Optic atrophy in Wolfram syndrome - Its pathophysiology and potential treatments

Open University dissertation by Georgina Carolan

Abstract

Introduction:

Wolfram syndrome (WS) is an extremely rare neurodegenerative disease, manifesting in four main conditions: diabetes insipidus (DI), diabetes mellitus (DM), optic atrophy (OA), and deafness (D) (DIDMOAD). Research has found the underlying cause of these conditions to be endoplasmic reticulum (ER) dysfunction and stress; the ER being an organelle responsible for functions in the cell such as protein folding and calcium storage. The localisation of the gene product of the WFS1 gene, called Wolframin, to the ER membrane has helped researchers to understand the pathophysiology of WS and identify targets for treatments.

WS has an autosomal recessive pattern of inheritance, and it is estimated that 1 in 770,000 individuals in the UK are affected. The WFS1 gene was localised to chromosome 4p.16.

Main:

The focus of this literature review is optic atrophy, whereby the optic nerves connecting the eye and the visual cortex in the brain are degenerated due to the ER stress in what are known as retinal ganglion cells (RGCs).

Understanding this pathophysiology has led to the development of ongoing drug trials which target different stages of the ER-stress cascade. New treatments, based on gene technology and stem cells, are currently being trialled using patient-derived fibroblasts, in order to provide both models on which to test treatments and potential replacement tissues for repair of damage.

The early results of one clinical drug trial, using the drug dantrolene, have not shown significant improvements in visual acuity following six months of treatment. Another drug trial, of sodium valproate, is still underway, lasting three years.

Conclusion:

Many hopes are being invested in gene technology to provide a treatment or even a cure for WS, with therapies such as MANF (mesencephalic astrocyte-derived neurotrophic factor) showing optimism. New treatments are needed to improve patients' quality of life.

295 words

Key terms: Wolfram syndrome, Optic atrophy, Retinal ganglion cells, Gene therapy, Stem cells

Contents:

List of acronyms:

4BPA= 4-phenylbutyric acid
AAV= Adeno-associated virus
ATP= Adenosine triphosphate
CRISPR= Clustered regularly interspaced short palindromic repeats
DIDMOAD= Diabetes insipidus, diabetes mellitus, optic atrophy, and deafness
ER= Endoplasmic reticulum
ERSE= Endoplasmic reticulum stress-response element
IP3R= Inositol triphosphate receptor
IPSCs= Induced pluripotent stem cells
LogMAR= Logarithm of the Minimum Angle of Resolution
MAMs= Mitochondria-associated membranes
MANF= Mesencephalic astrocyte-derived neurotrophic factor
NAD+= Nicotinamide adenine dinucleotide
NCS1= Neuronal calcium sensor-1
OCT= Optical coherence tomography
RGCs= Retinal ganglion cells
RyRs= Ryanodine receptors
TUDCA= Tauroursodeoxycholic acid
VPA= Valproic acid (sodium valproate)
WS=Wolfram syndrome
WSUK= Wolfram syndrome UK (charity)

List of figures and tables:

1. <u>Introduction:</u>

1.1- Background:

Wolfram syndrome (WS) is an extremely rare neurodegenerative disease that affects an estimated 1 in 770,000 of the adult UK population (Ganie et al, 2009). Its primary features are diabetes insipidus (DI) (an endocrine condition where the pituitary gland does not produce antidiuretic hormone), diabetes mellitus (DM), optic atrophy (OA) and deafness (D); hence its other name DIDMOAD (Mishra et al, 2021). Other signs and symptoms can include gastrointestinal problems and neurological dysfunction (Cagalinec et al, 2016).

Though it is such a rare condition, it is increasingly being researched as a "mechanistically homogenous" model of endoplasmic reticulum (ER) stress (Lu et al, 2014). Understanding the pathology of WS may lead to better treatments for more common ER diseases such as Alzheimer's and atherosclerosis. WS is an especially good model of ER stress as it is monogenic, meaning it is caused by a single gene, making it easier to study (Lu et al, 2014).

Research, such as that being undertaken by Abreu et al (2019) is seeking to identify targets and an effective treatment to slow down the progression of WS. One avenue of pursuit has been gene technology, especially targeting the retinal ganglion cells (RGCs) that are affected by the loss-of-function WFS1 mutation. Such gene therapy techniques have shown promise so far in induced pluripotent stem cell (IPSC) and rodent models of WS (Mishra et al, 2021). There are also ongoing clinical drug trials taking place internationally, including that of sodium valproate, being conducted in the UK (Clinicaltrials.gov).

1.2- Objectives:

1) To explain the pathophysiology that underlies optic atrophy specifically in WS (Contextualisation)
2) To describe what WS is: its pattern of inheritance, other signs and symptoms, cell biology and diagnosis (Contextualisation)
3) To describe and evaluate the current treatment options for OA in WS (Contextualisation, development)
4) To critically analyse available literature detailing potential treatments or therapies (Development, application, analysis)

5) To evaluate in which direction research and treatments could go in the future (Where next?)

1.3- Scope:

This review focuses on optic atrophy (OA), whereby the optic nerve is degraded, leading to declining levels of vision. The pathophysiology underlying OA is similar to that which causes other types of cell dysfunction in the other affected tissues in WS. The potential treatments for OA and WS in the pipeline are also explored.

1.4- Methodology:

I have made use of databases such as PubMed and the OU library to search for articles relevant to my literature review. Making use of Boolean terms such as 'AND' when searching, for example Wolfram syndrome AND optic atrophy, has refined my search results. I have also found many relevant articles through reading the references cited in articles, finding many of the authors in my reference list were co-authors in other papers.

I have made use of PROMPT to evaluate the usefulness and quality of articles I find, with the aim of making my literature review well researched and referenced.

2: What is Wolfram syndrome? (Obj2):

2.1- Signs and symptoms:

Wolfram syndrome (WS), also known by the acronym DIDMOAD, for its constituent conditions of diabetes insipidus, diabetes mellitus, optic atrophy and deafness, is an extremely rare neurodegenerative condition. It is estimated to have a prevalence of just 1 in 770,000 in the UK population (Ganie et al, 2009).

Feature	Prevalence
Diabetes mellitus	100%
Diabetes insipidus	72%
Optic atrophy	100%
Deafness	66%

Figure 1- The prevalence of the signs of WS (adapted from Barrett et al, 1995)

Affected individuals do not necessarily have all four of the main symptoms of WS, with an estimated 50% having the full DIDMOAD phenotype (de Heredia et al, 2013). As shown by the data in figure 1 collected by Barrett et al (1995), diabetes insipidus occurred in 72% of cases, out of a group of 29 patients with WS. 100% of cases have optic atrophy and diabetes mellitus, both of which are required for a clinical diagnosis of WFS1. Other conditions that can also occur in WS include neurological dysfunction and gastrointestinal issues (Abreu et al, 2019). DM or OA are not always the first clinical symptoms of WS to appear, with DM on average occurring in the first decade and OA before the second (de Heredia et al, 2013).

In a patient-reported survey conducted in 2018, optic atrophy and the resulting visual impairment was reported as the most important symptom affecting quality of life among 48 patients living with WS in the UK (Barrett, 2018 (unpublished)). Feedback from patients highlight how urgent a treatment or cure for OA in WS is.

Optic atrophy is one of the most progressive of the four conditions in terms of deterioration over time. It does not occur exclusively in WS, with other types being predominantly caused by mitochondrial dysfunction (Mishra et al, 2021). This has added to the argument that WS has underlying mitochondrial pathology.

2.2-Inheritance:

The causative gene for Wolfram syndrome, WFS1, was localised to chromosome 4p.16 (Inoue 1998, Takeda et al, 2001). The most common type of WS is caused by mutations in this WFS1 gene, accounting for 90% of cases, with the other 10% being WFS2 (Khanim et al, 2001). Wolframin is formed of 890 amino acids, of which 309 different mutations have been identified (Hu et al, 2021). Such mutations include missense and frame-shift mutations, where the reading frame is shifted due to the insertion or deletion of one or more nucleotide pairs (Delvecchio et al, 2021).

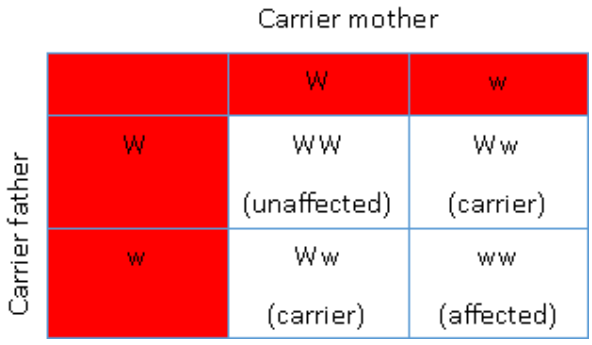

Figure 2- Punnett square showing autosomal recessive inheritance for WS

The syndrome comes with a spectrum of phenotypes, with no two patients affected in the same way. Its autosomal recessive mode of inheritance means that there is a 1 in 4 chance of two asymptomatic carrier parents having an affected child, as shown in the Punnett square in figure 2. Autosomal refers to any of the chromosomes other than the X and Y sex chromosomes (Hine, 2019). Unfortunately, despite updates in genetic technology, it is not possible to predict a phenotype and prognosis even if the genotype is known. Several studies are ongoing to try and establish a genotype-phenotype correlation (Chaussenot et al, 2011). Recent research has put the prevalence of heterozygous carriers of the recessive WS gene as 0.3-1% (Fischer et al, 2020).

Recently, it has been found that the pattern of inheritance of WFS1, together with the type of mutation, can affect both the onset and severity of the clinical features of WS (Delvecchio et al, 2021). In a recent study, it was found that patients with compound heterozygous nonsense mutations in the WFS1 gene had no detectable WFS1 protein expression (Hu et al, 2021). This was found to cause a more severe phenotype, with worse visual acuity. Conversely, those with missense mutations had greater Wolframin protein expression, leading to milder phenotypes.

3-What is optic atrophy? (Obj1):

3.1- Signs and symptoms:

Optic atrophy (OA) involves a degeneration of the axons of the optic nerve, which can be due to several causes (Barry et al, 2017).

Barrett et al. (1995) found an average age of onset of OA in WS patients to be 10-11 years old. The condition usually presents with deteriorating colour vision, visual field deficits (including scotoma- a 'blind spot') and a decrease in so-called 'best-corrected visual acuity', through the wearing of spectacles (Mishra et al, 2021). In a cohort study assessing visual acuity decline in WS, O'Bryhim et al (2022) found a mean slope of decline of 0.059 LogMAR per year. This is similar to Hoekel et al's (2018) study, which found a mean LogMAR score of 0.66 in their cohort of 23 patients. As shown in figure 4, LogMAR score decreased with age, indicating a decline in visual acuity.

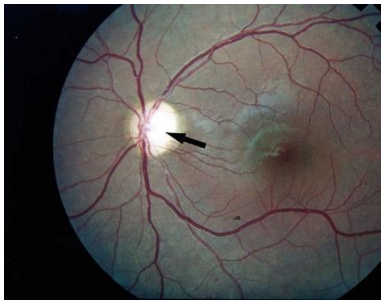

Figure 3- Photograph of the pale optic disc against the retina (wikimedia commons, 2009)

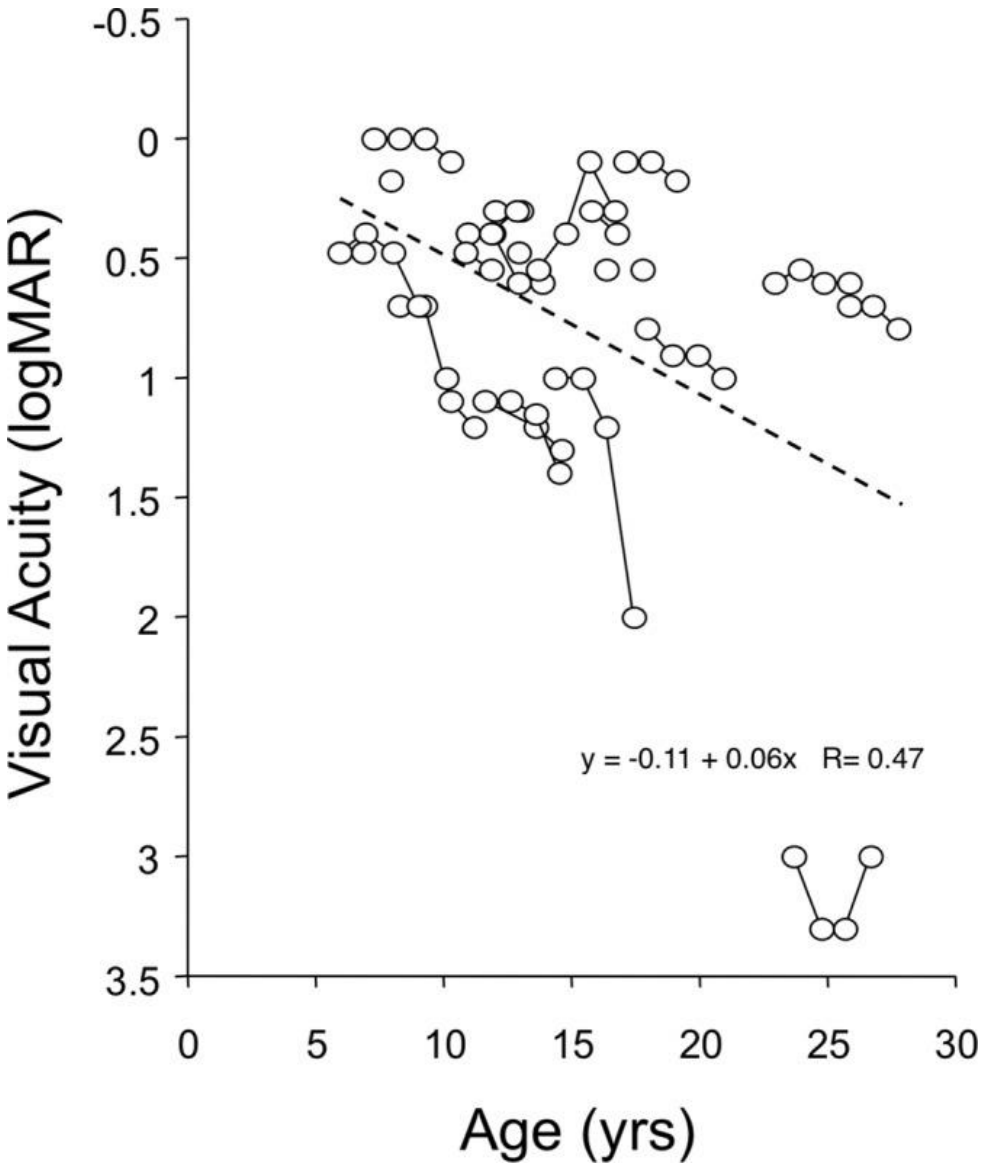

Figure 4- The decline of visual acuity (LogMAR score) with increasing age in a cohort of 23 patients (Hoekel et al 2018)

Other ophthalmic conditions can also occur in WS including nystagmus and cataracts. The nystagmus is a result of cerebellar degeneration in later stages of WS, because of neurodegeneration (Barrett et al, 1997). They also reported an absence of pupillary reflexes in blind patients and a surprising absence of diabetic retinopathy, despite the median duration of DM of 24 years.

Histologically, Schmidt-Kastner et al (2009) localised Wolframin to the human retina, notably the RGCs, optic axons and the proximal portion of the optic nerve. Interestingly, they found that Wolframin expression was 'virtually

absent' in the myelinated portion of the optic nerve, giving some clues as to the increased vulnerability of the unmyelinated optic nerve, where Wolframin would normally be expressed. Furthermore, a later study by Hoekel et al (2018) found that WS is associated with lower myelination throughout the brain. Whilst Schmidt-Kastner (2009) made used of histological techniques such as immunolabelling, Hoekel et al (2018) used diffusion tensor MRI-derived fractional anisotropy to investigate the microstructure of RGCs.

3.2- Diagnosis:

In addition to visual field analysis, fundoscopic examination, retinal nerve fibre layer (RNFL) imaging and optical coherence tomography (OCT) can also be used to diagnose and monitor OA in patients (Hoekel et al, 2018). RNFL thinning is associated with deterioration of visual acuity in WS as is the increasing pallor of the optic disc, as shown in figure 3, where the pale optic disc is clearly visible (Zmyslowska et al, 2017). Battista et al (2022) made use of OCTA (OCT angiography) to measure vascular parameters in the optic nerve head such as perfusion density.

Alongside these methods, Barboni et al (2022) also used MRI (magnetic resonance imaging) to look at the morphological features of the visual pathways in OA in WS. This allows imaging of areas such as the optic chiasm, where the two optic tracts cross. In a study by Zmyslowska et al (2019) it was found that the optic chiasm and visual tracts had significantly reduced intracranial thickness in patients with WS, compared to those with type one diabetes. This control group is beneficial as they share some of the pathophysiology of WS patients, such as varying blood glucose levels, without having the underlying optic atrophy.

In a study assessing the correlation between the degree of WFS1 gene expression and clinical progression of OA, milder visual acuity impairment was found in those who had some degree of Wolframin protein expression. Heterozygous nonsense mutations in WFS1 were found to have no WFS1 protein expression, leading to worse visual prognosis compared to those with

partial WFS1 expression. Milder visual acuity impairment was defined in this context as colour deficiency or asymptomatic, whilst severe visual acuity impairment had a LogMAR score >1.0 (Hu et al, 2021).

4- The underlying pathophysiology of optic atrophy in WS (Obj1):

4.1- The involvement of the endoplasmic reticulum:

With its monogenic ER dysfunction pathology, WS provides a model for understanding more common ER diseases such as Alzheimer's and atherosclerosis (Lu et al, 2014). Hence, if WS can be treated, there is potential for new therapies for more common diseases to also be developed. This has provided financial incentives for drug companies.

The ER are organelles in the cell which are involved in a number of critical functions including calcium storage, protein folding and redox regulation (Pallotta et al, 2019). The ER have been identified as being involved in the pathophysiology of WS through the identification of the WFS1 gene product Wolframin to the ER membrane, acting as an ion channel controlling the influx and efflux of Ca^{2+} ions in particular (Mishra et al, 2021).

In WS, OA results from the vulnerability of the retina at the back of the eye to the effects of ER stress. Retinal ganglion cells (RGCs) have been found to be particularly vulnerable to this pathological condition, due to their high expression of the protein Wolframin (Mishra et al, 2021). The protein is highly expressed in the retina, especially in the inner segments of the photoreceptors (rods and cones), the inner nuclear layer (INL) and the papillomacular bundle (PMB) (Mishra et al, 2021). The identification of cells and tissues in the eye affected by WS through histopathology has enabled both the monitoring of disease progression and the establishment of biomarkers for assessment of the effects of clinical trials (Zmyslowska et al, 2017).

In all cells, a mechanism of the ER known as the unfolded protein response (UPR) occurs in response to unfolded and abnormally folded proteins, causing their degradation (Mishra et al, 2021). Wolframin is involved in the UPR as it negatively regulates a protein called ATF6α (activating transcription factor 6α) (Pallotta et al, 2019). In WS, a deficiency of Wolframin causes increased ATF6α signalling, leading to upregulation of the UPR and increased apoptosis (Programmed cell death) (Mishra et al, 2021). This is summarised in figure 5, showing the different components of the UPR and the ER chaperones (Pallotta et al, 2019).

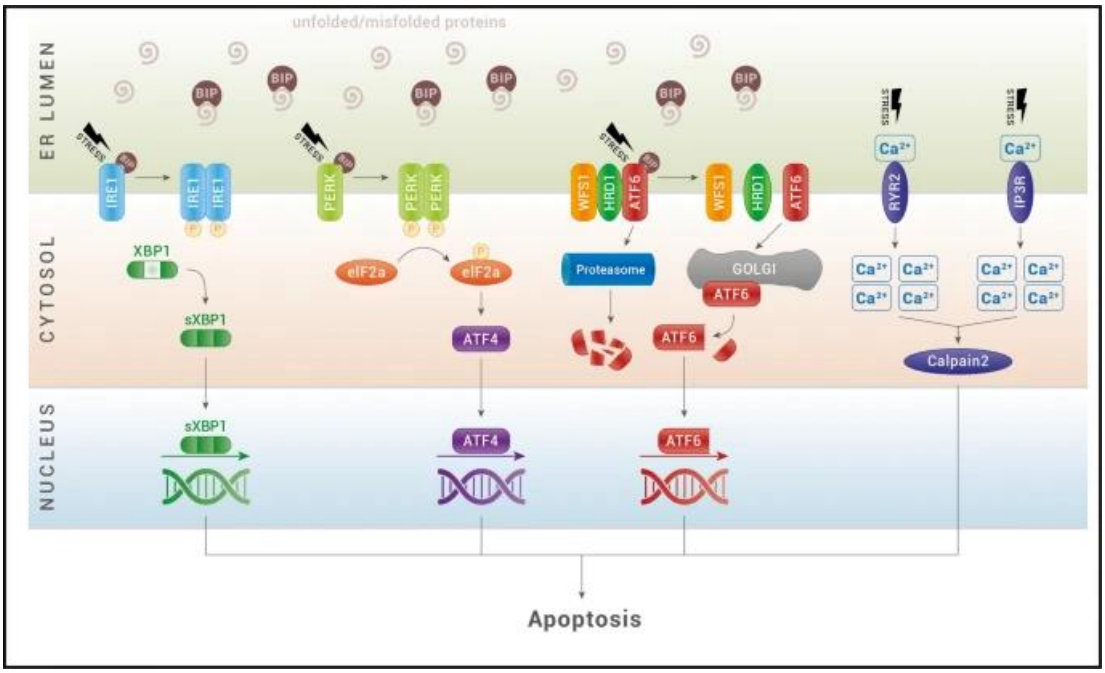

Figure 5- The unfolded protein response (UPR) in the endoplasmic reticulum under conditions of ER stress in WS (from Pallotta et al, 2019)

The UPR is normally beneficial in healthy cells, helping the cell avoid situations where protein folding capacity is exceeded. However, under situations of chronic and pathological stress, such as in WS, it is unable to achieve homeostasis, leading to apoptosis (Mishra et al, 2021).

Another protein, called calpain-2, is also involved in the UPR and apoptosis in WS, again shown in figure 5. Calpain-2 has been demonstrated to be hyperactivated in WFS1 knockout mice and neural progenitor cells (derived from IPSCs) in a study by Lu et al (2014). It is hypothesised that by targeting the pathway leading to the activation of calpain-2, it may provide a therapy for WS and other ER diseases.

Another organelle, the mitochondria, is closely linked to the ER via what are known as mitochondria-associated membranes (MAMs) (Delprat et al, 2018). These channels facilitate communication between the two organelles and allow Ca^{2+} transfer via channels called IP3Rs. A loss of Wolframin triggers IP3R dysfunction and a decrease in the production of the energy molecule ATP, manufactured by the mitochondria through respiration (Mishra et al, 2021). If WFS1 is present, it binds to the ATF6α transcription genes, reducing the activation of the ER stress response element (ERSE) (Fonseca et al, 2010). ER stress signalling is further reduced by WFS1, in healthy cells, by bringing ATF6α to the proteasome for degradation and ubiquitination. This process is shown in figure 5.

4.2- The argument for the involvement of the mitochondria:

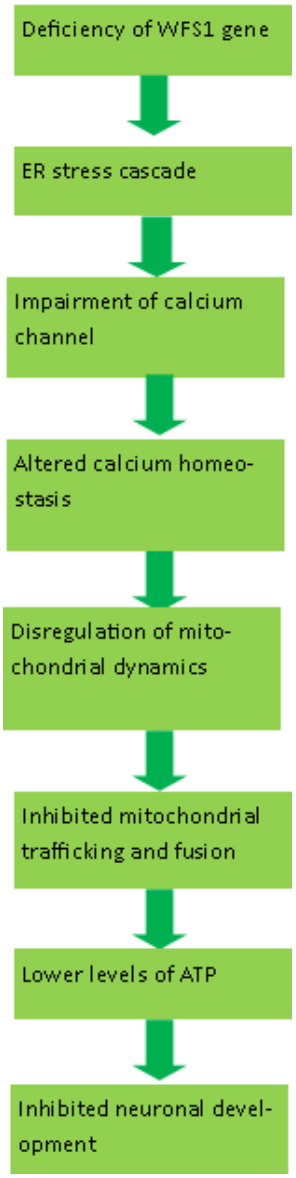

Figure 6- The proposed involvement of mitochondria in the pathophysiology of WS

Mitochondria are considered the energy factories of the cell, where respiration reactions take place to produce ATP. They are especially important in cells and tissues with a high energy demand, such as skeletal muscle and the RGCs of the eye. As noted by Cagalinec et al (2018), The tissues and organs affected in WS all have high energy demands, with Cagalinec et al (2016) taking this as evidence of an energy metabolism defect in WS. Further evidence of mitochondrial involvement in WS comes from the finding that ATP levels are reduced in WFS1-depleted cells (Zatyka et al, 2015).

Clinical features of WS resemble mitochondrial disorders and there have been reports of mitochondrial DNA (mtDNA) abnormalities in patients with WS (La Morgia et al, 2020). This has added fuel to the "long-standing controversy" that mitochondria have a role to play in the pathophysiology of WS.

With Wolframin being an ER transmembrane protein, and the ER and mitochondria being closely connected and interacting, via MAMs, it was not a surprise that Wolframin was found to be involved in the functioning of MAMs (Delprat et el, 2018).

Cagalinec et al (2016) found that down-regulating the WFS1 gene in neurons, grown from patient-derived fibroblasts, led to noticeable changes to mitochondrial dynamics, such as altered mitochondrial fusion and trafficking. As shown in figure 6, WFS1 deficiency can lead to a cascade, eventually causing inhibited neuronal development, like that seen in WS.

Such studies with patient-derived fibroblasts have led to the identification of a protein named NCS1 (neuronal calcium sensor 1) which forms a complex with WFS1 and the other ER calcium channel IP3R (inositol triphosphate). It was found that WFS1 deficient cells have reduced NCS1 protein expression, leading to reduced ER-mitochondrial interactions and calcium exchange (Angebault et al, 2018). NCS1 has therefore been identified as a possible therapeutic target,

as increasing its expression was found to restore ER-mitochondrial interactions and calcium transfer and reverse mitochondrial dysfunction.

In addition to NCS1, and supposing mitochondrial dysfunction does play a part in the pathophysiology of WS, a treatment using dietary supplements which contain NAD+ (nicotinamide adenine dinucleotide) precursors may prove beneficial, as such precursors have been shown in animal studies to block neurodegeneration, under conditions of compromised mitochondrial function (Rajman et al, 2018). Treatments like this can potentially protect vulnerable cells and tissues such as RGCs (Mishra et al, 2021).

5-The current treatment landscape (Obj3):

5.1- Treatments for individual conditions:

Currently, the only available approved treatments for WS are based on managing individual conditions, but not all the conditions have treatments (Asada Kitamura et al, 2021). Diabetes mellitus can be managed using insulin injections or a pump, alongside monitoring of carbohydrate intake. Diabetes insipidus is treated with the drug desmopressin, a synthetic version of ADH, which restores the individual's ability to regulate fluid levels and reverses the symptoms of polydipsia and polyuria.

Both optic atrophy and deafness cannot be reversed, but management options such as hearing aids and glasses can help improve quality of life. In later stages of optic atrophy, with declining levels of vision, input from a low vision clinic may be helpful, which can provide advice and magnification aids.

The lack of a treatment for optic atrophy often leaves patients despondent at their diagnosis, hence this condition has been a focus of efforts to find better treatments for WS.

5.2- Current drug trials:

There are currently two main drug trials which have been underway for about two years, one of which has released preliminary efficacy and safety results (Asada Kitamura et al, 2021). The clinical trial of dantrolene, which targets ER calcium leakage, has several secondary outcome measures, including changes in visual functioning (assessed using Snellen charts and best-corrected visual acuity), quality of life measures, neurological functioning and pancreatic β cell functioning (Abreu et al, 2021). This study in paediatric and adult WS patients was the first in the world using an open-label Ib/IIa trial design (Abreu et al, 2021). As shown in figure 7, after 6 months of dantrolene treatment, there were no significant improvements in the outcome measures.

Outcome measure	Result after 6months treatment
Pancreatic β cell functioning	Not significantly improved, but in-creasedresponsiveness of β cell s (P=0.004)
Visual acuity	No improvement
Neurological func-tions	No improvements

Figure 7- The outcome measures and results of Abreu et al (2021);s study of dantrolene

Another trial, targeting the ER stress response, is evaluating the safety and efficacy of sodium valproate (VPA), used to treat epilepsy and bipolar disorder. There are no published results yet, as recruitment for the trial ended in November 2021. Sites conducting the trial are based in England, France, Poland and Spain, with 63 participants enrolled on the trial (Clinicaltrials.gov, 2022). There are 27 secondary outcome measures for the trial, including brainstem volume (measured using annual MRI scans), RNFL thickness (measured using OCT) and balance. The trial lasts three years and has a double-blind placebo design, meaning neither the clinician nor the patient knows if the patient is taking the placebo or trial drug. VPA has been shown in animal studies to have neuroprotective properties, promoting neurite outgrowth by inhibiting apoptosis caused by the pathological ER stress in WS (Pallotta et al, 2019). Additionally, VPA has been shown to promote the expression of both WFS1 mRNA and ER chaperones (Kakiuchi et al, 2009).

A drug that has shown promise in other mitochondrial diseases, owing to its antioxidant effects is Idebenone (Mishra et al, 2021). This has shown improvements in visual acuity in other types of optic atrophy, namely LHON (Leber's hereditary optic neuropathy and DOA (dominant optic trophy) (Yu-Wai-Man et al, 2020). In one case report the drug was trialled in a patient with

WS who reported a subjective improvement in visual acuity after 6 months of treatment (Bababeygy et al, 2012).

6- Potential treatments and therapies for WS (Obj4):

With the identification of various pathways and proteins involved in the pathophysiology of WS, this gives researchers targets for any future treatments and therapies for WS patients. Figure 8, which summarises the pathophysiology of WS, shows that there are numerous targets for therapy.

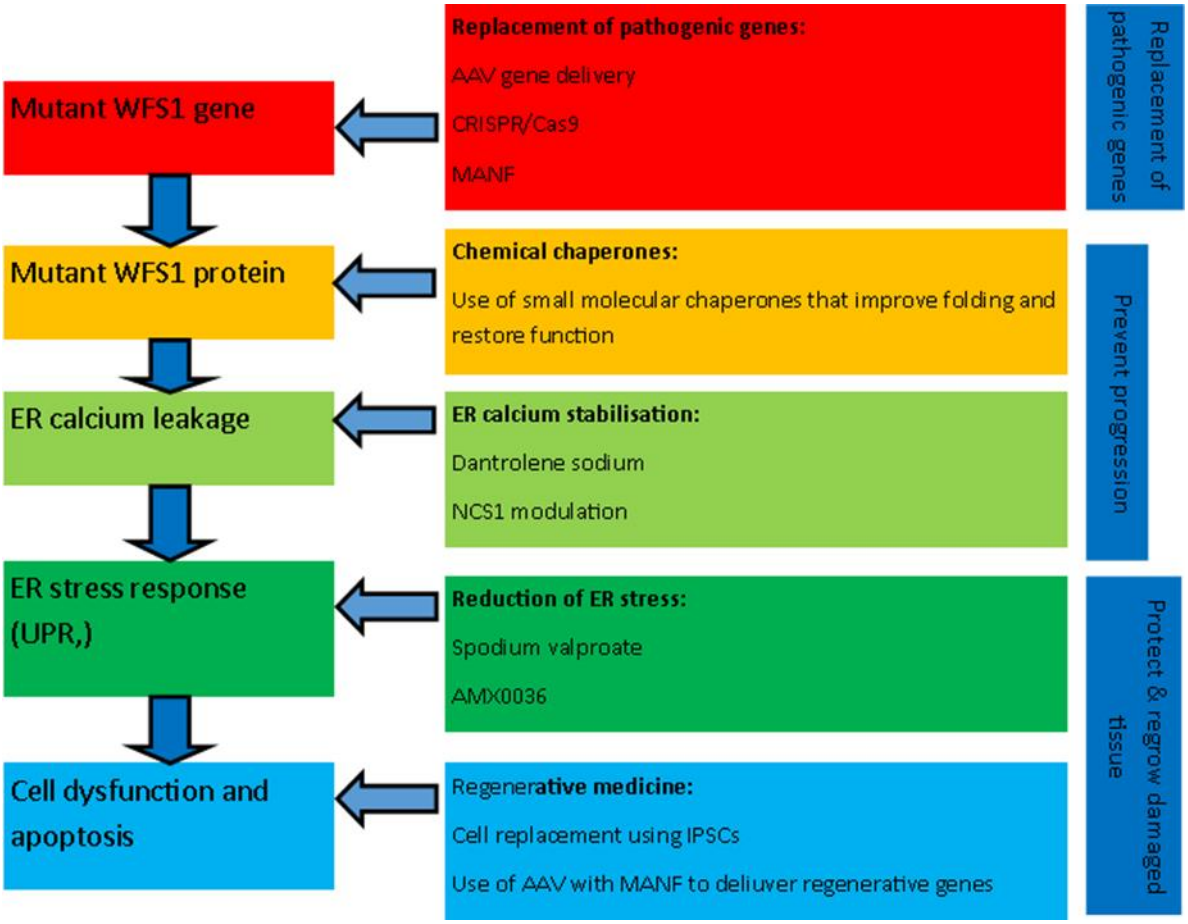

Figure 8- The targets and treatments for WS (adapted from Abreu et al 2019 and wsresearchalliance.com, 2022)

6.1- Drug trials:

Two chemical chaperone drugs, namely 4-phenylbutyric acid (4BPA) and tauroursodeoxycholic acid (TUDCA), have been demonstrated to mitigate ER stress through the stabilisation of the conformation of mutant WFS1 proteins, thereby leading to a reduction in ER stress, as fewer misfolded proteins accumulate in the ER (Abreu et al, 2019). These chemical chaperones can potentially slow the progression of WS through the reduction of apoptosis as a result of ER stress, thereby leading to the preservation of affected tissues such as RGCs.

In addition, another repurposed drug has been shown to target ER stress and delay progression of WS. Dantrolene sodium, which is licensed to treat muscle spasms, is currently in clinical trial in the US, following positive results from animal studies using mouse models of WS and patient-derived stem cells (Mishra et al, 2021).

Dantrolene reduces ER stress through inhibition of ryanodine receptors (RyRs) on the ER membrane, thereby reducing calcium leakage from the ER to the cytosol (Lu et al, 2014). As shown in figure 5, the RyRs act alongside IP3Rs to control the efflux of Ca^{2+} from the ER to the cytosol. Under conditions of ER stress, the increased cytosolic Ca^{2+} causes the activation of a calcium-dependent protease called calpain-2, which promotes cellular apoptosis (Pallotta et al, 2019). Therefore, by reducing the efflux of calcium and the cytosolic concentration of calcium, there is less apoptosis of cells such as RGCs and β cells.

6.2- Gene therapy:

Another possible therapy for WS is the use of MANF (mesencephalic astrocyte-derived neurotrophic factor) to both prevent cell death and promote the proliferation of remaining insulin-producing β cells, neurons and RGCs (Iafusco et al, 2022). Surprisingly, a study by Mahadevan et al (2020) even found that MANF is secreted by ER- stressed cells, further increasing its applicability for treating WS.

In addition, AAV (adeno-associated virus) can be used as a vector to transfer wild-type WFS1 into the RGCs (Mishra et al, 2021). This involves using the

ability of the virus, which is inactivated, to penetrate the cell nucleus to insert the functional WFS1 gene. This technology also makes use of an up-and-coming technology known as CRISPR (clustered regularly interspaced short palindromic repeats) which acts like molecular scissors to cut the desired gene for insertion into another cell (Hamel et al, 2017).

6.3-Stem cells:

Much of the research to find and develop such treatments and therapies has been conducted using both animal models, such as mice and zebrafish, and human induced pluripotent stem cells (IPSCs) derived from patient fibroblasts (Cairns et al, 2021, La Morgia et al, 2020). The IPSCs, being patient-derived, have the advantage of being accurate *in vitro* models on which to test treatments, and better understand the pathophysiology of WS (Mishra et al, 2021). Such fibroblasts can be relatively easily obtained using a patient skin biopsy, which can then be 'transformed' into various cell types, including RGCs. Making use of new gene technology is helping to create better treatments, rather than relying on commonly used animal models in the past such as zebrafish and mice (Cairns et al, 2021). It is notable, however, that a lack of patient tissues, such as RGCs is a limiting factor in WS research. zebrafish have previously been used as their eyes are comparable to humans' (Cairns et al, 2021).

IPSCs are most useful for screening potential drugs and therapies, with Urano (2014) also highlighting the ability of 4BPA and TUDCA to mitigate ER stress, with applicability to other ER diseases. Another potential use of stem cells is their transplantation to regenerate damaged tissues, explored by Urano et al (2014) with a focus on pancreatic β cells to stimulate insulin production in WS.

7. Discussion:

OA was reported as the most important symptom in a patient-reported outcomes measures survey (Barrett, 2018, unpublished). Achieving stable visual acuity has therefore been one of the main outcome measures for clinical trials, such as the ongoing VPA trial in England. One source of such patient feedback has been from the annual WS UK conferences, which allow patients, families, and medical professionals to discuss what patients would like to see as an outcome of research.

As mentioned in this literature review, research that has helped to better understand the underlying pathophysiology of WS and OA has helped to create new treatment ideas and assess their efficacy. The identification of the causative gene WFS1 in 1998 provided a significant breakthrough (Inoue et al, 1998). Furthermore, the establishment of both animal and IPSC models of WS has allowed researchers to better study the genotype-phenotype relationship, which may hopefully develop further in the future (Lu et al, 2014). Not only are IPSCs good for modelling the pathophysiology of WS, but they also provide models on which to test new treatments and potential replacement cells and tissues for repairing damage (Mishra et al, 2021). The potential to grow RGCs from IPSCs could potentially overcome the limitation that few WS patient specimens are available for research (Urano, 2014).

In addition to IPSCs as a potential treatment for WS and OA, Iafusco et al (2022) researched the potential held by gene technologies. In particular, MANF was thought to be promising, with its regenerative abilities holding particular hope. Mishra et al (2021) highlight, however, that more research is needed to fully understand MANF's applicability to WS as the receptors for MANF has not yet been identified.

As shown in figure 8, it may be that a combination of techniques can be employed to treat WS, such as the combination of AAV and MANF to deliver corrected genes (Abreu et al, 2019). As has been the case with much of the research into WS, owing to such a small patient population, it will be necessary for researchers to collaborate, often internationally, to pool their resources and knowledge. As is the case for the sodium valproate trial, the recruitment

of patients was across Europe to gain enough participants for the trial to have meaningful results (Clinicaltrials.gov,2022).

8. Conclusion:

This literature review has explained what WS is, considering most people will not have heard of it due to its extreme rarity. The focus has been on optic atrophy, itself relatively rare. Despite the rarity, however, the underlying pathophysiology of WS is relatively simple, and is common to several conditions, including diabetes and Parkinson's.

It has taken considerable time and effort for researchers to begin to understand OA in WS, and questions remain. However, the major benefit of such research has been the identification of biomarkers and cellular targets for the current and future treatments of WS. One such biomarker, RNFL thickness, has proven particularly useful in assessing visual acuity stability in the ongoing VPA trial.

Other sources of hope for a treatment or cure of WS, particularly for OA, include new technologies such as AAV and CRISPR. As has been the case in treatment for other genetic disorders, it is likely that a combination of these techniques is used.

In the future, I would expect there to be more research into finding an association between genotype and phenotype, which may facilitate the development of more personalised treatments. As is the case for many rare conditions, the small number of patients may limit any future research, however. It is notable however, especially with WS, that the monogenic nature of the condition proves beneficial as a model for understanding ER stress, attracting great interest, considering the rarity of WS.

I feel I have met my five objectives for this literature review, but given a greater word limit I would have liked to explore hypothetical treatments more, some of which are discussed at the annual WSUK conferences.

4673 words

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Glossary:

Autosomal [(1)]: The chromosomes in the cell other than the sex chromosomes X & Y

Frame shift mutation [(1)]: A shift in the reading frame caused by the insertion or deletion of one or more nucleotide pairs within the DNA sequence.

Genotype [(1)]: The genetic make-up of an individual, involving the combination of alleles.

Optical coherence tomography [(3)]: An imaging technique that provides high-resolution, cross-sectional images of the eye non-invasively.

Pathophysiology [(2)]: The physiological processes underlying disease.

Phenotype [(1)]: The outward observable characteristics of an individual, determined by genotype and the environment.

Retinal nerve fibre layer (RNFL) [(3)]: A layer within the retina which is formed of the axons of the ganglion cells, arranged horizontally. The axons travel from their cell bodies within the ganglion cell layer, joibg at the optic disc to form the optic nerve.

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