9th International Wolfram Syndrome Symposium
Berkshire, England 22nd-24th Oct 2024

Meeting Notes

Summary

These meeting notes have been developed to provide a helpful summary of the International Symposium for attendees, other professionals working in Wolfram Syndrome research and for the WS community of affected people and their families.

These notes include the following:

- Meeting agenda
- Abstracts provided by each researcher (where they can be shared)
- Points noted during the presentation (that does not represent a summary of the work – which has / will be published in peer reviewed journals).
- Meeting attendee list.

Agenda: Day 1 (23rd October 2024)

Presentations

(08:45-9:00) Welcome. Stephanie Gebel – CEO Snow Foundation & Tracy Lynch – CEO Wolfram Syndrome UK.

(9:00-9:20) Digestive Disorder in Wolfram Syndrome. Dr Christophe Orssaud – Ophthalmology Unit, Rare Disease Centre, Paris City University, Paris, France.

(9:25-9:55) Predictive Value of Genetics in Central Nervous System Neurodegeneration in Wolfram Syndrome. Dr Gema Esteban- Bueno, Ángel Miguel Roldán Molina. Spanish Multidisciplinary Wolfram Syndrome team. Almería, Spain.

(10:00-10:30) Preclinical Gene Therapy for Rare Nervous System Disorders: Progress on the Wolfram Syndrome Project. Dr Assumpcio Bosch - Institute for Neurosciences, Universitat Autònoma de Barcelona, Spain.

(11:05-11:25) Endoplasmic reticulum stress, mitochondrial morphology and calcium dynamics in cellular model of Wolfram syndrome. Michal Cagalinec[1], Silvia Borecka[2], Mohammad Adnan[1],3, Marek Sklenar[2], Martina Skopkova[2], Dominika Hromnikova[2], Alexandra Zahradnikova[1], Daniela Gasperikova[2]

[1]Dept. of Cellular Cardiology. [2]Dept. of Metabolic Disorders, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia. [3]Dept. of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia.

(11:30-12:15) Pharmacological intervention at the level of calcium homeostasis improves mitochondrial and axonal health in the neuronal models of Wolfram Syndrome. Dr Allen Kassik – University of Tartu, Estonia.

(12:30- 1.30) Lunch

(1:35- 2:05) Investigating Wolframin-dependent cellular dysfunctions and correct them by cell-type specific gene therapy. Dr Niccolò Vanni. Division of Neuroscience, San Raffaele Scientific Hospital & Vita-Salute San Raffaele University *(Unable to attend)*

(2:10-2:55) Targeting defective autophagy for the treatment of Wolfram syndrome. Dr. Sovan Sarkar - University of Birmingham, UK

(3:00 – 3:45) Clinical biomarkers for Wolfram syndrome. Prof Patrick Yu Wai Man - University of Cambridge and Moorfields Eye Hospital, UK

(4:15 – 5:00) Therapeutic Development for Wolfram Syndrome Oral medications, Gene therapy, Regenerative therapy. Dr Fumihiko Urano - Samuel E. Schechter Professor of Medicine, Washington University School of Medicine, St. Louis, USA

(5:05-5:30) AMX0035 HELIOS study updates. Dr Nathalie Erpelding, PhD - Amylyx Pharmaceuticals

(5:30-5:50) Rounding up of Day. Dr Fumihiko Urano

(7:00) Dinner

Agenda: Day 2 (24th October 2024)

Presentations

(9:00-9:45) Bridging Wolframin structural alterations, non-sense mediated decay and immunological outcomes in Wolfram Syndrome. Dr Raniero Chimenti - Vita-Salute San Raffaele University, Milan, Italy. Diabetes Research Institute (DRI), IRCCS San Raffaele Scientific Institute, Milan (Italy) ***(Presented via Zoom)***

(9:45-10:20) Neurodegeneration in Wolfram Syndrome: Casting a Wider Net. Dr Nayana Gaur PhD - University of Tartu, Laboratory Animal Centre. Tartu, Estonia.

(10:50-11:35) Genome editing in non-dividing neural cells, and creation of models to study CNS defects in WS. Prof Catherine Verfaillie - Stem Cell Institute, KU Leuven, Belgium.

(11:35-12:20) Characterization of the visual system phenotype of a novel knockin Wfs1 mouse line, and proof-of-concept study of base editing as a therapy to preserve vision. Prof Lies de Groef - Cellular Communication and Neurodegeneration Research Group, Biology Department, KU Leuven, Belgium.

(12:30- 1.30) Lunch

(1:30- 2:00) Paediatric onset gonadal dysfunction in Wolfram Syndrome Type 1. Dr Giulio Frontino - IRCCS San Raffaele Hospital, Milan, Italy. ***(Presented via Zoom)***.

(2:00- 2:20) TREATWolfram. Prof Timothy Barrett - Leonard Parsons Professor of Paediatrics, NIHR Senior Investigator. Director, Centre for Rare Disease Studies. Department of Cancer and Genomic Sciences - University of Birmingham, UK. Honorary Consultant Paediatric Endocrinology and Diabetes - Birmingham Women's and Children's Hospital NHS Trust

(2:25-2:45) Clinical Guidelines. Dr Fumihiko Urano MD, PhD - Samuel E. Schechter Professor of Medicine, Washington University School of Medicine, St. Louis, USA

(2:45-3:00) Rounding up of the day's sessions to create action points if any & closing comments.

Day 1 (23rd October 2024)

1. Digestive Disorder in Wolfram Syndrome. Dr Christophe Orssaud - Ophthalmology Unit, Rare Disease Centre, Paris City University, Paris, France.

Abstract: Type 1 Wolfram syndrome is an autosomal recessive neurodegenerative pathology leading to insulin-dependent diabetes, optic atrophy, hearing loss, diabetes insipidus, neurological manifestations and urinary disorders. A patient association alerted us about the existence of fecal incontinence disorders. To clarify its frequency, we addressed a questionnaire to our 37 patients with WS.

We received 25 answers (67.57%). Seventeen patients reported fecal incontinence and constipation (45.95 % of the whole group). On an analog scale, the severity of this disorder is rated at 8.23 +/- 2.86. In addition, to identify the mechanism of this trouble, we retrospectively analysed data from patients with or without fecal incontinence disorders. We noted the duration of the WS and the age at the last consultation, the existence of vesico-sphincter disorders, swallowing disorders, sleep apnoea and diabetes insipidus. Twenty-three patients were included in the retrospective study, 12 of them presented with fecal incontinence disorders. There is no correlation between the presence of fecal incontinence and the age of the patients ($p = 0.28$) nor with the mutations ($p = 0.066$). Ano-sphincteric disorders are significantly more frequent in cases of urinary disorders ($p < 0.001$) or, to a lesser degree, sleep apnoea ($p = 0.03$). No significant association could be demonstrated with the presence or absence of swallowing disorder ($p = 0.10$) or diabetes insipidus ($p = 0.16$).

In a preliminary study, we found that half of the WS patients complained with fecal incontinence disorders which was a severe handicap for most of them. This real symptom of WS and must be taken into account and treated. The origin seems to be a neurological attack as evidenced by the correlation between these ano-sphnicteric disorders and urinary disorders of the neurological bladder type. The management of these ano-sphincteric disorders is difficult and further studies remain necessary to better adapt the treatment.

It is important to make this symptom known in order to better manage it.

Points noted:

- Bowel disorders and faecal incontinence (FI) in WS patients are not widely reported in the literature.
- Patients who experience these issues are often reluctant to discuss them due to feeling of embarrassment or shame.
- The goal of this research was to highlight FI as a symptom of WS and consider how best to manage and treat these issues.
- The short questionnaire developed for this research received ethics committee approval and included only two simple questions – a) whether patients experience faecal issues (yes/no) and b) the severity of the issues experienced (scale -1-10). Patients who did not respond to the questionnaire were assumed not to experience FI or other bowel issues.

- Given the simplicity of the questionnaire, this study could potentially be repeated in other WS patient populations to increase the sample and provide further evidence of FI issues in WS patients. This could potentially involve WS patient organisations that have strong links to their local WS communities (e.g. The Snow Foundation and WSUK).

2. Predictive Value of Genetics in Central Nervous System Neurodegeneration in Wolfram Syndrome. Dr Gema Esteban- Bueno, Ángel Miguel Roldán Molina. Spanish Multidisciplinary Wolfram Syndrome team. Almería, Spain.

Abstract: *Main Objective: Highlight the relevance of central nervous system involvement in patients with Wolfram syndrome.

*Specific Objectives:

1. Analyse the main causes of death in WS patients.

2. Evaluate the possible correlation between neurological symptoms and genetic factors.

3. Correlate clinical findings with pontocerebellar atrophy.

Methods: A sample of 45 patients diagnosed between 1999 and 2024 was analysed, consisting of 25 males (55.5%) and 20 females (44.5%). Of these, 10 patients died, 7 due to causes related to the central nervous system at an average age of 33.43 years (standard deviation [SD]: 11.17) and 3 due to renal failure at an average age of 39 years. (SD: 9.67).

The average age of the 35 living patients in 2024 is 27.52 years (SD: 11.08).

The following neurological symptoms were evaluated: brainstem involvement (dysphagia, absence of the gag reflex, choking, sialorrhea, dysmetria, gait instability, and ataxia), cortical manifestations (dementia), and signs of peripheral nervous system involvement. It was noted that when assessing intellectual coefficients in childhood and adolescence, no significant cognitive decline was observed, except in one patient with developmental delay since childhood.

*Statistical Analysis: The Random Forest algorithm was used, employing decision trees to analyse the most relevant genetic variables and their correlation with neurological symptoms.

*Imaging Results: Magnetic resonance imaging (MRI) of 19 patients was evaluated. Due to the geographic dispersion of the sample and heterogeneity in the equipment used for MRIs, the analysis focused on four morphological aspects:

1. Diffuse restriction in periventricular white matter.

2. Increased signal intensity in subcortical white matter of the occipital lobes, visible on FLAIR sequence.

3. Multifocal restriction of diffusion in the pons and cerebellar peduncles.

4. Decreased anteroposterior diameter of the pons, this alteration being particularly consistent among patients with Wolfram syndrome.

It was observed that, while the space between the bony canal and the anterior surface of the pons is typically 7-8 mm in healthy subjects, it consistently measured between 10 and 12 mm in the analysed patients.

***Conclusions:** This study aims to complete an analysis in depth, that could allow the use of algorithms to cross-relate genetic variables, neurological symptoms, and morphological measurements of the brainstem, since the main cause of death is related to failures in this brain region. Thus, it is crucial the early identification of patients potentially predisposed to brainstem disorders.

The most predictive genetic mutation for central nervous system deterioration, in our population is the p.Val142fs* mutation in proteins 1 and 2 associated with the syndrome. This finding will enable preventive measures against symptoms such as dysphagia, recommending thickening agents and speech therapy exercises to avoid complications such as choking or aspiration pneumonia.

Points noted:

- The youngest patient who died was 16 years and the oldest was 50 years – highlighting the wide variation in this patient population. Brain stem failure was identified as the cause of death.
- The research focused on identifying which mutation was most linked with each of the symptoms studied using mathematical models. The researchers shared examples of their modelling results for several of the symptoms studied. The most predictive mutation of CNS impairment was p.Val142fs* mutation in proteins 1 and 2.
- During the discussion, it was noted that although there is correlation between the type of mutations in the WFS1 gene and the severity of the manifestation of symptoms, patients with the same genetic mutations (e.g. siblings, twins) can manifest differently due to differences in gene expressivity and / or environmental or genetic factors.
- The researchers have not assessed the spinal cord as part of this study.

3. Preclinical Gene Therapy for Rare Nervous System Disorders: Progress on the Wolfram Syndrome Project. A Bosch (1), M Page (1), I Fernandez (2), D Ramos (3), J Ruberte (3), M Chillon (2), A Consiglio (2), A Sanchez (1). (1) Institute for Neurosciences, Universitat Autònoma de Barcelona, Spain; (2) Idibell, Universitat de Barcelona, Spain; (3) CBATEG, Universitat Autònoma de Barcelona, Spain.

Abstract: Gene therapy offers a promising approach for treating chronic, progressive monogenic diseases by enabling targeted correction of specific organs or cell types through a single treatment. Our research group has extensive experience in developing preclinical gene therapy strategies for rare diseases affecting the central nervous system, primarily using adeno-associated viral (AAV) vectors. In the Wolfram Syndrome project, we are developing a gene therapy approach using three distinct models. These include two in vitro models -2D and 3D patient-derived

systems- generated from fibroblasts reprogrammed into induced pluripotent stem cells (iPSCs) and subsequently differentiated into neurons and organoids. In these models, we are investigating the molecular mechanisms underlying disease progression and evaluating the extent of correction following gene therapy. Additionally, the efficacy and safety of the vector will be tested in vivo using WFS1 knockout mice. Specific AAV vectors have been developed to assess biodistribution after various routes of administration in vivo. By integrating results from these three models, we aim to validate the proof of concept for gene therapy in Wolfram Syndrome.

Funded by the Alianza de Familias Afectadas por el Síndrome de Wolfram (AFASW), Spain.

Points noted:

- This group is based in Barcelona, Spain and is part of a research and academic institution with links to a local hospital. Their research is focused on several rare and ultra rare diseases, which is promoted by local family associations. They have been working on WS for the last 18 months.
- Research involving gene therapy has increased over time. There has been an increase in published papers since the 1990s and an increase in the number of clinical trials – particularly in the last 5 years. Some gene therapy-based treatments have been approved to date. This area of research is exciting and progressing very quickly. Many more clinical trials involving gene therapies for other diseases are expected in the future.
- The most common rare disease that the team work on is Amyotrophic lateral sclerosis (ALS), which causes loss of muscle control. Using gene therapy, the group has created modified ALS muscle cells which survive much longer and are more innervated than unmodified ALS cells. Delays in clinic onset are also seen in ALS animal models. A patent for this treatment has been filed and a clinical trial is planned to start next year, involving a commercial group.
- For WS, the group has developed stable WS cell models (iPSC) which differentiate as anticipated into neurons and organoids. The iPSCs are available to the research community in Spain through a BioBank. The group plan to explore the effect of gene therapy of these cell models.
- One challenge of gene therapy is to express the right amount of the modified protein at the right time. Over expression can be toxic due to the formulation of aggregated protein in cells. How much WFS-1 protein cells need to express is currently unknown.
- Studies have not yet been conducted in WS animal models, both the vector and the route of administration are being selected. The intention is to also investigate the eye and inner ear, but the dose of the vector will also need to be considered for safety reasons.
- One key challenge is how to translate gene therapy into a commercial drug as the cost of a new potential treatment will be very high. Companies are only likely to invest in a new drug if there are many patients to treat. Therefore, this represents a major challenge for rare diseases. Initiatives need to be

developed by the FDA (US regulator) and others to overcome / decrease these high treatment costs. One potential option is to create a bespoke platform which brings like rare diseases together and involves countries with the highest populations of patients.

4. Endoplasmic reticulum stress, mitochondrial morphology and calcium dynamics in cellular model of Wolfram syndrome. Michal Cagalinec[1], Silvia Borecka[2], Mohammad Adnan[1],3, Marek Sklenar[2], Martina Skopkova[2], Dominika Hromnikova[2], Alexandra Zahradnikova[1], Daniela Gasperikova[2]

[1]Dept. of Cellular Cardiology. [2]Dept. of Metabolic Disorders, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia. [3]Dept. of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia.

The abstract will be available following publication of the data presented.

5. Pharmacological intervention at the level of calcium homeostasis improves mitochondrial and axonal health in the neuronal models of Wolfram Syndrome. Dr Allen Kassik – University of Tartu, Estonia.

No abstract provided.

Points noted:

- The primary defect in WS is related to reduced calcium in the Endoplasmic reticulum (ER). Defects in the mitochondria drive disease progression. It is unclear why there are mitochondrial issues and associated calcium impairment and how relevant these are to cellular function.
- In WFS-1, is the reduction in mitochondrial calcium due to i) reduced calcium in the ER; ii) impaired transfer from the ER to the mitochondria or iii) does calcium leak out from the mitochondria? Over expression studies provide support for all 3 hypotheses.
- How relevant is the insufficiency in mitochondrial calcium? WS deficient cell models reveal that insufficient mitochondrial calcium leads to insufficient Kreb's cycle, reduced ATP (i.e. reduced energy production) and increased oxidative stress.
- Studies involving pharmacological interventions to increase mitochondria calcium restored mitochondrial density and morphology and partially rescued ATP production. In addition, most compounds tested also rescued axonal length, which is reduced in cells with reduced WFS-1. Therefore, compounds that increase mitochondrial calcium directly and indirectly improve mitochondrial and axonal health. WS can provide a disease prototype for other conditions with similar mitochondrial issues.
- WS type 2 is due to mutations in the CISD2 gene. Although the CISD2 protein is a very different from WFS-1, mutations in CISD2 lead to reductions in ER calcium and mitochondrial issues and reduced ATP. Overexpression of either

CISD2 or WFS-1 in cell models restores calcium levels and ATP, highlighting that these two proteins can compensate for each other. Could CISD2 therefore represent a target to improve ER calcium transfer?

- It was noted in the discussion that over-expression of CISD2 by gene therapy would provide rescue of calcium levels but is toxic. WFS-1 and CISD2 double knock out models also show toxicity, and an additive effect is not really seen.
- It was also noted that there are patients with variations in both WFS-1 and CISD2.

6. Investigating Wolframin-dependent cellular dysfunctions and correct them by cell-type specific gene therapy. Niccolò Natanaele Vanni[1], Valerio Castoldi[2], Serena Gea Giannelli[1], Vania Broccoli[1,3].

1) Stem Cell & Neurogenesis Unit, *Division of Neuroscience*, *San Raffaele Scientific Hospital*; *Vita-Salute San Raffaele University*, *Milan, Italy*

2) Experimental Neurophysiology Unit, Institute of Experimental Neurology, San Raffaele Scientific Hospital

3) CNR - Research National Council, Institute of Neuroscience, Milan, Italy.

Presenter Dr Niccolò Vanni was unable to attend. The slides and notes from the presentation are available on the WSUK website at https://wolframsyndrome.co.uk/.

Abstract: Progressive optic atrophy leading to relentless blindness is caused by the selective loss of retinal ganglion cells (RGCs) and their axons in the optic nerve and represents the major pathological burden of Wolfram syndrome. We have demonstrated that RGCs' degeneration is triggered by surrounding glial cells' dysfunctions, which compromise the correct transfer of bioenergetic molecules, such as lactate and pyruvate, fundamental for the function and survival of RGCs. This chronic hypometabolic state is particularly detrimental for RGCs' axons, which require very high levels of energy supplies given their long and myelinated axons. Through a single-cell analysis of RGC morphology before and during disease manifestation in Wolfram mice, we collected evidence that RGC axonal degeneration precedes cell bodies and dendritic degeneration, confirming the essential axonopathic nature of Wolfram syndrome. Moreover, we also confirmed and further extended Wolframin's role in regulating selected newly synthesized transmembrane proteins. Finally, our AAV-mediated gene replacement therapy consists in restoring a functional copy of the WFS1 gene in either retinal RGCs, glia or both, using different AAV serotypes and delivery routes, to understand which therapeutic intervention would promote the best physiological recovery.

7. Targeting defective autophagy for the treatment of Wolfram syndrome. Dr. Sovan Sarkar & Dr. Malgorzata Zatyka - University of Birmingham, UK.

No abstract provided.

Points noted:

- Dr Sarkar works with Prof. Tim Barrett at the University of Birmingham.
- Autophagy is a cellular process essential for the survival of cells. Autophagy occurs in the cytoplasm of cells by the formation of a double membrane structure termed phagophore, which elongates to form an autophagosome. During autophagosome biogenesis, unwanted cellular materials, termed autophagic cargo, comprising of damaged organelles (e.g. mitochondria) and undesirable macromolecules (e.g. aggregated proteins) are engulfed within the autophagosome. The autophagosome fuses with a lysosome (a cell organelle) to form an autolysosome. The enzymes contained within the lysosome degrades the cargo, thereby removing the cellular rubbish (i.e. the cargo) that would be otherwise toxic to the cell. The breakdown products are reused by the cell to generate energy. This process is responsible for cellular homeostasis ("housekeeping") to maintain normal functioning of the cell.
- Impairment of autophagy has been implicated in many diseases including various neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic lateral sclerosis (ALS)). The autophagy process degrades a wide variety of cellular debris including mutant proteins found in some diseases (e.g. Huntington's disease).
- Autophagy is regulated by several different pathways (mTOR and mTOR independent). There is therefore a wide choice of biomedical inducers of autophagy. The mTOR-independent inducers are clinically desirable because mTOR has critical cellular functions and therefore not ideal to inhibit for autophagy induction.
- In a study led by Malgorzata Zatyka (this research group), loss of WFS-1 was found to be associated with reduced autophagy. In WS cell models such as human neuroblastoma cell model with *WFS-1* knockdown and patient fibroblasts, there is a reduction in the number of autophagosomes, and consequently, an accumulation of autophagic cargo. This was also observed in clinically relevant cell models such as patient induced pluripotent stem cell (iPSC)-derived neurons (brain cells) neuronal stem cells (the precursors of brain cells; cell lines obtained in collaboration with Laetitia Aubry, France). Additionally, accumulation of autophagic cargo was found in different brain regions in a rat model of Wolfram syndrome (in collaboration with Mario Plaas, Estonia).
- Autophagy inducers was found to be cytoprotective in WS cell models and patient-derived neuronal cells by increasing autophagy, and reducing aggregate formation, mitochondrial dysfunction and cell death.
- Drugs known to increase autophagy that can cross the blood brain barrier could represent potential treatment candidates for WS. This current research is exploring both investigational and FDA approved drugs for repurposing in WS. To date, four test compounds that are FDA approved autophagy-inducing drugs have been shown to significantly improve the survival of patient-derived neuronal cells and rescue disease-relevant cellular phenotypes.
- In a separate study involving knockout of essential autophagy genes, loss of autophagy in human neurons has been shown to mediate neuronal cell death

via depletion of cellular levels of nicotinamide adenine dinucleotide (NAD), a coenzyme involved in metabolism. Since NAD depletion was identified as a key mediator of cell death, increasing NAD levels by NAD-boosting nutritional supplements was found to be cytoprotective in autophagy-deficient cells.

- Since WS is associated with autophagy malfunction, in another study led by Congxin Sun (this research group), NAD depletion was also observed in WS cell and neuronal models where NAD boosters were found to be cytoprotective (4 different NAD boosters were tested). These NAD boosters also rescued the hyperlocomotion phenotype in a zebrafish model of WS (in collaboration with Benjamin Delprat, France).
- Impairment in autophagy is therefore a major contributory factor in neurodegenerative diseases through NAD depletion and potentially other mechanisms. Therefore, drugs rescuing these cellular deficits could be an effective treatment in WS and other neurodegenerative diseases.
- Question during this disscussion (Mario Plass) – Insulin is known to be an autophagy inhibitor and may be toxic in some conditions. Could insulin treatment contribute to islet cell death in WS through an autophagy dependent mechanism?

8. Clinical biomarkers for Wolfram syndrome. Prof Patrick Yu Wai Man - University of Cambridge and Moorfields Eye Hospital, UK.

No abstract provided.

Points noted:

- Prof. Yu Wai Man presented his personal perspectives on the development of treatments for WS.
- The selective vulnerability of retinal ganglion cells (RGCs) in WS cannot be fully explained by a lack of ATP and impaired mitochondrial dynamics. Other neuronal cell populations with similarly high metabolic demands are not as vulnerable as RGCs. Their vulnerability is more complex and likely to involve other cells / processes (e.g. lack of myelin).
- As translational research takes considerable time and is very expensive, multi-centre collaborations involving industry partners will be needed.
- WS communities will need to be made aware that not all patients will benefit from any potential new treatment.
- To move potential new treatments into clinical practice, robust quality-of-life (QoL) and patient reported outcomes (PROs) will be needed, along with specific surrogate markers for identified structural and functional outcome measures.
- Another challenge is the reimbursement of new treatments, which are lengthy, convoluted processes that differ across countries

- The disease progression of WS is currently not fully understood. For example, there is heterogeneity in visual decline across WS patients. Those with recessive mutations in WFS-1 typically experience visual decline more quickly than patients with a dominant form. The cells and processes responsible for these differences need to be further explored.
- In the future, imaging techniques such as Flavoprotein fluorescence (FPF) will used as an indicator of mitochondrial oxidative stress in ocular disease.
- New AI technologies are being investigated that will enable patients to measure their vision at home using a smart phone. For example, a screening tool is being developed to distinguish between children who have normal vision from those with abnormal vision, to identify visual impairment at a very young age. Visual measurements made by patients at home could complement formal measurements taken by HCPs during hospital appointments.
- Other techniques are being developed to e.g. i) assess eye movements to identify specific abnormalities in the brain stem and cerebellum, and ii) collect tears as to measure markers of disease severity.
- Researchers agreed that a clinical trial methodologist is needed to help design future WS trials and help prepare the clinical data for the FDA / Europe.
- Specific markers of clinically relevant worsening need to be defined based on the natural history of the disease.

9. Therapeutic Development for Wolfram Syndrome Oral medications, Gene therapy, Regenerative therapy. Dr Fumihiko Urano - Samuel E. Schechter Professor of Medicine, Washington University School of Medicine, St. Louis, USA.

No abstract provided.

Points noted:

- In WS the median age for symptom onset is: - Diabetes Mellitus 6 years; optic atrophy 11 years; deafness 14 years; neurodegeneration later years of adolescence.
- Complications of WS include among others neurogenic bladder and bladder incontinence (very common); respiratory failure, choking and brain stem atrophy.
- Most autosomal recessive patients (i.e. classical or syndromic WS) have mutations in the WFS-1 gene. The current estimate prevalence of WS is between 1:250,000 – 1:700,000.
- WS type 2 patients have mutations in the CISD2. These patients also experience GI issues.
- Genotype and phenotype data are now available for approximately 500 autosomal recessive WS patients. In this population, female patients typically have milder symptoms, which manifest later compared with males. Specific

types of mutations are associated with milder manifestations (missense mutations) or more severe forms (frameshift mutations) of WS.

- There is also a variant seen in the Ashkenazi Jewish population, which is associated with milder manifestations of WS.
- A WS severity categorisation system is being developed from 1 most mild (both mutations are in frame) to 6 most severe (neither mutation is in frame). Work to further modify the severity scores is on-going.
- There are also several autosomal dominant forms of WS (often referred to as WFS-1 related forms), which are associated with different types of mutations in the WFS-1 gene.
- The goal of WS treatments is to 1) slow progression (oral medications); 2) halt progression and 3) revive damaged cells (gene editing).
- A clinical trial aimed to repurpose the existing marketed drug Dantrolene for use in WS. 19 WS patients completed the open label Dantrolene trial. There was no significant improvement in study outcomes, although some improvement was seen in pancreatic beta-cells in some patients. These findings were not consistent with preclinical data from animal models. It was noted that dose of dantrolene used in the clinical trial could not be increased due to the increased risk of side effects.
- In collaboration with Amylyx Pharmaceuticals, AMX-0035 is currently being investigated in a WS clinical trial of WS patients. AMX-0035 is a combination of two compounds sodium phenylbutyrate and taurursodiol (TURSO), which work through different mechanisms to reduce endoplasmic reticulum stress and mitochondrial dysfunction respectively.
- In pre-clinical cell models, AMX-0035 reduced cell death and improved glucose control and restored mitochondrial function.
- WS patients have syndromic monogenic diabetes, where a single gene abnormality causes Diabetes Mellitus. Most patients (98%) diagnosed with Diabetes Mellitus have either Type 1 or Type-2, which are polygenic forms, involving multiple genes. WS is one form of monogenic diabetes.
- Many WS patients experience a delayed glucose spike and c-peptide spike after food. By measuring C-peptide levels, the level of insulin produced by the pancreas can be assessed.
- In the clinical trial of AMX-0035, C-peptide levels are measured using a mixed meal tolerance test. Other measures of glucose control are also included in the trial such as the time in target glucose range, level of HbA1c and exogenous insulin dose (for more details on this clinical study refer to the next presentation by Amylyx Pharmaceuticals).
- The sigma 1 receptor agonist Pridopidine could also be potentially beneficial in WS based on current preclinical data. (The biotech company Prilenia are providing Pridopidine for preclinical studies).

- Base / prime editing is also being investigated in collaboration with other researchers. The best combination of base editor and signal guide RNA (sgRNA) is currently being tested.
- The neurotrophic factor MANF is also being exploring as a potential regenerative therapy for optic atrophy.
- The aim is to treat patients very early with gene therapy e.g. at the time when they are diagnosed with Diabetes Mellitus.

10. AMX0035 HELIOS study updates. Dr Nathalie Erpelding, PhD - Amylyx Pharmaceuticals.

No abstract provided.

Points noted:

- AMX0035 is an investigational product that has not yet received regulatory approval in any market.
- AMX-0035 is a combination of sodium phenylbutyrate and taurursodiol (TURSO), which work through different mechanisms to reduce endoplasmic reticulum stress and mitochondrial dysfunction, two connected central pathways that lead to cell death and neurodegeneration.
- In preclinic models, AMX-0035 increased insulin secretion and improved beta cell viability in patient derived cells and delayed progression of Diabetes Mellitus in mice.
- Helios is a single arm, open label, 96-week US clinical trial of AMX-0035 of 12 adults living with WS. The primary endpoint is the change in baseline of C-peptide levels following a 240 min mixed meal tolerance test. Secondary endpoints include HbA1c levels, level of exogenous insulin dose, time in target glucose range and visual acuity.
- Key trial inclusion criteria include: - 17+ years of age, a confirmed diagnosis of WS with a homozygous mutation; receiving insulin, but no GLP-1 and C-peptide of a least a defined minimum level (>0.2ng/ml). All patients were using a continuous glucose monitor (CGM) before starting the study.
- Of the 12 patients, 1 patient did not quite meet the genetic inclusion criteria for the study (confirmed mutation in 1 allele). Therefore, there are two groups in the analysis– intent to treat (12 patients) and per protocol (11 patients).
- There was improvement in pancreatic cell function, as measured by the C-peptide response at week 24, the primary efficacy endpoint. A stronger effect was seen at later timepoints (not all patients have reached the later timepoints at the time of the analysis).
- There was overall improvement or stabilization of all secondary endpoints including HbA1c levels, time in target glucose range and visual acuity.

- Patient and physician-reported global impressions of change were either stable or improved from baseline in all participants, meeting predefined responder criteria.
- AMX-0035 was well tolerated, with good safety profile. 0 patients discontinued the study due to side effects and no severe adverse events were reported. All side effects were mild or moderate and the most common was diarrhoea.
- The limitations of the study include the open label study design, the small sample size of 12 patients.
- Analyses will be conducted at 48 weeks and later timepoints, which will provide further insights and help inform a Phase III programme, which is currently being developed.

11. Rounding up of Day. Dr Fumihiko Urano

Points noted:

- At the time of the first Symposium 15 years ago, there were no drug candidates for WS – now several have been identified or are under investigation.
- The WS research community now needs to consider how to design new WS clinical trials. An expert in clinical trial design needs to be identified and invited to join the WS research community

Day 2 (24th October 2024)

12. Bridging Wolframin structural alterations, non-sense mediated decay and immunological outcomes in Wolfram Syndrome. Dr Raniero Chimenti - Vita-Salute San Raffaele University, Milan, Italy. Diabetes Research Institute (DRI), IRCCS San Raffaele Scientific Institute, Milan (Italy) ***(Presented via Zoom)***

Abstract: Wolfram Syndrome 1 (WS1) is a rare genetic disorder causing childhood-onset diabetes mellitus and optic nerve atrophy due to mutations in the *WFS1* gene. These pathogenic variants affect the endoplasmic reticulum (ER)-associated protein Wolframin, leading to a wide range of symptoms. Understanding genotype-phenotype correlations is crucial for predicting disease outcomes and developing targeted therapies. We investigated iPSC-derived β cells from a WS1 patient with compound heterozygous WFS1 mutations: c.316-1G>A, affecting the splice site upstream of exon 4, and c.757A>T, introducing a premature termination codon in exon 7. The c.316-1G>A mutation caused alternative splicing, producing both nonsense-mediated decay (NMD)-regulated transcripts and open reading frame (ORF)-conserving mRNAs, resulting in truncated but partially functional Wolframin proteins. These proteins retained the C-terminal domain essential for ER stress response but failed in proper insulin processing and calcium dynamics, correlating with altered gene expression. Notably, Wolframin defects led to a systemic pro-inflammatory status in this patient, exacerbating ER stress, inhibiting NMD, and

causing further accumulation of defective WFS1 transcripts, which promoted β cell apoptosis. Additional analysis of immune cells from WS1 patients with missense and/or frameshift mutations revealed a WFS1 variant-dependent pro-inflammatory profile, characterized by elevated cytokine production, CD4+/CD8+ T cell activation, along with alterations in Natural Killer (NK) cell subsets. These immune changes underscore the link between WFS1 mutations, protein structural alterations, and chronic inflammation, which impair β cell function and survival. Moreover, the inflammation-dependent NMD inhibition exacerbates the accumulation of mutated transcripts and truncated proteins, highlighting a novel pathogenic mechanism and potential target for therapeutic intervention.

Points noted

- There are multiple pathogenic variants of WFS-1 with differing severity in manifestations. There is no absolute classification of these variants.
- A heterozygous variant of WFS-1 was investigated to understand the potential functional implications of the specific mutations.
- Using patient derived iPSC cell models, variant transcripts and proteins were identified, which impaired insulin processing and calcium flux mechanisms, increased inflammatory status and beta cell death.
- Chronic inflammation and immune cells dysfunction may be a new feature of a cohort of WFS-1patients.
- Is inflammation the trigger for beta cell death and could nonsense-mediated decay (NMD) dysfunction represent the link between inflammation and cell damage? Could inflammation and NMD therefore represent a new target for treatment? This requires further exploration in animal models.

13. Neurodegeneration in Wolfram Syndrome: Casting a Wider Net. Dr Nayana Gaur PhD - University of Tartu, Laboratory Animal Centre. Tartu, Estonia.

The abstract will be available following publication of the data presented.

14. Genome editing in non-dividing neural cells, and creation of models to study CNS defects in WS. Prof Catherine Verfaillie - Stem Cell Institute, KU Leuven, Belgium.

No abstract provided.

Points noted:

- An aim of the research is to explore which cell type(s) should be targeted in the WS retina. Base editing aims to correct the gene at the target level and reduces some of the major risks associated with conventional gene therapy such as cell toxicity.
- Base editing was successful in studies using a non-dividing cell line HepG2.

- iPSCs derived wild type non-dividing neuronal cells (including RGCs and astrocytes) were successfully mutated (85-100%) using base editing.
- In iPSC WFS-1 patient derived cell line (provided by Dr Urano), although base editing was achieved, it was not as successful. Therefore, a new vector with a narrower base editing window was selected (ABE9). Using this vector, base editing was successful in both oligodendrocytes and RGCs (78-96%) derived from the iPSCs WFS-1 cell line.
- Immature oligodendrocytes, developed from iPSC cell lines (provided by Dr Urano), that were corrected with base editing, showed no obvious signs of ER stress, mitochondrial dysfunction or changes in calcium flux. These immature cells were not yet making myelin.
- More complex in vitro models of myelinated cells using myelin spheres and 3D models are currently being developed to explore the interactions between the neural cell types and identify which cells drive optic atrophy in WS.

15. Characterization of the visual system phenotype of a novel knockin Wfs1 mouse line, and proof-of-concept study of base editing as a therapy to preserve vision. Prof Lies de Groef - Cellular Communication and Neurodegeneration Research Group, Biology Department, KU Leuven, Belgium.

No abstract provided.

Points noted:

- A Knock-In (KI) WS mouse model has been developed by introducing DNA point mutations that humanise a section of mouse DNA and therefore, will more closely reflect human disease.
- At 6 months of age, +50% of WS KI males experience sudden death (not due to wasting). This effect is less pronounced in females.
- WS KI mice are smaller than wild type and these difference in weight can easily be seen by eye.
- WS KI mice have high blood glucose levels, which decline more slowly than wild type, and develop Diabetes Mellitus.
- Visual acuity is normal in WS KI mice for the first 3 months and begins to decline from 4 months onwards. This provides a good therapeutic window for research i.e. therapies can be administered in the 8 weeks before the start of visual decline at 4 months. The visual decline precedes the degeneration of RGC. A longer time-period is therefore needed to assess RGC decline. The retinal dysfunction is mostly a result of degradation of RGCs.
- Microglia are stressed in WS KI mice, with increased proliferation and changes in morphology, and increases in other markers of stress (e.g. activation of the unfolded protein response and increase expression of BiP). No clear differences in CHOP were detected, suggesting that the cells have not yet reached pathological levels of stress.

- The next important goal is to establish proof of concept of base editing as a potential therapy to preserve vision using the WS KI mouse model. Two initial challenges are to identify the most appropriate target cell type and the most efficient base editing vector. Studies have identified that oligodendrocytes are not the key cell type to target (these cells are largely able to conserve their function), while two ABE9 AVV vectors are needed (too large for 1 vector). Further studies are being conducted to assess the transfection rate. The aim of these studies is to explore the potential of base editing as a therapy to preserve vision in WS.
- A SWOT analysis of base editing as a potential therapy in WS highlighted the following:
    - Strengths – i) KI is based on specific gene corrections. (There is a risk cell toxicity if gene is over expressed); ii) A pilot study will represent an important milestone – a measure that can potentially be used in the clinic; iii) Studies will increase the understanding of WS disease mechanisms, which can be leveraged by others. The researchers are willing to share the WS KI mouse model with other groups.
    - Weaknesses – The development and manufacturing costs will be extremely high, which will require public and private partnerships, potentially through its applicability to other neurodegenerative diseases.
    - Opportunities – Potential to explore hearing phenotype and heterozygous WS carriers.
    - Threats – i) Requires broad stakeholder involvement. The regulatory environment is not yet ready to evaluate these types of therapies. The WS research community will need positively influence regulators; ii) Years of work are required to develop a potential treatment that is ready for clinic (a single intravitreal treatment); iii) Better diagnosis of WS will increase the number of patients (e.g. WS 1:100,000) and the number of different types of WS, leading to increased demand for treatment with potentially greater complexity.

16. Paediatric onset gonadal dysfunction in Wolfram Syndrome Type 1. Dr Giulio Frontino - IRCCS San Raffaele Hospital, Milan, Italy. ***(Presented via Zoom).***

No abstract provided.

Points noted:

- Hypogonadism (i.e. low levels of sex hormones) is a common feature of WS, which is typically underestimated. 1-3 out of 10 adults are reported to experience hypogonadism.
- Hypogonadism is also seen in other neurogenerative diseases such as Alzheimer's, Huntington's, Parkinson's diseases and Multiple Sclerosis. There is a sex bias – prevention and progression differ between sexes. Oestrogens help to protect against oxidative stress through mitochondrial biogenesis and

mitochondrial bioenergetics. By contrast, androgens may exacerbate neurodegeneration at the mitochondrial level.

- As sex hormones play a role in oxidative stress and mitochondrial function and health, hypogonadism could worsen progression of WS.
- In a small sample of 8 children / young people with WS, with different mutations in WFS-1, including 1 with dominant (non-classical, WS related forms), 50% experienced primary hypogonadism (67% males and 40% females). These findings support the hypothesis that hypogonadism is underdiagnosed in WS patients and may be more frequent than currently reported.
- Early diagnosis of gonadal deficiency in WS patients will enable patients to be treated with hormone replacement therapy (HRT) and thereby prevent delayed puberty age, a clinical feature of WS. HRT may help to slow down WS progression in these patients by helping to reduce ER stress and improve mitochondrial health.
- Ovarian tissue from a young WS female patient with low levels of LH, FSH and oestradiol has been frozen to help her fertility in later life. A study to assess the structural characteristics of these tissues is on-going.
- A phase 2 clinical trial is to assess tirzepatide, a dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 receptor (GLP-1) agonist, as a monotherapy in WS patients has been initiated in Italy. The study will assess whether tirzepatide can improve glucose control and preserve beta cell function.
- The primary outcome measure is C-peptide response and change from baseline following mixed meal tolerance test. The secondary outcome measures include additional glucometrics and other clinical manifestations of WS, including gonadal dysfunction.
- During the discussion, it was noted that there are some differences between the proportions of WS affected males and females who experience gonadal dysfunction. No sex bias was seen in one US study, but the sample size was small (n=10), while typically more WS affected males with gonadal dysfunction are seen in the UK compared with females.

17. TREATWolfram. Prof Timothy Barrett - Leonard Parsons Professor of Paediatrics, NIHR Senior Investigator. Director, Centre for Rare Disease Studies. Department of Cancer and Genomic Sciences - University of Birmingham, UK. Honorary Consultant Paediatric Endocrinology and Diabetes - Birmingham Women's and Children's Hospital NHS Trust.

No abstract provided.

Points noted:

- The rationale for selection of sodium valproate in the TREATWolfram trial includes: – sodium valproate i) reduces cells death; ii) is licensed for use in children; iii) there is freedom of use to operate; iv) crosses the blood brain barrier.

- TREATWolfram is a phase 2 efficacy randomised, double blind, multi-centre clinical trial, funded by the Medical Research Council (MRC). Patients were randomised 2:1 and were required to complete 11 visits in 3 years.
- The primary outcome is the effect of sodium valproate on progression of visual acuity loss. The biomarker for visual acuity in the trial is the rate of progression in visual acuity loss over time. A positive outcome will be considered as better than 50% reduction in rate of loss. The aim is to provide patients with useful vision into early adulthood and a meaningful clinical outcome for families.
- The secondary outcomes include various clinical manifestations of WS such as Diabetes Mellitus and C-peptide, sleep, mood, balance and bladder, and patient reported Quality of Life.
- In total 63 patients were recruited across 4 centres (25 UK, 18 Spain, 15 France and 5 Poland). The last patient last visit was conducted in Oct 2024. The timings for the remaining study milestones are: - i) Trial end Nov 2024, (once the final patient telephone call has occurred); ii) database lock mid-Jan 2025, iii) delivery of the draft report to funders in mid-March 2025; iv) delivery of the final report and submission of publications April 2025.
- The possible study outcomes are: i) there are no significant differences following treatment with sodium valproate; ii) there are differences between sodium valproate and placebo – but the differences are not statistically significant; iii) there are statistically significant differences between sodium valproate and placebo.
- If the trial is successful (i.e. point (iii) above is achieved), WS patients, who are not contraindicated, will be able to access the treatment through the Early Access to Medicines Scheme (EAMS) in the UK and in the EU through the EMA Compassionate Use programme. The trial team will partner with a commercial company to develop the data package need to secure a regulatory licence for use of the sodium valproate in WS patients. This is likely to take 12 – 18 months.
- If there some differences are seen in the trial (i.e. point (ii) above is achieved), that are trending favourably in secondary outcomes, it may still be possible to licence the medication for WS patients.
- If the trial is not successful (i.e. point (i) is achieved), the researchers will focus on working with other groups to develop new clinical trials (e.g. partner with Amylyx, develop a new trial(s) using GLP-1 receptor agonist).
- When developing future clinical trials for WS, the following key points are recommended: i) secure patient involvement at an early stage; ii) confirm there is freedom to operate; iii) secure advice from regulators early; iv) involve multiple trial centres; iv) design the trial to incorporate all data needed for marketing authorisation.
- Standard clinical trial approaches are too slow and are typically better suited to larger numbers of patients. Therefore, better trial design is needed to

provide new treatments to patients with WS or other rare disease more quickly.

- There are new initiatives to explore / develop new clinical trial designs for rare diseases:
    - MRC and NIHR – Changing Clinical Practice Through Innovative Trial Designs, which is one part of the Rare Disease Research UK Platform. This initiative will consider how i) the whole trial pathway can be included in a single trial; ii) to derive the maximum benefit from existing information and historical patient data for clinical research; iii) trials can be designed to include small samples - how low can we go?
    - LifeArc LifeArc Centre for Acceleration of Rare Disease Trials, part of the LifeArc Translational Centres for Rare Disease. This initiative aims to i) provide a platform to recruit rare disease patients, ii) work with patient groups to develop patient relevant outcomes; iii) boost the capacity of clinical trials for rare diseases and iv) develop innovative trial design e.g. platform trials.
- For innovative trial designs in rare diseases, discussions with regulators will be needed to agree clinical trial requirements that help to reduce the potential barriers and utilise same data package (as much as possible) for regulatory approval in different countries (e.g. both EMA and FDA).
- Platform trials could be developed for WS clinical trials and have been successfully used in rare childhood cancers. Platform trials require a co-ordinated network of clinical trial centres and can remove the need for a placebo arm. WS patients would be asked to provide their consent for sharing their data and samples, which could be stored and managed through a regulated BioBank. This would enable researchers to leverage patient data and samples following completion of the trial. Patient consent can also be secured post-trial if needed.
- Existing WS patient placebo data can be utilised (with appropriate patient consent) in other studies to provide detailed natural history data, which would help to inform new WS clinical trials and other future research.

18. Clinical Guidelines. Dr Fumihiko Urano MD, PhD - Samuel E. Schechter Professor of Medicine, Washington University School of Medicine, St. Louis, USA

The group discussed the development of new clinical guidelines for WS. The following points were noted:

- The last WS clinical guidelines were developed in 2014 – led by Prof. Barrett.
- New updated WS clinical guidelines need to be developed to i) reflect the latest research and disease understanding, not included in the current guidelines, which are almost 10 years old; ii) to provide guidance to physicians who are not experts in WS, including in countries where there are no recognised expert centres / HCPs.
- A second patient friendly version should also be developed.

- The clinical guideline package delivered by Wolfram Syndrome UK (WSUK) as part of a WS awareness initiative (that includes the 2015 WS clinical guidelines) will provide a useful starting point for this new update.
- The process for developing the new clinical guidelines was agreed as follows:
    - Dr Josie Elliott and Prof. Tim Barrett will take the lead in Europe while Dr Fumi Urano will take the lead in the US.
    - To include feedback from the different specialties involved in WS, a spreadsheet of all the consultants from the different countries will be developed.
    - The consultants in each specialty will be grouped and asked to review / develop the guidelines for their specialty area.
    - Each specialty team will meet online to confirm their agreement to be included and agree a timetable for their contribution.
    - The final guidelines will be submitted for open access publication.
    - The release of the new WS clinical guidelines will be linked to the WS Global Awareness Day (1st Oct). The aim is to promote and potentially release the new WS clinical guideline on 1st Oct 2025.
    - Representatives from WS patient organisations will be copied into emails to help drive progress [i.e. The Snow Foundation (Stephanie Gebel and Dr Sarah Gladstone) and WSUK (Tracy Lynch)].

19. Rounding up of the day's sessions to create action points if any & closing comments.

No specific action points were agreed.

Meeting attendee list

Name	Hospital/Institution
Dr Lahar Mehta	Amylx Pharmaceuticals
Dr Nathalie Erpelding	Amylx Pharmaceuticals
Ryan Miller	Amylx Pharmaceuticals
Dr Niccolò Vanni	Division of Neuroscience, San Raffaele Scientific Hospital & Vita-Salute San Raffaele University
Dr Ludo Vanden Bussche	EyeHope Foundation
Dr Marianne Van de Kerckhove	EyeHope Foundation
Dr Assumpcio Bosch	Insitute for Neurosciences, Universitat Autònoma de Barcelona, Spain.
Dr Giulio Frontino	IRCCS San Raffaele University, Milan, Italy
Dr Lieve Moons	KU Leuven, Belgium
Prof. Lies De Groef	KU Leuven, Belgium
Prof. Catherine Verfaillie	KU Leuven, Belgium
Dr Michal Cagalinec	Slovak multidisciplinary Wolfram Syndrome team. Bratislava. Slovakia
Dr Silvia Borecka	Slovak multidisciplinary Wolfram Syndrome team. Bratislava. Slovakia
Dr Gema Esteban Bueno	Spanish Multidisciplinary Wolfram Syndrome team. Almería. Spain
Stephanie Gebel	The Snow Foundation
Dr Sarah Gladstone	The Snow Foundation
Prof Timothy Barrett	University of Birmingham
Dr Malgorzata Zatyka	University of Birmingham
Dr Sovan Sarkar	University of Birmingham
Josephine Elliott	University of Birmingham
Dr Xuehao Cui	University of Cambridge
Prof. Patrick Yu Wai Man	University of Cambridge and Moorfields Eye Hospital
Dr Mario Plaas	University of Tartu, Estonia
Dr Nayana Gaur	University of Tartu, Estonia
Dr Toomas Jagomäe	University of Tartu, Estonia
Dr Allen Kaasik	University of Tatu
Dr Raniero Chimienti	Vita-Salute San Raffaele University, Milan, Italy
Dr Fumihiko Urano	Washington University Hospital
Tracy Lynch	WSUK
Dr Gina Isherwood	WSUK
Dr Christophe Orssaud	
Dr Filipe Chicani	