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Wolfram Syndrome: a Monogenic Model to Study Diabetes Mellitus and Neurodegeneration

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Abstract

Wolfram syndrome (WS) is a rare, progressive disorder characterized by childhood-onset diabetes mellitus, optic nerve atrophy, hearing loss, diabetes insipidus, and neurodegeneration. Currently, there is no effective treatment for WS, and patients typically die between 30 and 40 years of age. WS is primarily caused by autosomal recessive mutations in the *Wolfram syndrome 1* (*WFS1*) gene (OMIM 222300), which encodes for wolframin (WFS1). This disorder is therefore a valuable monogenic model for prevalent diseases, particularly diabetes mellitus and neurodegeneration. Whereas reduced survival and secretion are known cellular impairments causing WS, the underlying molecular pathways and the physiological function of WFS1 remain incompletely described. Here, we characterize WFS1 as a regulator of intracellular calcium homeostasis, review our current understanding of the disease mechanism of WS, and discuss candidate treatment approaches. These insights will facilitate identification of new therapeutic strategies not only for WS but also for diabetes mellitus and neurodegeneration.

Keywords

Orphan disease; WFS1; neurodevelopment; pancreatic β -cells; intracellular calcium signaling; ibudilast

Introduction: Ca²⁺ and Cell Function in Disease

Calcium (Ca²⁺) is a universal second messenger, and intracellular Ca²⁺ homeostasis is a key determinant of cell health and viability. Intracellular Ca²⁺ signaling is a highly versatile process that regulates numerous cell functions – including metabolism, gene transcription, and apoptosis – in all tissue types (for reviews see [1, 2]). As such, the Ca²⁺ concentration

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Competing interests

B.E.E is a founder of Osmol Therapeutics, a company that is targeting NCS1 for therapeutic purposes.

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in different cellular compartments must be tightly regulated through a network of Ca²⁺ channels and transporters, which, in turn, are regulated by multiple signaling molecules and interacting partners. Generally, resting free cytosolic Ca²⁺ levels are low (approximately 100nM) compared to the concentration in organelles (endoplasmic reticulum (ER): approximately 1mM, mitochondria: approximately 0.1–500μM) or extracellular space (approximately 1mM) [2, 3]. It is well established that disruption of intracellular Ca²⁺ homeostasis underlies several pathologies, including Alzheimer's disease [4], cancer progression [5], and diabetes mellitus [6]. In addition, emerging evidence suggests that intracellular Ca²⁺ dyshomeostasis contributes to the pathology of the rare disease called Wolfram syndrome.

Wolfram Syndrome

Wolfram syndrome (WS, OMIM 222300) is an orphan, autosomal recessive endocrinological and neurodegenerative disorder that affects 1:160,000 to 1:700,000 people worldwide [7]. In 1938, WS was first described by Wolfram and Wagener [8]. This disorder is characterized by the “**DIDMOAD**”-phenotype in the majority of patients: diabetes insipidus (average age of onset: *14 years*), diabetes mellitus (*6 years*), optic nerve atrophy (*11 years*), and sensorineural deafness (*12.5 years*) (Figure 1A) [9]. Other common symptoms of WS include widespread neurodegeneration, cerebellar ataxia, autonomic symptoms, urological complications, and psychiatric illness (Figure 1A) [9]. At the moment, there is no effective treatment for this devastating disease, and patients typically die in their 30s, most often because of respiratory failure due to brain stem atrophy [10, 11].

Around 90% of WS cases are caused by inactivating mutations in the Wolfram syndrome 1 gene (*WFS1*) [9], which encodes for wolframin (WFS1), a transmembrane protein localized to the ER [12]. The remaining WS cases are attributed to sequence variants in the *CISD2* gene (also known as *WFS2*) or other unknown genes [13]. The heterozygous carrier frequency of *WFS1* variants is 0.3–1% [11], and these carriers show increased risks of psychiatric illnesses [14]. Moreover, some *WFS1* variants can cause autosomal dominant low-frequency sensorial hearing loss (DFNA6/14) [15], autosomal dominant optic atrophy with hearing impairments [16, 17], and autosomal dominant diabetes mellitus [18]. Single nucleotide polymorphisms (SNPs) in the *WFS1* gene locus can predispose patients to type 2 diabetes [19]. Still, despite the emerging importance of the *WFS1* gene locus, the physiological functions of the protein WFS1 remain elusive.

This review summarizes our current understanding of the functions of WFS1 and the disease mechanism of WS. We focus on the role of intracellular Ca²⁺ signaling and describe candidate strategies to treat WS. Moreover, we propose future directions for studies on WS and discuss how a better understanding of WS may provide further insights into the pathology of diabetes mellitus and neurodegeneration.

The *WFS1* Gene Product: Wolframin (WFS1)

The *WFS1* gene is localized on chromosome 4p16.1 and encodes for wolframin (WFS1), an 890-amino-acid protein with nine putative transmembrane segments [20]. WFS1 is

ubiquitously expressed, but particularly high expression levels are observed in the brain, heart, and pancreatic β -cells (β -cells) [21]. Intracellularly, WFS1 is predominantly localized to the ER with a $N_{\text{cytosol}}/C_{\text{ER lumen}}$ topology [12, 21, 22] and appears to be enriched in mitochondria-associated ER-membranes (MAM) [23, 24]. WFS1 is also present in the membranes of secretory granules [25, 26]. The crystal structure of WFS1 has not been solved. Over 200 pathogenic variants in the *WFS1* gene locus have been identified in WS patients, but genotype-phenotype correlations are not well established [9]. Typically, these genetic variants are loss-of-function mutations resulting in depletion of WFS1 mRNA or protein or expression of defective WFS1 protein [9]. Whereas *in vivo* and *in vitro* disease models of WFS1-deficiency may not reflect the numerous different mutations in patients, they recapitulate cardinal symptoms of WS [27–30].

WFS1 as a Regulator of Intracellular Ca²⁺ Homeostasis

The current body of evidence suggests an essential role for WFS1 in the regulation of ER homeostasis and intracellular Ca²⁺ signaling. In recent studies on β -cells, neurons, and patient-derived fibroblasts, loss of WFS1 function results in a global disruption of intracellular Ca²⁺ homeostasis, affecting Ca²⁺ signaling both at the resting state and under stimulation (Figure 1C) [23, 24, 29, 31, 32].

Elevation of cytosolic calcium.

Free resting cytosolic Ca²⁺ $[Ca^{2+}]_{\text{cyto}}$ is elevated due to loss of WFS1 [24, 29, 31, 33]. Normally, $[Ca^{2+}]_{\text{cyto}}$ is kept at low concentrations because sustained $[Ca^{2+}]_{\text{cyto}}$ elevation results in cellular impairments and ultimately, apoptosis [34]. The molecular mechanisms that lead to elevation of $[Ca^{2+}]_{\text{cyto}}$ in WS have yet to be determined. On one hand, dysfunction of the ER, the main intracellular Ca²⁺ store, may be an underlying cause. WFS1 likely regulates ER-Ca²⁺ sequestration through its interaction with the sarcoendoplasmic reticulum Ca²⁺ transport ATPase (SERCA), but it remains uncertain how WFS1-deficiency alters SERCA expression and activity [31, 35]. Additionally, some studies indicate an ER-Ca²⁺ leak in WFS1-deficient cells [31, 33, 36], although we and others did not see a difference in ER-Ca²⁺ loading [24, 29, 32]. On the other hand, the increase of $[Ca^{2+}]_{\text{cyto}}$ due to loss of WFS1 may result from increased Ca²⁺ influx or decreased Ca²⁺ efflux across the plasma membrane. Mechanistically, it has been suggested that WFS1 positively regulates the expression of sodium/potassium ATPase β 1 subunit (Na^+/K^+ -ATPase β 1), which would result in reduced Na^+/K^+ -ATPase and sodium-calcium exchanger (NCX) activity in WFS1-deficient β -cells [37]. The effect of WFS1-deficiency on voltage-gated calcium channels and store-operated calcium channels should also be explored in future studies.

Reduction in stimulus-evoked ER-Ca²⁺ release.

Stimulus-evoked ER-Ca²⁺ release via the inositol 1,4,5-trisphosphate receptor (InsP3R) is impaired due to loss of WFS1 [23, 24, 29, 32]. The InsP3R can release Ca²⁺ from the ER into the cytosol and – at interorganellar signaling hubs called mitochondria-associated ER membranes (“MAMs”) – into mitochondria [38, 39]. Proper InsP3R activity, which is regulated by several interacting partners of the receptor, is integral to cellular signaling [38]. WFS1 appears to be a positive regulator of InsP3R-dependent ER-Ca²⁺ release into the

cytosol and mitochondria [23, 24, 29, 32], but further studies on the underlying mechanisms are necessary. It has been described that the expression of InsP3R1 and InsP3R3 is not changed in WFS1-deficient β -cells [24]. Co-immunoprecipitation experiments suggest an interaction between InsP3R and WFS1, but it remains unclear whether they interact directly [32]. Alternatively, neuronal Ca²⁺ sensor-1 (NCS1) may bridge the two proteins as NCS1 interacts directly with InsP3R and WFS1 [32, 40]. Overexpression of NCS1 rescued Ca²⁺ signaling in WFS1-deficient fibroblasts and β -cells [24, 32], suggesting a WFS1-NCS1-InsP3R signaling complex which is disrupted in WS. Finally, InsP3R dysfunction may be a secondary result of other cellular signaling impairments due to loss of WFS1, such as unresolved cell stress [41].

Collectively, WFS1 has been characterized as a versatile regulator of intracellular Ca²⁺ homeostasis, likely through interaction with several key players of intracellular Ca²⁺ signaling, including InsP3R [32], SERCA2 [35], and NCS1 [32]. One study also suggested that WFS1 itself may act as a cation-selective ion channel [42]. This seems unlikely because in all recent studies, WFS1-deficiency results in elevated $[Ca^{2+}]_{cyto}$ [24, 29, 31, 33], and we did not observe an increase in ER-Ca²⁺ release with WFS1 overexpression [24]. However, a better understanding of the WFS1 structure and tests for channel activity with purified WFS1 are necessary to clarify this matter. Additionally, we have posed several other questions that need future investigations in order to develop a global understanding of intracellular Ca²⁺ signaling in WS. Nevertheless, the impairments of intracellular Ca²⁺ signaling that have been described in WS thus far point to potential treatment targets as discussed in the section “Treatment Approaches for Wolfram Syndrome”.

Molecular Mechanisms of Wolfram Syndrome

Consistent with the neuroendocrinological symptoms in WS patients, *in vivo* and *in vitro* studies have established that WFS1-deficiency causes reduced cell viability and secretion primarily in β -cells and neurons (Figure 1B). This tissue selectivity is likely because β -cells and neurons are hypersusceptible to ER-dysregulations due to their high rates of protein synthesis as secretory cells [13, 43]. Here, we summarize current mechanistic insights and delineate the role of altered Ca²⁺ signaling in producing the WS phenotypes.

Secretion.

Impairments of secretion in WS have mostly been studied in β -cells. Glucose-stimulated insulin secretion (GSIS) from β -cells is required to facilitate glucose uptake into adipose tissue, muscle, and liver following a meal. Elevated plasma glucose concentrations stimulate ATP production in β -cells. Consequently, ATP-sensitive potassium channels close which causes depolarization of the plasma membrane. Voltage-gated calcium channels open and Ca²⁺ influx results in a rapid elevation in $[Ca^{2+}]_{cyto}$ that ultimately triggers insulin exocytosis (for review see [44]). In contrast, in WFS1-deficient cells, this glucose-stimulated rise in $[Ca^{2+}]_{cyto}$ is diminished, resulting in reduced GSIS [27]. Mechanistically, the rapid increase in $[Ca^{2+}]_{cyto}$ is likely inhibited by the permanently elevated $[Ca^{2+}]_{cyto}$. This is supported by our observation that drug interventions that normalize $[Ca^{2+}]_{cyto}$ in WFS1-KO cells also restore GSIS [24]. Moreover, ER Ca²⁺ release and mitochondrial Ca²⁺ transients,

both of which are impaired in WFS1-deficient β -cells [24], are important for the fine-tuning of insulin exocytosis [45]. ER- Ca^{2+} release via the InsP3R – for example, stimulated by the parasympathetic neurotransmitter acetylcholine – potentiates insulin secretion by elevating $[\text{Ca}^{2+}]_{\text{cyto}}$ [46]. Ca^{2+} uptake into mitochondria can stimulate ATP synthesis [47], which amplifies insulin release.

Overall, WFS1-dependent Ca^{2+} signaling appears to be crucial for GSIS (Figure 1C). Additionally, it was proposed that WFS1 positively regulates insulin expression and processing [25, 48]. Because proper Ca^{2+} signaling is as essential for secretion in neurons as for GSIS, and WFS1 was found to interact with vacuolar proton (H^+)-ATPase in neuroblastoma cells [26], it can be expected that WFS1-deficient neurons show secretory impairments similar to β -cells. Consistently, dopamine release is decreased in the striatum of WFS1-deficient mice [49]. However, findings are sparse, most likely due to technical challenges and regional heterogeneity when studying secretion in the CNS.

Survival and Cell Death.

WFS1 has been characterized as a regulator of cellular homeostasis that promotes cell health [48] and inhibits ER stress [50]. Whereas overall cell viability appears to be similar between wild-type and WFS1-deficient β -cells *in vitro* [24], β -cells mass is reported to be reduced in WFS1-deficient animal models [28, 30, 48, 51]. This is consistent with studies showing that WFS1-deficiency renders β -cells and neurons hypersusceptible to cell death induced by stress, such as ER stress, age, and hyperglycemia [24, 27, 48, 52]. Decreased pro-survival and increased proapoptotic signaling are likely responsible for this phenotype.

We and others observed that the phosphorylation – and hence activity – of Akt, a well-known pro-survival effector [53], was decreased in WFS1-deficient β -cells [24, 48]. This seems to be a shared downstream consequence of decreased activity of the insulin receptor and increased expression of tribbles homolog 3 (Trib3) [24, 48]. Additionally, Akt activity may be downregulated by elevated $[\text{Ca}^{2+}]_{\text{cyto}}$ either directly [54] or through Ca^{2+} -dependent activation of protein phosphatase 2 A (PP2A) [55]. This hypothesis needs further elucidation. Mitochondrial energy metabolism and dynamics, which are also important for cell survival, are likely impaired due to loss of WFS1 in neurons and fibroblasts [29, 32]. Indeed, because WFS1 is enriched at mitochondria-associated ER-membranes (MAM) [23], it is well-positioned to regulate mitochondrial function. However, the nature of mitochondrial impairments in WFS1-deficient cells remains controversial [23], and reports about mitochondrial function in WFS1-deficient β -cells are lacking.

Hyperactivation of calpains is one of the alterations that increase proapoptotic signaling in WFS1-deficient β -cells and neurons [24, 31]. As calpains are Ca^{2+} -activated cysteine proteases, their hyperactivation is consistent with elevated $[\text{Ca}^{2+}]_{\text{cyto}}$ in WS [24, 31]. Additionally, WFS1-deficient cells show increased levels of ER stress because WFS1 is a negative regulator of this pathway [29, 50]. Ultimately, unresolved, chronic ER stress in WS results in apoptosis (for review see [56]). Perturbations of ER- Ca^{2+} homeostasis are among stress signals that can trigger ER stress [57]. In turn, ER stress can also impair InsP3R-dependent Ca^{2+} signaling [41], suggesting a vicious cycle between disrupted Ca^{2+}

signaling and ER stress. Lastly, increased levels of caspase activity, which can be triggered by Ca²⁺ dyshomeostasis [34], were observed following loss of WFS1 in β -cells [28, 48].

In sum, impaired Ca²⁺ signaling appears to be an underlying cause for the observed dysfunctions in WS, but further elucidation of the precise disease mechanism, especially in neurons, are required. Future studies should focus on recently developed patient-derived stem-cell models of WS for in-depth mechanistic studies [58, 59]. Thereby, distinct molecular consequences due to different disease-causing mutations in the human *WFS1* gene may be identified.

Trajectories of Wolfram Syndrome

The natural history of WS is characterized by the childhood onset of endocrinological and neuronal symptoms associated with progressive degeneration. On a cellular level, this phenotype can be attributed to reduced secretion and survival – as described in the previous section. With non-autoimmune, insulin-dependent diabetes mellitus typically being the first diagnosed symptom of WS, hyperglycemia may accelerate the loss of neurons due to their hypersusceptibility to stress-induced cell death [27, 48, 52]. Therefore, strategies that aim to treat diabetes mellitus may delay the onset of other symptoms in WS. Furthermore, recent neuroimaging studies in WS patients suggest that impaired neurodevelopment precedes neurodegeneration (for review see [60]). Alterations in early brain development were primarily characterized by hypomyelination [60], necessitating studies on the function of WFS1 in oligodendrocytes. Interestingly, WFS1 expression is strongly upregulated in the human brain during development, with the highest levels occurring between 8 and 15 years of age [60]. Of note for further studies, reduced brain development and the trajectory of WFS1 expression are recapitulated in WFS1-deficient mice [29, 61]. Future research endeavors should leverage functional neuroimaging and explore underlying molecular pathways, connecting impaired neurodevelopment and neurodegeneration.

Treatment Approaches for Wolfram Syndrome

Currently, there is no effective treatment for WS, and patients typically die in their 30s [9]. Disease management focuses on alleviating the symptoms in order to improve patients' quality of life; for example, insulin treatment to control diabetes mellitus [13]. Candidate treatment strategies include the repurposing of existing drugs and development of regenerative medicine tools (Table 1). Most drug tests in WS animal models have focused on diabetes mellitus as an outcome measure. However, it is also essential to measure brain volume and assess neuronal functions because patients usually die due to brain stem atrophy [9].

Targeting Disrupted Ca²⁺ Signaling.

The drugs we summarized in this group likely act through normalization of [Ca²⁺]_{cyto} or ER Ca²⁺ homeostasis. As such, dantrolene, which is used to treat muscle spasticity and malignant hyperthermia [62], restores [Ca²⁺]_{cyto} and calpain activity in WFS1-deficient β -cells, neurons, and patient-derived neuronal progenitor cells [31]. This compound stabilizes ER-Ca²⁺ homeostasis through the inhibition of ryanodine receptors [62]. Currently, a

clinical trial that assesses safety, tolerability, and efficacy in WS patients is ongoing (Clinical Trial Number: [NCT02829268](#)). Recently, we investigated the potential of ibudilast, a PDE4-inhibitor that is approved for treating asthma and post-stroke dizziness in Japan [63], in the context of WS [24]. We observed that this drug rescues $[Ca^{2+}]_{cyto}$, GSIS, and survival in WFS1-deficient β -cells [24]. Ibudilast may restore Ca^{2+} homeostasis through its interaction with NCS1 [64] or cAMP- Ca^{2+} cross-talk [65], but further mechanistic studies are needed. This drug may also reduce neurodegeneration in WS, as it showed promising effects on neuropathy and multiple sclerosis [66, 67]. Because hyperactivation of calpain is observed in WFS1-deficient cells, we also tested the effect of calpain inhibitor XI (also known as AK295) [24]. Calpain inhibitor XI rescues $[Ca^{2+}]_{cyto}$, GSIS, and survival in WFS1-deficient β -cells [24]. Most calpain inhibitors are relatively toxic and produce broad effects, but new, more specific calpain inhibitors are under clinical trial [68] and could potentially be repurposed for the treatment of WS. The potential of oral antidiabetic drugs has also been investigated in the context of WS. For example, pioglitazone can restore ER- Ca^{2+} homeostasis, likely by enhancing SERCA levels [33, 69]. WFS1-deficient agouti (Avy) mice were partially protected against diabetes following pioglitazone treatment [51]. Another antidiabetic drug, sitagliptin, a dipeptidyl peptidase-4 inhibitor, can also restore ER Ca^{2+} signaling and may be tested in WS [70]. Furthermore, we suggest testing verapamil, a calcium channel blocker, because blocking Ca^{2+} influx may help to normalize $[Ca^{2+}]_{cyto}$ and associated pathways in WS [29]. Similarly, verapamil reduced neurodegeneration in a mouse model of ALS by inhibiting calpains, ER stress, and autophagy [71]. Additionally, a recent clinical trial found that this drug preserved β -cell function in patients with type 1 diabetes mellitus [72].

Targeting ER Stress.

Because ER stress is another established contributor to WS, associated pathways have been targeted for candidate treatments. Valproate (VPA) is a mood stabilizer [73], that can inhibit histone deacetylase (HDAC) [74], reduce ER stress [75], and induce WFS1 expression [76]. In a mouse model of WS, valproate improved blood glucose tolerance, potentially through enhancement of insulin sensitivity [77]. Valproate is currently under assessment in a clinical trial in WS patients (Clinical Trial Number: [NCT03717909](#)). Exenid-4 and liraglutide, glucagon-like-peptide 1 (GLP1)-receptor agonists, can restore glucose tolerance in WFS1-deficient animal models likely by alleviating ER stress [78, 79]. Liraglutide also showed promising effects on glycemic control in a WS patient [79].

Regenerative Medicine.

Future advancements in the fields of gene therapy and replacement medicine may ultimately lead to a cure for WS [13]. Using gene editing, *WFS1* mutations could be corrected in induced pluripotent stem cells (iPSCs) generated from patients' skin cells. These iPSCs could then be differentiated and transplanted into patients [59]. Alternatively, wild-type WFS1 could be increased in affected organs with adeno-associated virus systems (AAV) [13]. Another approach involves stimulating the proliferation of remaining β -cells and neurons. For this purpose, restoring Akt activity in WS may be a promising new avenue [24, 53, 80]. Similarly, it has been reported that mesencephalic astrocyte-derived neurotrophic factor (MANF) can enhance the proliferation of remaining β cells in WS mice [81].

Overall, there are multiple promising avenues for the treatment of WS that should be further explored. Still, the multiorgan nature of this disorder presents significant challenges, and may necessitate a combination of treatment strategies.

Wolfram Syndrome as Monogenic Disease Model

WS represents a monogenic model for diabetes mellitus, neurodevelopment, and neurodegeneration. On one hand, monogenic models cannot replicate the complexity of multifactorial diseases. On the other hand, studies on single-gene forms of diabetes mellitus and neurodegeneration have provided many mechanistic insights (for reviews see [82, 83]). Furthermore, WS was recently used as a diabetic disease model to improve approaches of gene editing and replacement medicine for the therapy of diabetes mellitus [59]. Mechanistically, diabetic and neurodegenerative pathology shares several molecular impairments with WS, particularly unresolved ER-stress, Akt dysregulation, and impaired Ca^{2+} signaling [6, 39, 56, 80, 84]. A recent review described WS as a prototype of ER stress-associated disorders [85]. We noted that several impairments of intracellular Ca^{2+} homeostasis in WS had been similarly observed in diabetes mellitus. First, $[\text{Ca}^{2+}]_{\text{cyto}}$ is elevated in human and rat wild-type pancreatic islets following culture in hyperglycemia [86, 87]. Second, we observed that hyperglycemia reduced stimulus-evoked ER- Ca^{2+} release in wild-type cells [24]. Lastly, the MAM-signaling axis appears to be disrupted in diabetes mellitus [88, 89]. Therefore, drugs that restore Ca^{2+} homeostasis and reduce ER stress in WFS1-deficient cells may hold similar therapeutic potential for diabetes mellitus and neurodegeneration. This hypothesis is further supported by the recent observation that WFS1 was transcriptionally downregulated in pancreatic islets from type 2 diabetes mellitus patients, suggesting a role for WFS1 in the pathology of common forms of diabetes [48].

Concluding Remarks

Recent studies *in vivo* and *in vitro* have enriched our understanding of WS which represents a monogenic model for diabetes mellitus and neurodegeneration. WFS1 is an emerging regulator of cellular homeostasis and particularly intracellular Ca^{2+} signaling. Signaling impairments caused by the loss of WFS1 culminate in impaired secretion and survival. A more detailed view of how WFS1 regulates development will further elucidate the trajectories of WS. Future studies are necessary to determine the molecular mechanism of this rare disease and will simultaneously provide new insights into more complex diseases. We are optimistic that currently developed strategies will contribute to the treatment of WS and, eventually, diabetes mellitus and neurodegeneration.

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Highlights

- Wolfram syndrome (WS) is a rare, incurable disease caused by mutations in the *WFS1* gene
- Clinical hallmarks of WS include childhood onset of diabetes mellitus and neurodegeneration
- WFS1 protein is an emerging regulator of cellular homeostasis including calcium signaling
- Loss of WFS1 function results in reduced secretion and survival primarily in pancreatic β -cells and neurons
- Studies on WS will facilitate the development of therapeutics for diabetes mellitus and neurodegeneration

Wolfram syndrome

A - Clinical presentation:

Neurodegeneration and psychiatric symptoms
Optic nerve atrophy
Hearing loss
Diabetes insipidus
Diabetes mellitus
 Urological symptoms
 Peripheral neuropathy

B - Cellular dysfunction:

Decreased insulin secretion
Hyper-glycemia
 Loss of β -cells
 (● → ○)
 Pancreas, Islet of Langerhans

C - Molecular impairments

Symptoms of characteristic
 "DIDMOAD"-phenotype in **bold**

● β -cell,
 ● blood vessel

WFS1 loss of WFS1, ● calcium (Ca^{2+}), ↓ decreased, ↑ increased,
 P = phosphorylation, PM = plasma membrane, *grey italicized and dashed lines* indicate questions that require further investigations.

Figure 1:

Overview of Wolfram syndrome pathology. (A) Clinical presentation. Symptoms most frequently observed in Wolfram syndrome patients are listed with labels indicating affected organs (schematic, list not exhaustive). The characteristic "DIDMOAD"-phenotype is shown in **bold**. (B-C) These two subpanels illustrate the pathomechanism of WS, focusing on pancreatic β -cells because diabetes mellitus is the first diagnosed symptom in WS and has been most frequently studied. (B) Cellular dysfunction due to the loss of WFS1 in the endocrine pancreas (Islet of Langerhans) is characterized by decreased insulin secretion and increased loss of β -cells. (C) Molecular impairments due to the loss of WFS1 are characterized by disrupted intracellular Ca^{2+} homeostasis, ER stress, and reduced mitochondrial functionality – resulting in reduced secretion, enhanced apoptosis, and reduced survival. Disruption of intracellular Ca^{2+} homeostasis, including elevation of resting free cytosolic Ca^{2+} [Ca^{2+}]_{cyto} and impaired ER- Ca^{2+} release into the cytosol and mitochondria, appears as a likely underlying cause for WS pathology.

Table 1:

List of candidate treatments for Wolfram syndrome (WS). In black are drugs that have been tested in *in vivo* or *in vitro* models of WS. In blue are proposed drugs and hypothesized mechanisms that should be assessed in future studies. FDA: U.S. Food and Drug Administration; NCT: [Clinicaltrials.gov](https://clinicaltrials.gov) identifier; ER: endoplasmic reticulum; Ca²⁺: calcium; T2: type2; SERCA: sarcoendoplasmic reticulum Ca²⁺ transport ATPase; GLP1R: glucagon-like peptide-1 receptor; MANF: mesencephalic astrocyte-derived neurotrophic factor; Akt: protein kinase B.

Drugs	Approved in humans? (FDA?; indication)	Clinical trial for WS	Proposed mechanism of action in WS
Targeting disrupted Ca ²⁺ signaling (drug repurposing)			
Dantrolene [31]	yes (FDA; malignant hyperthermia, muscle spasticity)	yes (NCT02829268)	Normalizing [Ca ²⁺] _{cyto} and calpain activity by inhibiting ER Ca ²⁺ efflux via ryanodine receptors [31, 62]
Ibuprofen [24]	yes (Japan; asthma, post-stroke dizziness)		Normalizing [Ca ²⁺] _{cyto} [24] through interaction with NCS1 [64] or the cAMP-Ca ²⁺ link [65]
Calpain inhibitor [24]	no		Normalizing calpain activity and [Ca ²⁺] _{cyto} [24]
Pioglitazone [51]	yes (FDA; diabetes mellitus T2)		Restoring ER Ca ²⁺ homeostasis by enhancing SERCA levels [33, 69]
Sitagliptin	yes (FDA; diabetes mellitus T2)		Potentially restoring ER Ca ²⁺ homeostasis [70]
Verapamil	yes (FDA; arrhythmia, angina, hypertension)		Potentially normalizing [Ca ²⁺] _{cyto} and calpain activity by blocking plasma-membrane Ca ²⁺ -influx and alleviating ER stress [29, 71, 72]
Targeting ER stress (drug repurposing)			
Valproate [77]	yes (FDA; seizures)	yes (NCT03717909)	Alleviating ER stress [75, 76]
GLP1R agonists [78, 79]	yes (FDA; diabetes mellitus T2)		Alleviating ER stress [78, 79]
Stimulating proliferation (regenerative medicine)			
MANF [81]	no		Stimulating proliferation and alleviating ER stress [81]
Akt activators	no		Potentially inducing proliferation and survival by stimulating Akt pathway [80]