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A novel mutation of *WFS1* gene in a Japanese infant of diabetes mellitus, deafness and congenital cataract.

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Abstract

Background: Wolfram syndrome (WS) is a rare autosomal recessive disorder defined by the combination of early-onset, insulin-dependent diabetes mellitus (DM) and progressive optic atrophy (OA). Mutations of *WFS1* are identified in 90% of WS patients.
Patient and Methods: We encountered a young female Japanese patient with early onset insulin-dependent DM. She was found to have a cataract at 7 months old and DM was diagnosed at 11 months. *WFS1* direct sequencing and the functional consequence of the mutant *WFS1* identified in this study was analysed using GPR78-luciferase vector *in vitro*.
Results: We identified a heterozygous twelve base deletion in exon 8 (c.973_984del12), resulting in an in-frame deletion of 3 amino acids. *In vitro* analysis demonstrated that the mutant WFS1 had reduced ability to protect against ER stress compared with wild type WFS1.
Conclusion: We demonstrate that a novel heterozygous mutation of *WFS1* is a previously unidentified cause of WS.

Introduction

Wolfram Syndrome (WS)

❖ **Clinical features**[1)]

Known as "**DIDMOAD**"

- Diabetes insipidus (**DI**)
- Diabetes mellitus (**DM**)
- Optic Atrophy (**OA**)
- sensorineural Deafness (**D**)

❖ **Etiology**

☐ **WFS1**[2)]

- Protein coded by ***WFS1*** (4p16.1)
- Resident component of the endoplasmic reticulum (ER).
- Protective function against "**ER stress**".
- Pancreatic islet β-cells are major site of expression.

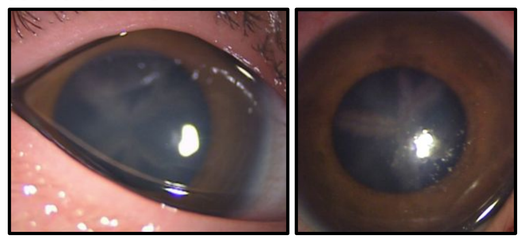
☐ **ER stress**[3)]

- **Cell apotosis** caused by the accumulation of misfolded and unfolded proteins in the ER.

ER stress caused by abnormal WFS1 is the major mechanisim underlying the development of symptoms in WS.

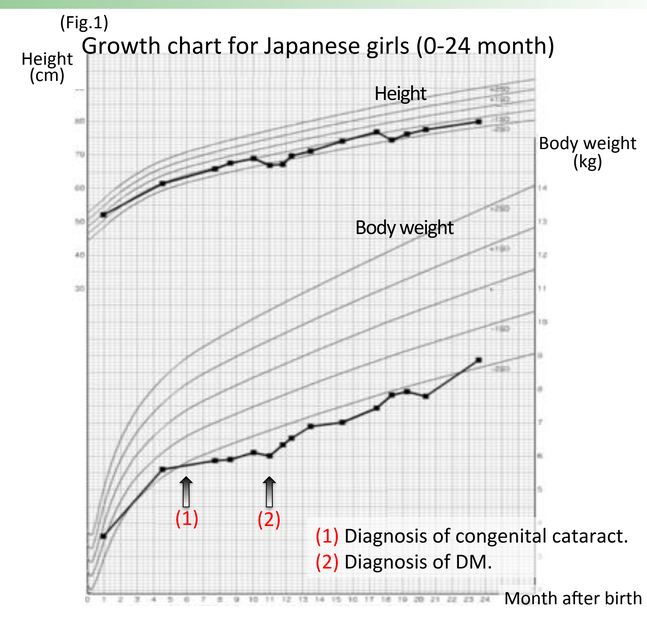
Case report

The Japanese female patient was admitted to our hospital for poor weight gain (Fig.1)and diabetes mellitus (DM). Her growth failure was evident at 3 months old and congenital cataract was noticed at 7 months old (Fig.2). In addition, auditory brainstem response (ABR) test revealed her severe bilateral hearing loss. Her psychomotor development was also delayed. Based on these findings, she was suspected to have WS.



(Fig.2)
Bilateral congenital cataract noticed at 7 month old.

(Fig.1)



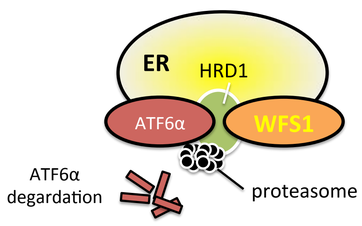
Methods

❖ Direct sequencing of *WFS1*.

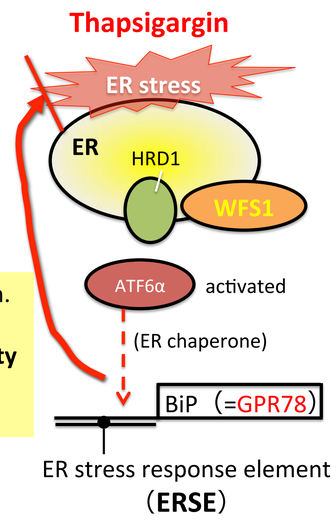
❖ Analysing the functional consequence of the mutant *WFS1* using GPR78-luciferase vector *in vitro*.

- COS-7 cell
- Transfection
  - *WFS1*
    - wild type (WT)
    - p.N325_M328del (our case)
    - p.H313Y (previously reported) [4)]
  - GPR78 promoter luciferase reporter
- Twenty-four hours after transfection, cells were treated with 10nM thapsigargin for 6h.[5)]

Normal



Under ER stress [6)]

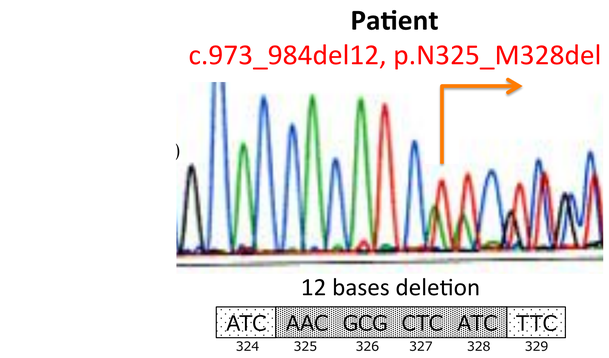


❖ ER stress is induced by Thapsigargin.

❖ **Measure the ERSE luciferase activity** which reflects the strength of ER stress.

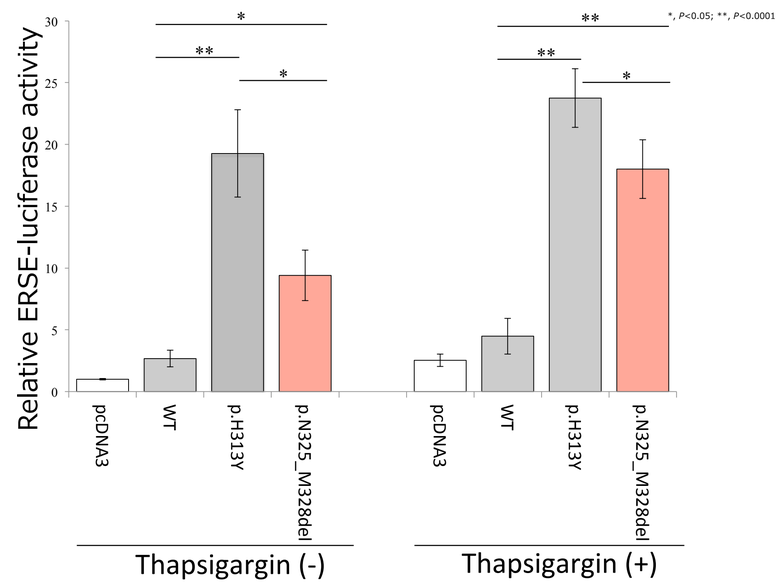
Results

❖ Sequence analysis revealed a novel heterozygous twelve base deletion in *WFS1* exon 8. This deletion resulted in an in-frame deletion of four amino acids.



❖ Even in the absence of thapsigargin, the reporter activity of our mutant activated the ERSE reporter, indicating that it is a constitutively active mutation.

❖ After inducing ER stress with thapsigargin, our mutant increased the ERSE activity.



Discussion

❖ Our mutant (c.973_984del12, p.N325_M328del) impaired the capacity of WFS1 to suppress ER stress. This mutant is considered to be the cause of WS.

❖ Early-onset symptoms in our case suggests the severity of WS. Careful follow-up for DI and optic atrophy is necessary.

Clinical features	Our case	Previous report [1) 7)]	
		Onset age (range)	Incidence
Diabetes insipidus	Not developed	14y (3m～40y)	73%
Diabetes mellitus	Noticed at 10m	6y (3w～16y)	100%
Optic atrophy	Not developed	11y (6w～19y)	100%
Sensorineural deafness	Noticed at 10m	16y (5y～39y)	62%
Congenital cataract	Noticed at 7m	---	66%

❖ **Autosomal dominant (AD) mutations of *WFS1***

Most *WFS1* mutations in WS patients are detected on both alleles and the inheritance of WS is considered to be autosomal recessive. However, AD mutations of *WFS1* have been identified recently [4) 5)].

❖ **Dominant negative effect**

WFS1 is a multimer and is likely to exist as a homo-oligomer of WFS1 monomers [2)]. Previous report suggested that the function of WFS1 is impaired through dominant negative effect [5)].

Our mutant acts as a dominant negative mutant.
(Underlying mechanism must be further studied.)

Conclusion

❖ We report a Japanese patient with early onset WS caused by a novel heterozygous mutation of WFS1 (c.973_984del12, p.N325_M328del) .

❖ As WS is characterized by a wide spectrum of clinical features, it should be considered in the differential diagnosis of a toddler with DM and accompanying features such as hearing impairment, growth failure and cataracts.

Reference

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