Spinocerebellar ataxia type 7 in South Africa: Epidemiology, pathogenesis and therapy

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Disorders of the nervous system represent a significant proportion of the global burden of non-communicable diseases, due to the trend towards ageing populations. The Department (now Division) of Human Genetics at the University of Cape Town (UCT) has been involved in pioneering research into these diseases since the appointment of Prof. Peter Beighton as Head of Department in 1972. Beighton's emphasis on understanding the genetic basis of disease laid the groundwork for investigations into several monogenic neurodegenerative conditions, including Huntington's disease and the polyglutamine spinocerebellar ataxias (SCAs). In particular, SCA7, which occurs at an unusually high frequency in the South African (SA) population, was identified as a target for further research and therapeutic development. Beginning with early epidemiological surveys, the SCA7 project progressed to molecular genetics-based investigations, leading to the identification of a founder effect in the SA SCA7 patient population in the mid-2000s. Capitalising on the founder haplotype shared by many SCA7 patients, UCT researchers went on to develop the first population-specific gene-silencing approach for the disease. More recently, efforts have shifted to the development of a more accurate model to decipher the precise mechanisms of neurodegeneration, using induced pluripotent stem cells derived from SA SCA7 patients. In many ways, the SA SCA7 journey reflects the legacy and vision of Prof. Peter Beighton, and his efforts to establish world-class, collaborative research into diseases affecting the African continent.

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Inherited neurodegenerative diseases, characterised by progressive symptoms and a lengthy disease course, present a significant economic and psychological burden to affected families. The Department (now Division) of Human Genetics at the University of Cape Town (UCT) has a long history of research into these conditions, beginning under the leadership of Prof. Peter Beighton in 1972. This research has focused primarily on the polyglutamine (polyQ) diseases, a subset of monogenic neurodegenerative diseases with a well-defined genetic aetiology, which includes Huntington's disease (HD) and six of the spinocerebellar ataxias (SCA1, 2, 3, 6, 7 and 17).[1] Complementing the work done in the research laboratories, an internationally accepted counselling and testing protocol for these conditions has been offered by the Division's clinical service, and latterly, in conjunction with the National Health Laboratory Service (NHLS), since 1995.[2]

Seminal work by Michael Hayden, under Beighton's mentorship, provided the first comprehensive survey of HD in South Africa (SA) in 1979.[3] This laid the foundation for a number of MSc and PhD projects exploring the molecular basis of HD, including that of Jacquie Greenberg, herself now Professor of Human Genetics at UCT. In 2013, UCT researchers, in collaboration with Hayden (now University Killam Professor at the Department of Medical Genetics, University of British Columbia) and others, identified novel, ethnically distinct haplotypes among SA HD patients, providing the first evidence for multiple origins of the disease-causing mutation within the black SA population.[4] These findings are likely to have significant implications for future therapies.[5] (The history of HD research in Africa is reviewed elsewhere in this issue by Baine *et al.*)

Research into the polyQ SCAs at UCT was initiated by Alan Bryer, who completed his PhD on 'The Inherited Ataxias in SA' in 1994. Subsequent work, by Bryer and others, traced the origin and epidemiology of the polyQ SCA mutations in SA, identifying founder effects in two of the SCAs (SCA1 and 7), which may extend to neighbouring countries.[6-9] SCA7, in particular, has been the focus of concerted local research efforts over the past 10 years. The unusually high frequency of this condition in the SA population, coupled with unique phenotypic characteristics, make it an attractive prospect for therapeutic development. Research into the disease has since progressed from early molecular genetics-based investigations to the development of cutting-edge stem cell models and gene-silencing therapies, in an effort to better understand and treat the disease.

Spinocerebellar ataxia type 7

The inherited polyQ diseases all arise from a common type of mutation – an expanded trinucleotide CAG repeat tract within the coding region of the disease-causing gene, which is translated into an abnormally long polyglutamine tract in the mutant protein.[10] Although heterogeneous, a number of common pathogenic mechanisms have been proposed to account for polyQ protein toxicity, including proteolytic cleavage (leading to the production of toxic fragments), impairment of the ubiquitin-proteasome pathway, aggregation of the mutant protein (involving the sequestration of wild-type polyQ protein and other important cellular components), dysregulation of transcription, and mitochondrial dysfunction.[1]

SCA7 is a dominantly inherited polyQ disease, caused by a CAG repeat expansion within the *ATXN7* gene. Normal *ATXN7* alleles typically contain between 4 and 35 CAG repeats, while pathological alleles range in length from 36 to over 400 repeats.[11] Intermediate alleles in the 28 - 35 CAG repeat range may undergo further expansion into the pathogenic range during vertical transmission (particularly in the case of paternal transmission), resulting in the appearance of disease in the offspring of asymptomatic carriers.[12]

Clinically, SCA7 is characterised by cerebellar ataxia, dysarthria, dysphagia and exaggerated deep tendon reflexes.[13] It is distinct from other polyQ diseases by virtue of the presence of progressive macular degeneration, which results in severe visual impairment, in addition to the gait and speech difficulties characteristic of cerebellar degeneration.[14] The neuropathology of SCA7 shows a strong correlation with disease phenotype. Within the cerebellum, SCA7 patients typically exhibit extensive loss of Purkinje cells, with only minimal alterations to the cerebellar granule layer.[15] The retinal phenotype results primarily from the dysfunction of the cone, and later, rod photoreceptors, hence its classification as a cone-rod dystrophy.[16]

Wildtype ATXN7 protein is ubiquitously expressed, and has been shown to play an important role in transcriptional regulation, as a component of several transcriptional co-activator complexes.[17] Mutations in ATXN7 have been shown to inhibit the histone acetylation function of these complexes, leading to transcriptional dysregulation, even before the onset of disease symptoms.[18,19] However, the precise mechanism by which mutations in this gene cause specific degeneration of the cerebellum and retina remains incompletely understood.

A uniquely South African therapy?

SCA7 is one of the rarer polyQ disorders, with a prevalence of less than 1 per 100 000 worldwide.[10] In SA, however, SCA7 is the second most prevalent dominantly inherited ataxia, after SCA1.[20] This unusual pattern was first described by Bryer and colleagues[8] at UCT, who reported an increased prevalence of SCA7 in the SA population, compared with the rest of the world (then 22.2% of total dominant ataxia cases, compared with a frequency of less than 5% elsewhere). A follow-up survey by UCT researchers in 2012 reported a similar trend, with SCA7 now accounting for 26.6% of polyQ ataxia cases.[20] (An updated review of the unique epidemiology of the SA SCAs is provided elsewhere in this issue, by Smith *et al.*)

SCA7 occurs almost exclusively in individuals of black African origin, hypothesised to be the result of a founder event[7] similar to those proposed for coloured and white SCA1 patients in SA.[9] SA SCA7 patients are thus presumed to have descended from a common ancestor, and should consequently share a common haplotype surrounding *ATXN7*, including alleles of common single nucleotide polymorphisms (SNPs) within the region. Indeed, the observation that over 50% of SA SCA7 patients are heterozygous for a common SNP (rs3774729) in the 3' region of *ATXN7*, such that the A and G alleles of the SNP are associated with the mutant and wildtype transcripts, respectively, has provided the foundation for the development of RNA interference (RNAi)-based therapy for this patient population.[7,21]

In 2009, researchers at the UCT Division of Human Genetics, together with collaborators at the Universities of Oxford and the Witwatersrand, published the first evidence for an effective allele-specific RNAi-based therapy for SCA7.[21] Multiple short hairpin RNAs (shRNAs) were screened, in order to identify sequences capable of selectively targeting the *ATXN7*-linked SNP. Incorporating a single nucleotide mismatch to the wildtype RNA target sequence at position 16 from the 5' end of the active shRNA guide sequence was shown to confer maximal mutation selectivity in a heterozygous model, in which HEK293 cells were cotransfected with mutant and wildtype full-length *ATXN7*. Treatment with the allele-specific shRNA resulted in efficient knockdown of the mutant *ATXN7* transcript to 7% of control levels (p=0.05) with minimal effects on the wildtype allele, and led to a marked reduction in ATXN7 aggregation. A similar trend was observed when the allele-specific guide sequence was incorporated into a microRNA (miRNA) mimic.

Although significant, this study was nonetheless hindered by its use of an artificially heterozygous system, reliant on overexpression of exogenous *ATXN7* alleles. To evaluate therapeutic efficacy in a more genetically accurate disease model, this work was followed up by a 2014 study, using cultured fibroblasts from SCA7 patients.[22] The same RNAi effectors were shown to discriminate between endogenous wildtype and mutant alleles, using a novel qPCR assay, with primers capable of discriminating between alleles of the disease-linked SNP. Selective silencing of mutant *ATXN7* was also demonstrated to ameliorate a mild transcriptional phenotype in these cells. Consistent with previously described SCA7 models, a twofold increase in the heat shock protein gene *DNAJA1* and a twofold decrease in the de-ubiquitinating enzyme *UCHL1* were observed in SCA7 patient fibroblasts at the transcriptional level, relative to control lines. Sustained knockdown of mutant *ATXN7* resulted in restoration of both transcripts towards normal levels, suggesting that patient fibroblasts may serve as useful models for evaluating both dosage and efficacy of new therapies.

Disease modelling using induced pluripotent stem cells

Despite these promising results, however, concerns remain regarding the utility of non-central nervous system (CNS) tissues for modelling complex neurodegenerative diseases. Indeed, the lack of suitable disease models remains a major barrier to the study of these conditions. Although several SCA7 mouse models have been generated, none of these successfully recapitulate all facets of the disease.[23] This is largely due to challenges inherent in modelling neurodegeneration in mice, including delayed onset of symptoms, slow disease progression, and species-specific differences in physiology.

Recently developed induced pluripotent stem cell (iPSC) technology offers the opportunity to generate stem cells from somatic cells, through the exogenous expression of stem cell-specific transcription factors (so-called 'reprogramming factors') *OCT3/4, SOX2, c-MYC* and *KLF4*.[24] The resultant stem cells can then be differentiated into any cell type of the body, allowing the study of disease-relevant CNS tissues without the need for invasive surgical techniques.

As part of an ongoing collaboration between researchers at the University of Oxford and UCT, our research group has pioneered the use of iPSCs to study SCA7, generating the first iPSCs from SA SCA7 patients in 2012.[25] These cells have subsequently been differentiated into neurons and retinal cells,[25,26] and used to identify transcriptional and electrophysiological aberrations, which may be linked to the presence of mutant ATXN7. These results provide important preliminary evidence of a disease phenotype in SCA7 iPSC-derived cells, establishing a valuable model for the study of neurodegenerative disease. A number of challenges remain to be addressed, however. Chief among these is the differentiation of iPSCs into cerebellar-specific neurons, in order to generate more accurate models of the disease – a task made particularly difficult by the complex molecular mechanisms underpinning cerebellar development.[27] Future work will seek to

further develop these cells as a resource for the development of population-specific therapies.

SA SCA7 patient cells also played a key role in a breakthrough publication in 2014, in which a large collaboration headed by researchers at the University of Oxford identified a role for long non-coding RNAs in directing the tissue specificity of SCA7 pathogenesis.[28] Based on the observed correlation between expression of *ATXN7* and a conserved retropseudogene, *lnc-SCA7*, in human and mouse CNS, Tan and colleagues[28] proposed a regulatory loop, in which STAGA is required for the transcription initiation of miR-124, the most abundantly expressed miRNA in the CNS. This miRNA in turn mediates the post-transcriptional cross-talk between *lnc-SCA7* and *ATXN7*, which is itself a component of STAGA. SCA7 patient fibroblasts were used to validate the hypothesis that mutations in *ATXN7* may disrupt these regulatory interactions, leading to a neuron-specific increase in *ATXN7* expression, thereby contributing to the selective neurodegeneration observed in SCA7.

Conclusion and perspectives

The SA SCA7 story spans nearly two decades, combining the expertise of a large number of clinical and basic science researchers. Beginning with descriptive molecular genetics investigations, the project has expanded to incorporate cutting-edge stem cell and gene-silencing technologies. In many ways, this reflects the leadership of Prof. Peter Beighton, and his vision for a world-class Human Genetics Department at UCT, capable of fostering collaborative research of the highest quality. As mentor to several of the senior scientists who would go on to pioneer SCA7 research in SA, Prof. Beighton laid the foundation for the current generation of neurogenetics researchers. Current work on SCA7 is thus a reflection of his legacy, unravelling the complexities of SCA7 pathogenesis and developing patient-specific therapies.

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