/likely to arise from this research:

We have gone a long way to establishing a powerful cell model using stem cell derived neurones that could be effectively used for the screening of therapeutic molecules (not just RNAi-based compounds) in a patient-specific manner. We hope that this will be a valuable resource for the entire SCA7 community. In addition, in preparation for work in SCA7 animal models we have identified effective RNAi molecules that silence the gene in the mouse, which is essential to show that such studies are feasible. The Ataxia Investigators Meeting (AIM) was essential in forming collaborations with important SCA7 investigators. Importantly, discussions at this meeting have enabled us to plan work *alongside* other SCA7 investigators, rather than unnecessarily replicating complex experiments. This should go a long way to accelerating the project towards a Phase I clinical trial in SCA7 patients within a few years. Finally, as mentioned earlier, we have initiated similar investigations into other ataxias, namely SCA1. Thus we hope to be able to have an impact on a broader group of ataxia patients in the years to come.

Publications arisen from this project:

Scholefield J, Watson L, Smith D, Greenberg LJ, Wood MJ. Therapeutic silencing restores transcriptional dysregulation in SCA7 patient derived fibroblasts. Manuscript in preparation

Scholefield J, Greenberg LJ, Weinberg MS, Arbuthnot PB, Abdelgany A, Wood MJ. Design of RNAi hairpins for mutation-specific silencing of ataxin-7 and correction of a SCA7 phenotype. *PLoS One*. 2009; 4 (9):e7232

Scholefield J and Wood MJA. Therapeutic gene silencing strategies for polyglutamine disorders. *Trends in Genetics*. 2010;26(1):29-33.

Sibley CR, Scholefield J and Wood MJA. RNA Interference and Neurological Disorders. *ACNR* 2010; 10(5):17- 20.

Conferences/ meetings where this research has been presented:

Ataxia Investigators Meeting, Chicago USA, March 2010.

EuroAtaxia meeting, Italy, October 2011

Any other relevant information:

Both Dr Scholefield and I supervise students who are working on linked aspects of the project. Two PhD students are working on the complex task of differentiating these patient stem cells into retinal cells (photoreceptors). These are being used to compare patient neuronal cells with control neuronal cells in an attempt to better understand the mechanism of degeneration. Another project has lead to the preparation of a manuscript on allele-specific silencing for spinocerebellar ataxia-1 (SCA1). In this paper we show a method for screening populations for common single nucleotide polymorphisms which can be used to silence the mutant and not the wild-type gene of ataxin-1.

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