Review

ANNALS OF MEDICINE & SURGERY

OPEN

A comprehensive review of iPS cell line-based disease modelling of the polyglutamine spinocerebellar ataxias 2 and 3: a focus on the research outcomes

Narasimhan Raghunathan, MSc[a], Sathyanarayanan Sankaran, BSc[b], Goshen D. Miteu, MSc[a,c,d,*]

Abstract

Spinocerebellar ataxias (SCAs) are a rare autosomal dominant neurodegenerative disorder. To date, approximately 50 different subtypes of SCAs have been characterized. The prevalent types of SCAs are usually of PolyQ origin, wherein the disease pathology is a consequence of multiple glutamine residues being encoded onto the disease proteins, causing expansions. SCAs 2 and 3 are the most frequently diagnosed subtypes, wherein affected patients exhibit certain characteristic physiological manifestations, such as gait ataxia and dysarthria. Nevertheless, other clinical signs were exclusive to these subtypes. Recently, multiple molecular diagnostic methods have been developed to identify and characterize these subtypes. Despite these advancements, the molecular pathology of SCAs remains unknown. To further understand the mechanisms involved in neurodegenerative SCAs 2 and 3, patient-derived induced pluripotent stem cell (iPSC)-based modelling is a compelling avenue to pursue. We cover the present state of iPSC-based in-vitro illness modelling of SCA subtypes 2 and 3 below, along with a list of cell lines created, and the relevance of research outcomes to personalized autologous therapy.

Keywords: induced pluripotent stem cells, molecular diagnostic methods, neurodegenerative disorders, personalized autologous therapy, polyglutamine diseases, rare diseases, SCAs 2 and 3, spinocerebellar ataxias

Introduction

Spinocerebellar ataxia (SCA) is a degenerative disease of the nervous system that follows an autosomal dominant genetic inheritance pattern. Approximately 50 different subsets of SCAs have been identified, which can be distinguished based on associated mutations[1]. Six of these identified subtypes, namely SCAs 1, 2, 3, 6, 7, and 17, have been noted to arise from the translation of (CAG)n repeat expansions. The result of repeated expansions was profound neuronal damage. The disease proteins of these subtypes have been identified as Ataxin-1, Ataxin-2, Ataxin-3, α1A-Subunit of voltage-dependent calcium channels, Ataxin-7, and TATA-box-1-binding proteins, respectively. These SCAs have been classified as polyglutamine SCAs (PolyQ SCAs) because multiple glutamine (Q) residues are encoded within the corresponding disease proteins in each case, contributing to disease pathology. The clinical symptoms of PolyQ SCA-affected individuals include tremors, lack of coordination, and cognitive

HIGHLIGHTS

- Spinocerebellar ataxias (SCas) are rare autosomal dominant neurodegenerative disorders.
- The prevalent types of SCAs (2 and 3) are usually of PolyQ origin, wherein the disease pathology is a consequence of multiple glutamine residues being encoded onto disease proteins, causing expansions.
- Of late, multiple molecular diagnostic methods have been developed for the purpose of identifying and characterizing the subtypes. Despite these advancements, molecular pathology of SCAs is still unknown.
- Patient-derived induced pluripotent stem cells (iPSCs) capture disease characteristics *in vitro*, and therefore are valuable for studying SCA types 2 and 3 and serve as a platform for testing novel drugs.
- With their ability to be tailored to individual patient needs, iPSCs have the potential to emerge as personalized autologous therapeutic modalities.

[a]School of Biosciences, Biotechnology, University of Nottingham, England, UK, [b]School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur, Tamil Nadu, India, [c]Department of Biomedical Sciences, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, North Dakota, USA and [d]Department of Biochemistry, Caleb University, Lagos, Nigeria

All co-authors share first authorship.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

*Corresponding author. Address: 750 South 43rd street, #323A, Grand Forks, North Dakota 58201, USA. Tel.: + 701 885 0692. E-mails: goshendavids@gmail.com; goshen.miteu@und.edu (G.D. Miteu).

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Annals of Medicine & Surgery (2024) 86:3487–3498

Received 3 January 2024; Accepted 7 March 2024

Published online 19 March 2024

http://dx.doi.org/10.1097/MS9.0000000000001984

impairment[2]. Even within these PolyQ SCAs, each subtype seems to follow distinct degenerative patterns, presumed to be due to the diseased protein-specific PolyQ expansion. The molecular pathology of SCAs is not yet completely understood owing to the heterogeneity and complexity of disease forms[3]. Furthermore, despite best efforts, existing interventions (both prophylactic and therapeutic) have failed to alleviate SCA disease conditions or manage the severity of symptoms[4]. Several animal models, including murine and non-murine models, have been developed and have achieved positive outcomes. These models have limitations such as incomplete SCA neuropathology, genetic instability, and varied expression of mutant disease proteins in the affected brain tissue, translating these results impractical[5]. The aforementioned factors and animal models have become challenging for further research in SCA, highlighting the need for reliable and potent in-vitro disease modelling tools to effectively investigate the pathophysiology. As a tool and technology, induced pluripotent stem cells (iPSCs) have revolutionized regenerative medicine and drug screening. iPSCs are terminally differentiated somatic cells of patients in a pluripotent state (dedifferentiated), which can then be differentiated into any cell type. This technology has paved the way for future research on several neurodegenerative disorders, such as SCAs, Huntington's disease (HD), Parkinson's disease (PD), and frontotemporal dementia[6]. Herein, we review the current status of iPSC-based in vitro disease modelling of SCA subtypes 2 and 3, including a catalogue of cell lines generated and research outcomes.

Methodology

To gather relevant literature, a comprehensive search was conducted using electronic databases including PubMed, Embase, Web of Science, and Scopus. The primary keywords used in the search process included: "induced pluripotent stem cells",: "iPSCs",: "spinocerebellar ataxia 2",: "SCA2",: "Spinocerebellar ataxia 3",: "Machado-Joseph disease",: "SCA3",: and "polyglutamine disease modelling". These keywords were combined using Boolean operators (e.g. AND and OR) to refine the search and retrieve relevant articles. Relevance was considered using the inclusion criteria of the studies that were: (a) published in peer-reviewed journals, (b) focused on the use of iPSCs in the modelling of SCA2 and/or SCA3, and (c) reporting research outcomes related to disease modelling, phenotypic characterization of the disease, and therapeutic interventions. Non-peer-reviewed articles, conference abstracts, editorials, and articles not published in English were excluded from the literature analysis.

An initial search was performed in the selected databases using the above search techniques. The titles and abstracts of the retrieved articles were then used to filter them for a full-text review, and only papers that satisfied the inclusion requirements were chosen. According to the established selection criteria, full-text papers underwent further stringent evaluations to determine their potential for inclusion in the review. A co-author (G.D.M.) was consulted to settle any disagreements that arose during the selection process.

A thorough summary of the research findings on iPSC-based modelling of SCA2 and SCA3 was presented by extracting and synthesizing pertinent data from the selected papers. The goals, methods, major discoveries, and conclusions of the study were all included in the gathered data. An understandable and cogent narrative of the study findings was provided by thematically organizing the synthesized information.

The findings were synthesized and presented in a structured manner, highlighting the key observations and significant contributions. Relevant figures, tables, and illustrations were incorporated to enhance clarity and understanding of the current research outcomes.

Spinocerebellar ataxias 2 and 3: an overview

Genetic causality

SCA2 is caused by aberrant amplification of CAG repeats in the *ATXN2* gene mapped to chromosome 12q23-q24.1. This amplification results in the expression of an excessively long polyglutamine (PolyQ) sequence in the corresponding protein. Although it is still unclear how expanded Ataxin-2 protein results in the SCA2 disease phenotype, one of the known consequences of this addition is a mutation in the protein, which leads to abnormal cerebellar Purkinje cell (CPC) morphology and subsequent loss, contributing significantly to neurodegeneration[7]. A similar CAG expansion caused by SCA3/Machado–Joseph disease (MJD) has been noted in exon 10 of the *ATXN3* gene mapped onto human chromosome 14q32.1[8]. The lengths of CAG repeats under normal conditions in *ATXN2* are 14–31[9] and 12–44 in *ATXN3*[10]. In the case of neurodegenerative SCA ailments, these numbers increase significantly, ranging between 62 and 84 units in MJD and anywhere between 33 and 200 units in SCA2, with the bare minimum number of expanded units capable of causing disease progression being just 33[9]. In SCA2 and SCA3, an increased length of the enlarged allele repeat exacerbates disease severity[11]. Similar rates of survival in patients were noted after a 10-year follow-up period in a cohort study of both ataxias (74% and 73% for SCA2 and SCA3, respectively)[12]. The Scale for Assessment and Rating of Ataxia (SARA) has been the cornerstone of disease analysis since its development and validation in 2004. The eight parameters used to determine the extent of ataxia using SARA are gait, stance, sitting, nose–finger test, rapid alternating arm movements, heel–shin slide test, speech impediment test, and finger chase test. Unlike older methodologies, SARA allows efficient and consistent analyses[13]. Besides the longer repeat length, several other variables have also been linked to a lower chance of survival in patients, including older age at inclusion or higher scores on the SARA[12]. SCAs are believed to affect 1–5 people per 100 000 people; however, this number varies greatly depending on the chosen area and ethnicity of the subjects[14]. In a study consisting of 361 ataxia families, SCA3 had the highest frequency (20.8%), closely followed by SCA2 (15.2%)[15]. In unrelated Korean populations, SCA2 has the highest expansion frequency[16,17], whereas in Taiwan and Singapore, SCA3 was observed to be the most prevalent[18]. However, most of these population surveys are conducted in discrete geographic locations, making an unbiased assessment of the worldwide prevalence of SCAs complex[14].

Diagnosis of SCAs 2 and 3

Diagnostic screening and methods of subtype identification

Molecular genetic tools, such as polymerase chain reaction (PCR) assays followed by either sequencing or high-resolution

electrophoresis-based partitioning, have emerged as reliable methods for identifying SCA subtypes[19]. However, expanded repeats pose specific challenges, such as reduced amplification efficiency and skewed results when run on gels. In standard PCR cases, the products display a ladder artifact characteristic of reduced amplification efficiency, making labelling necessary for their detection[20]. Another drawback of standard PCR is the possibility of failed amplification of the expanded allele. Several strategies have been proposed over the last decade to overcome these barriers, leading to better diagnostic potential. Touchdown PCR is a variant of standard PCR wherein the annealing temperature is set higher and then gradually lowered in subsequent cycles to just above the primer annealing temperature (Tm) (Fig. 1A). Condorelli *et al.*[21] developed a nonradioactive protocol using this method to facilitate the detection of SCA2 instances in large groups of ataxia patients. To simplify the detection process, a methodology involving PCR-based amplification of mutated repeats (CAG)n followed by Sanger sequencing was successfully tested and identified as an SCA1 pedigree[22]. In 2018, Cagnoli *et al.* developed another novel approach called tethering PCR, in which a reverse primer consisting of 15–16 base pairs of the disease gene-specific region, as well as the final five CTG units of the PolyQ encoding repeat, was 'tethered' to a forward fluorescent-labelled primer. This protocol was tested successfully for the rapid identification of specific SCA subtypes 1, 2, 3, 6, and 7 by trial and error-based optimization of the amount and mix of PCR reagents. In addition to its rapid nature, since tethering PCR results consist of an array of peaks whose pattern is indicative of zygosity, even carriers of the enlarged allele can be readily distinguished from normal homozygous individuals using this approach[23]. Despite these improvements, PCR-based diagnostic screening methods are relatively expensive. Considering these flaws, in 2016, Melo *et al.* proposed triplet repeat primer PCR (TP-PCR) as a complementary technique. The proposed TP-PCR reaction of 35 cycles involved the action of three primers, each with a specific function. The 1st primer was locus-specific, that is, MJD gene-specific (5′-TGG CCT TTC ACA TGG ATG TGA A-3′), providing directionality for DNA synthesis. Primers 2 and 3 were designed to have a common sequence (5′-TAC GCA TCC CAG TTT GAG ACG-3′), with primer 2 having an additional (CAG)5 sequence. The principle is that primer 2 anneals to numerous sites within the ATXN3 locus CAG repeat alleles during the first few cycles of TP-PCR until it runs out of the annealing sites, resulting in the amplification of various fragments. Owing to the stabilizing effect of the 5′ tail sequence, primer 3 binds to the ends of the products from the prior amplification cycles with high specificity[24]. A schematic of the tethering and triplet-primed novel PCR methods is presented in Figure 1.

To improve the cost-effectiveness parameter, a screening technique for SCA subtypes 1, 2, and 3 was developed by coupling the TP-PCR and Melting Curve Analysis (MCA) techniques, allowing for 100% assay sensitivity in the detection of the expanded repeats in the disease proteins Ataxin-1, Ataxin-2, and Ataxin-3 involved in SCAs 1, 2, and 3, respectively. Moreover, the specificity of these assays was near absolute, with the SCA2 and SCA3 assays being 99.46% and 99.32% specific, respectively[25]. The significance of these new techniques is paramount in the pre-symptomatic diagnosis of genetic diseases to facilitate better family planning options for at-risk individuals and to aid them in better preparation for future ailments[26]. In addition to these protocols, a clinical application of the diagnostic techniques was described by Moutou *et al.*, wherein the research group successfully developed a duplex PCR methodology to diagnose embryos obtained for in-vitro fertilization, establishing a pre-implantation genetic diagnosis of SCA2[27].

Novel biomarkers – the emerging importance of ophthalmological examination

Numerous neuroimaging and electrophysiological indicators of ataxic disease progression have been discovered using tools such as brain magnetic resonance imaging (MRI) and electromyograms, following significant research into the clinical and diagnostic biomarkers of ataxia[28,29]. Studies utilizing SCA2 patient fibroblasts have found a considerable increase in mitochondrial superoxide generation, further leading to increased intracellular hydrogen peroxide levels, implying that these cells exhibit a higher baseline level of chronic oxidative stress. This information is potentially useful as a marker of SCA2 at the cellular level[30]. Similarly, the assessment of several enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in serum samples collected from SCA3 patients has led to the establishment of a pro-oxidative stress state in SCA3[31]. Other unusual biomarkers, such as alterations in walking patterns and increased sensory latency periods, have also been identified in SCA2[32,33]. In subjects with pre-ataxic SCA2, subtle timing-specific speech perturbation and swallowing impairments were observed, making dysarthria and dysphagia potential pre-ataxic clinical markers. In addition, it is noteworthy that the existing SARA speech disturbance tests failed to detect any impairments in these pre-ataxic subjects[34]. In SCA3, differential expression of an exosomal microRNA (mir-7014) in the cerebrospinal fluid has been detected. In the analysis of this variability, mir-7014's downstream target gene regulatory capability was found specifically in the axonal guidance pathway (involving outgrowth, repulsion, and attraction of axons) and ER protein processing, both of which have been implicated in MJD pathogenesis. This discovery led to the examination of exosomal miRNA 7014 as a biomarker[35]. Cytoskeletal neurofilament (NfL) proteins present in neurons have been detected in serum and CSF after nerve damage. The levels of these neurofilament light proteins are elevated in both SCA2 and SCA3 subtypes. Moreover, in patients with SCA2, higher NfL levels were associated with worsening disease conditions. In addition, serum NfL quantification is a relatively simple assay that requires only a peripheral blood sample, making it a promising marker[36,37]. Although the severity of degeneration varies between distinct SCAs, a type-specific cerebellar cortex degradation pattern has been identified in SCAs 2, 3, and 7, even in the early stages. In SCA2, the cortex degeneration was limited to lobules VII–IX, whereas in SCA3, the medial anterior lobe (IV–V) and lobule VI were also affected. These results implicate the potential use of type-specific cerebellar cortex degradation as a unique biomarker[38]. In recent years, the emerging role of ophthalmological examinations in diagnosing and detecting SCA 2 and 3 has been recognized due to the presence of many visual abnormalities. This has been hypothesized to be due to neurodegeneration of the optic nerve and retina caused by ailments. In the causal diagnostic decision tree developed by Degardin *et al.*, ophthalmic examinations have been of great significance[39]. Saccades are quick, simultaneous movements of both eyes that aim to orient

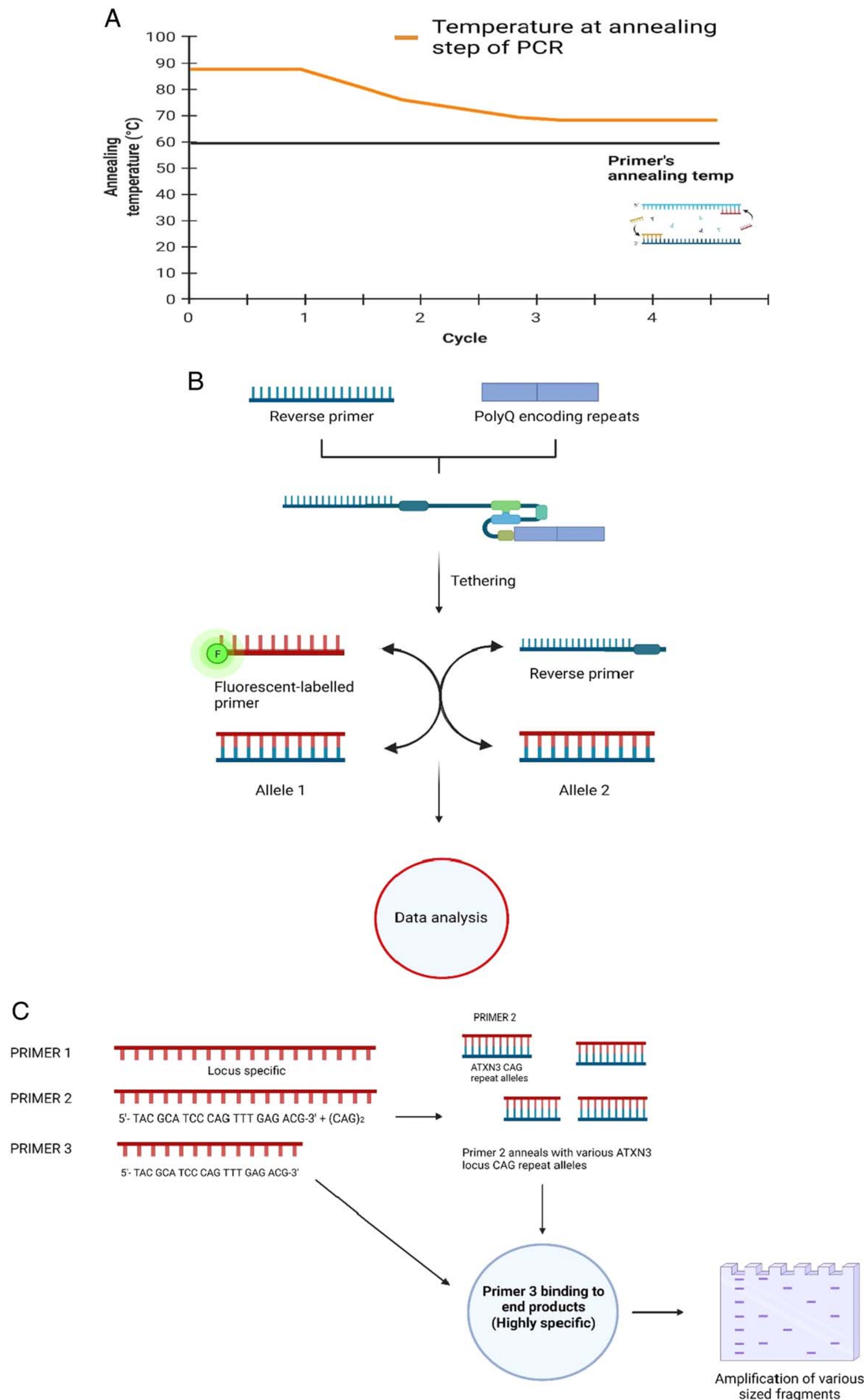

Figure 1. (A) A graphical depiction of the Touchdown PCR technique, wherein the primer's annealing temperature is used as a reference for setting up the temperature of annealing step and titrating down as the number of cycles increases. (B) Tethering PCR reaction schematic where a forward primer fluorescently labelled is tethered to a reverse primer, and both alleles are assayed. (C) Triplet primer PCR technique for ATXN3 alleles with the described primers 1, 2, and 3.

the fovea at any specific point, making it clearer[40]. In SCA2 patients, the maximum saccadic velocity (MSV) measured by electronystagmography was found to be ~149°/s compared to 415°/s in a normal control population. In addition, unlike ataxia scores, MSV measurements are altered even during the preliminary stages of the disease[41]. In another study, saccadic latency was demonstrated to be prolonged in SCA2 conditions. It has also been proven that saccadic velocity and latency are inversely related in SCA2 patients, suggesting that the delay in saccadic onset negatively impacts MSV readings[42].

Furthermore, in a cohort study of SCA2 patients, the heritability of increased saccade velocity was predominant within families with affected ancestry, indicating that this trait may be used as an endophenotype marker specific to SCA2[43]. Similarly, in SCA3, unique oculomotor deficits such as impaired smooth pursuit eye movement (SPEM), square-wave jerk (SWJ), and gaze-evoked nystagmus (GEN) have emerged as preclinical biomarkers. Pre-SCA3 patients also exhibited frequent gaze-evoked eye movements (GEEM), lower saccade velocity, and greater rates of anti-saccade mistakes, further highlighting the need to test visual acuity[44]. Ataxic dysarthria, the deficit in speech and swallowing caused by damage to cerebellar neural inputs[45], has been the cornerstone of pre-symptomatic manifestation in patients with SCA2. Similarly, the recognition of nystagmus as a pre-symptomatic manifestation of the SCA3/MJD condition[46] further reinforces the importance of ophthalmologic examination.

Clinical presentations

Characteristic manifestations of SCA2

Clinical symptoms, age of onset, and duration of illness differed significantly within and between SCA2-afflicted families. However, it is still unclear how ethnicity influences these parameters in SCA type 2 single-gene ailments[47]. Initially, patients often present with gait ataxia, tremor, and disrupted or slow speech patterns, as reported by Yang *et al*.[48]. Early SCA2 disease has also been recently characterized by white matter impairments, such as reduced axial diffusivity (AD) and fractional anisotropy (FA), and increased mean and radial diffusivities (MD and RD). These in-vivo modifications suggest the involvement of nerve damage, as white matter fibres enable the efficient transmission of information signals to neurons[49]. As the disease progressed, a broad range of symptoms began to appear. Several neuropathological indicators of SCA2, including nuclear degeneration, spinal cord demyelination, and loss of neurons, have been studied in depth and extensively reviewed by Magaña in 2012[50]. Other non-motor and non-ataxic presentations of SCA2, such as sleep disorders, chorea, redox imbalance, parkinsonism, dystonia, and depression, have been identified[51–53].

Characteristic manifestations of Machado–Joseph disease (MJD)

Similar to SCA2, the influence of ethnicity on disease presentation is apparent in MJD. However, in contrast to SCA2, the impact of patient ethnicity has been studied and characterized in MJD. After accounting for the aberrant trinucleotide repeat counts in *ATXN3*, Asians were 4.75 and 6.64 years older at illness onset compared to Caucasians and African Americans, respectively. In addition, African Americans suffered from much worse ataxia than Asians and Caucasians. Caucasian patients with SCA3 were more likely to suffer from depression than African Americans. These results pave the way for a better clinical study design[54]. Similar to SCA2-afflicted individuals, homozygous SCA3 patients also exhibit gait ataxia, tremor, dysarthria, peripheral neuropathy, and dysphagia[55]. Specific to the MJD condition, some significant presentations have been noted, including a disruption in the blood–brain barrier (BBB) as observed by a five-fold increase in the Evans blue dye concentration in the cerebellum of MJD transgenic mice when compared to wild-type mice. In addition, the tight junction proteins occludin and claudin-5 were abnormally cleaved and aberrantly oligomerized, respectively. Furthermore, a similar BBB disruption was noted in SCA3 human postmortem fixed tissue using fibrinogen extravasation, followed by immunofluorescence[56]. Sleepiness and obstructive sleep apnea (OSA) were also identified as specific manifestations of MJD[57].

iPSC lines generated to model SCAs 2 and 3: a compendium

iPSC lines have recently garnered the attention of researchers because of their ability to recapitulate the disease characteristics of SCAs *in vitro*[58]. Various sampling sites have been identified and somatic cells of different types have been successfully differentiated into pluripotent stem cells using numerous methods. The initial method of iPSC generation by reprogramming factor introduction involved the possibility of incorporating viral genes into the patient genome, leading to the development of either malignant or benign tumours[59]. Since then, great strides have been made in designing protocols for integration-free methods of iPSC generation and have been successfully utilized[60–62]. Herein, we compiled a catalogue of the existing pluripotent SCA2 and SCA3/MJD-specific stem cell lines, as shown in Table 1 and Table 2, respectively.

Research outcomes of iPSC-based modelling – uncovering impaired processes and proposing interventions

Since the pathogenic mechanisms of SCA types 2 and 3 are not well understood, patient-derived iPSCs provide an excellent base platform for testing novel drugs. In particular, SCA2-iPSCs can produce enhanced neurons, 70% of which express markers TUJ1 or MAP2, making them suitable for studying the mechanisms of pathophysiology *in vitro*[70]. To that end, Xia *et al*. generated neural stem cells (NSCs) from SCA2 patient-derived iPSCs and compared the differentiation process to that in control iPSCs. They found an interesting development of neurosphere clumping into cyst-like structures instead of forming neural rosettes as the control iPSCs did. Surprisingly, when the expression levels of Ataxin-2 were examined in SCA2 iPSC-derived NSCs, they were found to be significantly lower than those in normal NSCs[79]. This finding is significant because the reduced level of Ataxin-2 expression has been hypothesized to be one of the mechanisms involved in SCA2, even during embryonic developmental stages[80]. Several pathways, such as pain signalling and potentiation, are disorganized in SCA2 iPSC-derived neuronal populations. One of the significantly affected pathways was found to be the glutamate signalling pathway. Koch *et al*. first demonstrated the role of glutamate as an excitatory neurotransmitter that causes an increase in intracellular calcium ion flux, which further leads to calcium-dependent proteolysis of the disease protein Ataxin-3. The consequence of proteolysis is SDS-insoluble aggregate formation, which is a hallmark of SCA3[81]. This discovery led to a deeper investigation into the glutamate signalling pathway in MJD iPSC-derived neurons by Chuang *et al*., using the transcriptome profiling method. The analysis revealed a severe downregulation of glutamate receptor genes (GRIA4 and GRM3 in SCA2 neurons). Additionally, long-term (60 days) treatment of the cells with glutamate led to detrimental effects, as evidenced by higher cell death in the iPSC-derived neural cell population, for which the cause was reported to be mitochondrial damage owing to their bioenergetic capability. These discoveries

Table 1

Spinocerebellar ataxia 2 patient-specific pluripotent stem cell lines.

iPSC lines generated from spinocerebellar ataxia 2 patients:

Cell line name + unique ID	Sex of the cell	Age at sampling (years)	Sampling site	Hierarchy of cell line	Repeat length of mutant allele	Reference and remarks
H196 C7, CVCL_AB81	Male	52	Skin (fibroblasts)	Children: CVCL_DQ63 (H196 C7 GC)	36	[63]An intact genome with no abnormalities was confirmed by karyotyping.
H196 C7 GC, CVCL_DQ63	Male	52	N/A: Gene corrected line	Parent: CVCL_AB81 (H196 C7)	22	[64]The 36 CAG repeats are replaced by a normal 22 repeats by using the CRISPR/Cas9 system of gene correction. Control cell line established.
H266 C10, CVCL_AB82	Female	25	Skin biopsy	Children: CVCL_DQ64 (H266 C10 GC)	44	[65]Upon embryoid differentiation, the generated iPSCs were capable of forming derivatives of ecto, endo, and mesoderms.
H266 C10 GC, CVCL_DQ64	Female	25	N/A: Gene corrected line	Parent: CVCL_AB82 (H266 C10)	22	[66]The 44 CAG repeats are replaced by a wild-type 22 repeat by using the CRISPR/Cas9 system of gene correction. Control cell line established.
H271 C1, CVCL_9U78	Male	33	Skin biopsy	Children: CVCL_DQ65 (H271 C1 GC)	44	[67]
H271 C1 GC, CVCL_DQ65	Male	33	N/A: Gene corrected line	Parent: CVCL_9U78 (H271 C1)	22	[68]The 44 CAG repeats are replaced by a wild-type 22 repeat by using the CRISPR/Cas9 system of gene correction. Control cell line established.
(1) CHOPi002-A, CVCL_UL69 and (2) CHOPi003-A, CVCL_UL70	Males	58, 28 respectively	Peripheral blood	N/A	33, 43 respectively	[69]Method of generation: transduction with a Sendai virus.
(1) iSCA2-17, CVCL_XI51 and (2) iSCA2-28, CVCL_XI52	Male	57	Skin (Dermis)	Originate from the same individual	35	[70]Retrovirus-based method of generation.
(1) iSCA2E209-6, CVCL_XI53 and (2) iSCA2 E209-9, CVCL_XI54	Female	36	Peripheral blood	Originate from the same individual	44	[70]Sendai virus-based reprogramming of PMBC parent cells.

Table 2

MJD/SCA3 patient-specific pluripotent stem cell lines.

iPSC lines generated from Machado–Joseph disease (SCA3) patients:

Cell line name + unique ID	Sex of the cell	Age at sampling (years)	Sampling site	Hierarchy of cell line	Repeat length of mutant allele	Reference and remarks
ZZUNEUi002-A, CVCL_YB85	Male	22	Urine (urinary epithelial cell)	N/A	84	[71]The generated cell line seems to be integration-free after 20 passages.
(1) SCA3.A8, CVCL_VE47 and (2) SCA3.A11, CVCL_DQ98	Male	17	Dermal fibroblasts	Derived from the same individual	83 in both	[72]The expression of pluripotency genes namely OCT4, NANOG, SOX2, and LIN28 was upregulated in these iPSCs compared to patient fibroblasts.
(1) SCA3.B11, CVCL_DQ99 and (2) SCA3.B1, CVCL_VF01	Male	58	Dermal fibroblasts	Derived from the same individual	74, 78 respectively	[73]Method of reprogramming: Electroporation with 3 episomal plasmids.
CSUXHi005-A, CVCL_A4GP	Female	32	Urine (exfoliated epithelial cells)	–	76	[74]At the sampling time, the patient presented with progressive SCA3 symptoms.
CSUXHi001-A, CVCL_XI42	Female	12	Urine cells (UCs)	N/A	74	[75]At sampling, the donor was not affected with SCA3 yet. UCs reprogrammed by transfection with oriP/EBNA episomal plasmids carrying a combination of reprogramming factors.
(1) iSCA3 E149-10, CVCL_XI57 and (2) iSCA3 E149-12, CVCL_XI58	Female	39	Skin	Derived from the same individual	73	[70]The other allele has only 14 repeats.
IBCHi002-A, CVCL_XC41	Male	44	Skin	Parent: CVCL_7452 (GM06153)	70	[76]Sendai virus-based reprogramming of human SCA3 fibroblasts to provide a platform for initial drug efficacy testing and analysis.
(1) iSCA3-1, CVCL_XI55 and (2) iSCA3-36, CVCL_XI56	Female	38	Dermis	Derived from the same individual	70	[70]The other allele has 37 repeats Method: Sendai virus-based reprogramming of dermal SCA3 fibroblasts.
ZZUi014-A, CVCL_WN18	Female	65	Epidermis of forearm	N/A	64	[77]Episomal plasmids used in the generation of this cell line seem to have vanished after passage 15.
MUSIi004-A, CVCL_VN11	Female	48	Dermis (HDF)	–	Not mentioned	[78]The HDFs were reprogrammed by nucleofection with episomal plasmids, which express OCT4, SOX2, KLF4 and LIN28, hL-MYC, EBNA-1 and also shRNA against p53.

are significant as they suggest a possible therapeutic opportunity – targeting the glutamate pathway using anti-glutamate drugs[70]. A schematic representation of these findings is shown in Figure 2.

Likewise, iPSC modelling of SCA3 has been successful owing to the presence of mutant *ATXN3* (same as the parental somatic cells) and perfect expression *in vitro*, encouraging pathophysiological studies[79]. In SCA3, the mutant of Ataxin-3 has been investigated for its ability to aggregate into neuronal nuclear inclusions. The molecular chaperone and ubiquitin-proteasome systems might remove misfolded proteins in cells, and upon failure of these processes, Ataxin-3 aggregation is triggered[82]. In addition, chaperone-mediated autophagy is a key process in the degradation of almost 30% of cytosolic proteins[83]. In a cohort of primary fibroblast cells isolated from MJD patients, the protein and mRNA levels of Beclin-1, which plays an essential role in the initial vesicle nucleation step of autophagy, were suppressed[84]. Dysregulation of autophagic pathways has been discovered in differentiated and undifferentiated SCA3 patient-derived human IPSCs, and upon addition of rapamycin to the neural differentiation medium to promote autophagy, the amount of mutant protein was found to be significantly reduced, demonstrating an analeptic role for the upregulation of autophagy in SCA3[82]. Further scrutiny of autophagic dysregulation has revealed the overexpression of miR-340 and miR-543 in iPSC-derived self-renewing neuroepithelial-like stem cells (termed It-NES cells), both of which work in tandem to reduce the expression of DNAJB1, a co-chaperone that aids in minimizing PolyQ protein toxicity[85]. In a similar setting, glutamate treatment has been shown to induce PolyQ aggregates in neurons, but not in neural progenitor cells, suggesting a central role for chaperone network reorganization upon differentiation in the pathogenesis of SCA3. In addition, the expression of DnaJ homologue subfamily B member 6 (DNAJB6) in iPSC-derived neurons was profiled and found to be almost nonexistent. siRNA-mediated knockdown of DNAJB6 in neural progenitor cells made them susceptible to aggregate formation, whereas re-expression of DNAJB6 in neurons had an antagonistic effect on PolyQ aggregate formation[86]. These studies imply a pivotal role of chaperone dysregulation in the pathophysiology of SCA3. In an attempt to explain the neuron-specific nature of SCA3, Koch *et al.* stimulated MJD-derived neurons with L-glutamine and monitored the influx of Ca^{2+} into neurons. As a result, they observed cleavage of the Ataxin-3 protein upon influx of ions, leading to the formation of SDS-insoluble aggregates. A treatment strategy was developed to detect the involvement of specific proteases. The calpain inhibitors ALLN and Calpeptin were administered at molar concentrations of 100 mM and 200 mM, respectively, to the neurons. Consequently, these agglomerations disappeared. Moreover, this SDS-insoluble aggregate formation seems to be limited to neurons, as attempts to induce SDS-insoluble aggregate formation in other iPS-derived cells such as fibroblasts or glia were unsuccessful[81]. Regarding the treatment of SCA3, progress has been made in identifying some therapeutic opportunities using patient-derived iPSCs, one of which is CRISPR/Cas9-based gene editing as shown in Figure 3. He *et al.* designed and produced two small guided RNA nucleases, one for each protospacer adjacent motif (PAM) region on either stream of the abnormal CAG repeats located on exon 10 of *ATXN3* and succeeded in mutant allele correction, consequently producing corrected SCA3-iPSCs. In these corrected cell lines, several disease characteristics such as increased Ca^{2+} levels, higher amounts of reactive oxygen species generation, and increased MDA levels were rescued, pioneering an approach for autologous stem cell-based treatment for SCA3[87]. However, an issue regarding this finding is that there are no studies exploring whether the truncated Ataxin-3 generated via the nonhomologous end joining (NHEJ) method is

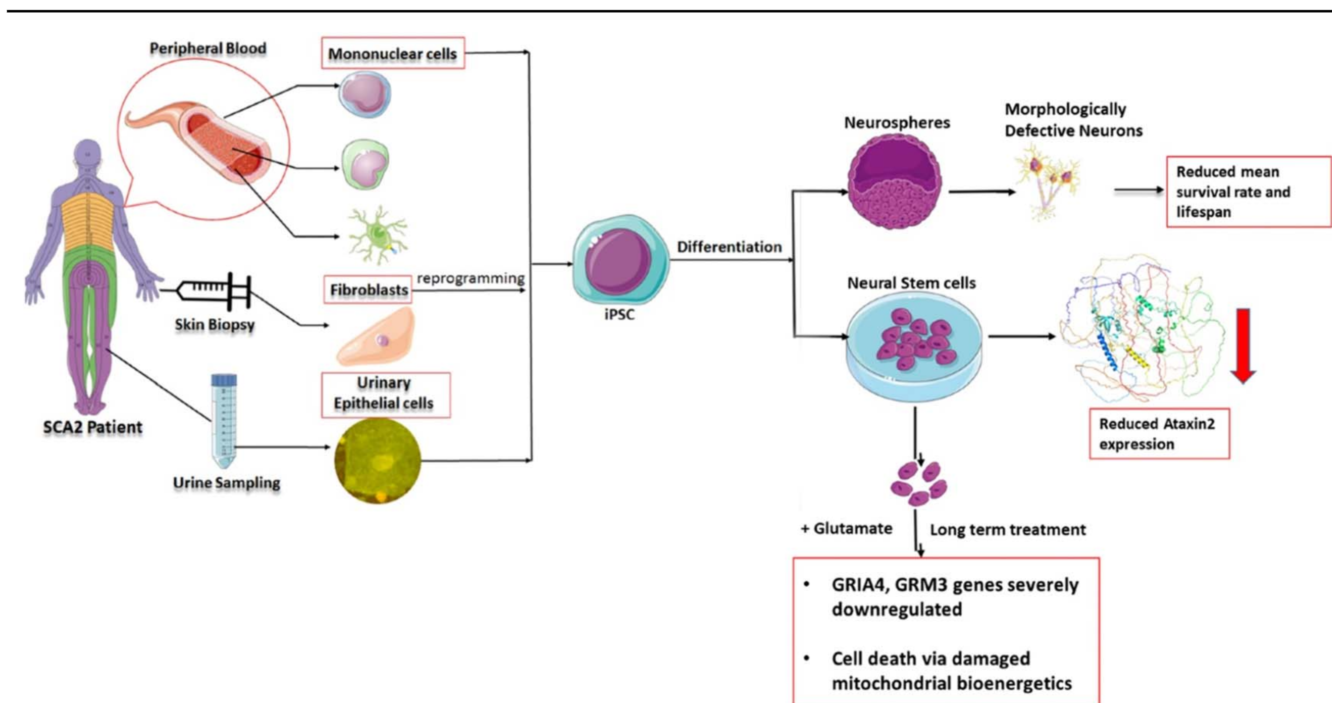

Figure 2. A graphical representation of the outcomes of SCA2 patient-specific iPSC-based modelling.

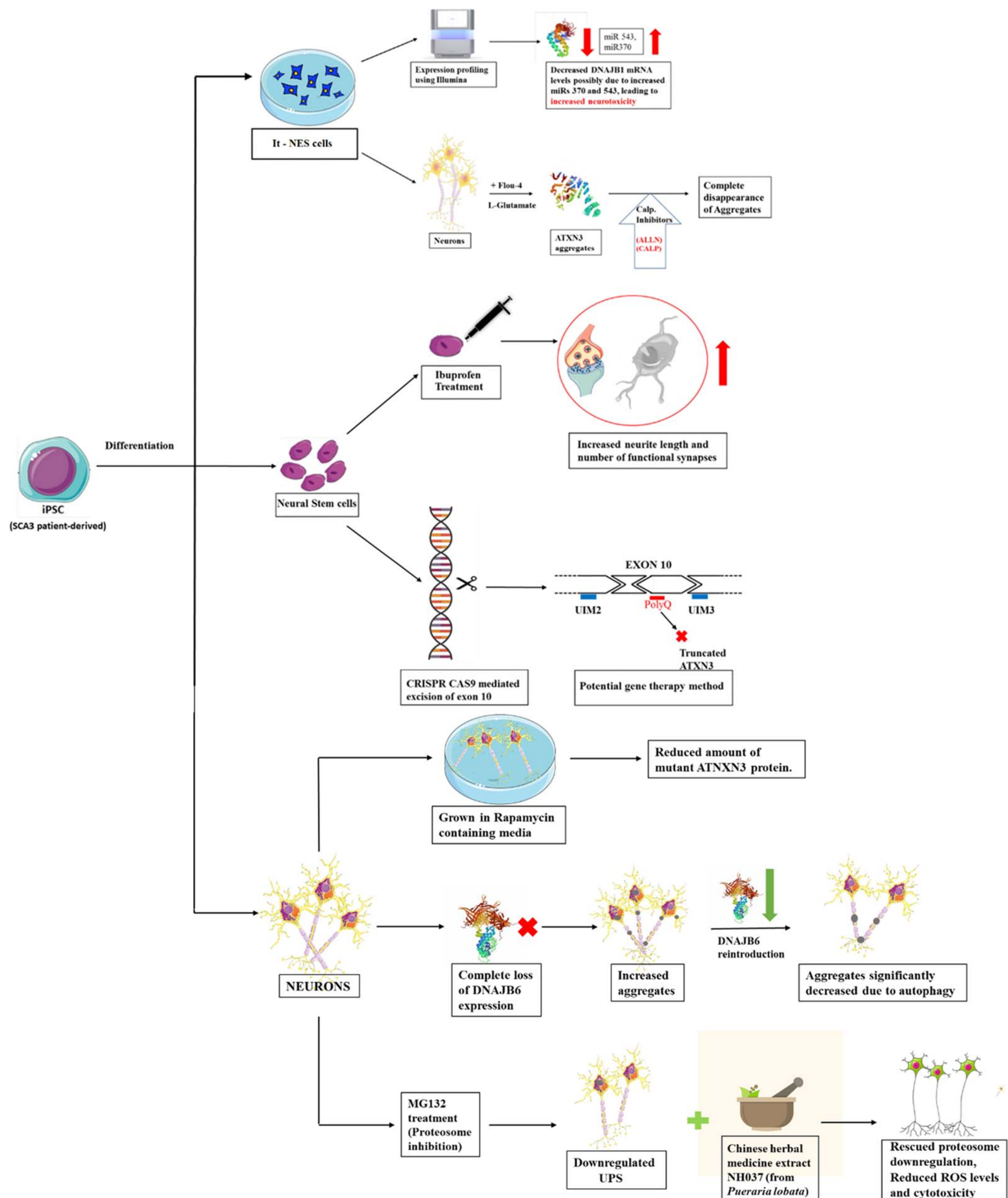

Figure 3. Illustration of some impaired processes discovered in various cell types and potential ways to rescue them in case of Machado–Joseph disease (MJD).

capable of and is performing the normal functions of Ataxin-3. To improve upon this technique, Ouyang *et al.* deleted the pathogenic expanded CAG repeats using two guide gRNAs inserted into the pSpCas9 (BB)-2A-GFP cloning vector and joined the remaining sequences, producing two cell lines. Glutamate treatment of SCA3-NCs derived from these lines did not cause agglomerate formation, suggesting that these cells may have an increased cleavage/clearance rate of Ataxin-3[88]. Furthermore, neuroprotective applications of some repurposed drugs have been tested in iPSCs, resulting in positive outcomes. Treatment of MG132 proteasome inhibited MJD iPSC-derived neurons with the Chinese herbal medicine extract NH037 obtained from the

plant species *Pueraria lobata* and daidzein, an element of the extract, led to normalized proteasome activities as well as reduced cytotoxicity and ROS levels[89]. Neural cell cultures incubated with ibuprofen had 2.08–0.36 times more excitatory synapses than control[90].

Hurdles in iPSC-based modelling and future scope of research

Although numerous studies have successfully used iPSC lines as SCA disease models and investigated the pathophysiology of SCA types 2 and 3, the methods used are far from perfect. For instance, SCA2 is linked to an increased APP exon 7 inclusion, as visualized in a human postmortem SCA2 cerebellum. However, the inclusion patterns were identical in both control and SCA2iPSC APP exon 7[91]. This observation is indicative of undiscovered phenotypic differences between iPSCs and diseased cells. Aging induces elevated macroaggregates in SCA, but the present in-vitro models may not have been long enough to create detectable amounts of agglomerations. In addition, no studies have explored the effects of mutant *ATXN3* on glutamate receptor genes such as GRIA4 and GRM3[70]. CRISPR/Cas9-based treatment also has some disadvantages, such as diminished cell viability and lower gene-targeting efficiency, making it more difficult to visualize phenotypic amelioration in treated cells[87]. Experiments using human iPSCs for disease modelling could have some reproducibility issues owing to gene function heterogeneity arising from donor genetic differences[88]. The unavailability of live neurons from patients to study the molecular aspects of neurodegeneration might also be a hindrance to therapeutic development[89]. Moreover, the establishment of standardized biomarkers for the purpose of being use in clinical trials in biofluids such as cerebrospinal fluid or plasma remains elusive[92]. Gene editing to produce shortened coding sequences of diseased genes could potentially contribute to mitochondrial dysfunction, as seen in a truncated variant of *ATXN3*. However, if gene-editing products using CRISPR/Cas9 systems are lethal, they are unknown[93]. As with many other multifaceted disorders, the effects of SCAs on organ systems have not yet been studied using iPSC lines. Despite some of these barriers, iPSC modelling could become a revelation in the near future. If successfully generated, brain organoids derived from iPSCs with relevant disease mutations will likely provide greater information on developmental pathways and selective neuronal sensitivity. Furthermore, because they deliver patient-specific effectiveness, screening compounds with iPSCs has significant benefits over immortalized cells or small animal-based screening approaches[94]. Overall, current research suggests a pivotal role for iPSCs in autologous personalized therapy for SCA types 2 and 3 in the imminent future.

Ethical approval

Ethics approval was not required for this review.

Consent

Informed consent was not required for this review.

Sources of funding

No funding was received for this review.

Author contribution

All authors contributed equally to this paper and equally approved the submission. N.R.: conceptualization and writing of initial draft; G.D.M.: writing, editing, and revision of drafts; S.S.: visualization and writing.

Conflicts of interest disclosure

The authors declare no conflicts of interest.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Goshen David Miteu.

Data availability statement

Not applicable.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Acknowledgement

Images were created using BioRender. Servier Medical Art (distributed under Creative Commons Attribution 3.0 Unported license) was also used to create illustrations.

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