A novel mutation of WFS1 gene in a Japanese man of Wolfram syndrome with positive diabetes-related antibodies

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Abbreviated title: WS with diabetes-related antibodies

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Wolfram syndrome (WS), also referred to as DIDMOAD syndrome, is an autosomal recessively inherited syndrome first described by Wolfram and Wagener [1]. It is a progressive neurodegenerative syndrome characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness. WS is rare with an estimated general prevalence of 1: 770,000 and a carrier frequency of 1: 354 [2]. In 1998, a nuclear gene responsible for WS, *WFS1*, was identified and mapped to chromosome 4p16.1 using positional cloning [3].

The following report is of a patient diagnosed with WS having positive glutamic acid decarboxylase (GAD) and insulinoma-associated antigen-2 (IA-2) antibodies. The diabetogenic mechanism in this syndrome has apparently not been recognized as an autoimmune process. Furthermore, we identified a novel mutation in the WFS1 gene of the patient.

A 47-year-old Japanese man was admitted to our hospital because of frequent severe hypoglycemic episodes. Diabetes was diagnosed and insulin treatment was started when he was 6 years. Progressive loss of vision was observed at the age of 11 and hearing loss at 19 years. At the age of 24, he was aware of difficulty in urinating, which required daily bladder

catheterizations. His parents, who are healthy as is the older brother, are consanguineous. No hereditary disease in the ascendants was found.

On physical examination, height was 165.1 cm and weight was 37.3 kg. Blood pressure was 108/62 mmHg, and pulse rate was regular at 66/min. There were no remarkable findings in heart, lung and abdomen. Neurological abnormalities such as cerebellar ataxia and myoclonus were not found except for the absence of Achilles tendon reflex. Psychiatric disorders such as depression and psychosis were not found.

In general laboratory findings, urine sugar was detected. Other general biochemical markers including renal function, electrolytes and lipid profiles were within normal range as were serum concentrations of lactate and pyruvate.

Diabetes related findings are shown in Table 1. Diabetic neuropathy was found, but diabetic nephropathy and diabetic retinopathy were not. A high level of glycohemoglobin A_{1c} (HbA_{1c}), an extremely low level of urinary output of C peptide reactivity (CPR) and unresponsiveness of CPR to glucagon loading test were comparable with insulin-dependent diabetes mellitus commonly seen in patients with WS. However, GAD and IA-2

antibodies were detected, being uncommon in WS. Autoantibodies to islet cells were negative. Human leucocyte antigen (HLA) typing of DR was DR-4 and DR-8. The mitochondrial tRNA Leu (3243) mutation was absent.

Optic atrophy was confirmed by fundascopy and a bilateral symmetric sensorineural hearing loss prevalent for the medium-high frequencies was demonstrated by audiometry. Urodynamics testings showed bladder atony regardless of no upper urinary tract abnormalities. Brain magnetic resonance imaging (MRI) showed the absence of posthypophysis signals, but other MRI abnormalities such as cerebellar and brain stem atrophy were not found. To confirm the presence of diabetes insipidus (DI), hypertonic saline test was done. Arginine vasopressin (AVP) was not responsive to 5% hypertonic saline infusion (from 1.48 pmol/l to 1.48 pmol/l), which confirmed the diagnosis of DI.

Genetic analysis was made under the approval of the institutional review board and written informed consent. Using genomic DNA extracted from peripheral blood mononuclear cells, all exons of *WFS1* gene were amplified by polymerase-chain-reaction (PCR) and directly sequenced, as described in a previous report [3]. The patient has a homozygous 5 base pairs (AAGGC)

insertion at position 1279 in exon 8 which causes a frameshift at codon 371 leading to premature termination at codon 443. Family analysis demonstrated that his parents but not his brother are heterozygous for the same mutation as the patient. Family members other than the patient did not show any signs suggesting WS.

There are several clear distinctions between WS-associated diabetes and classic type 1 diabetes. It is well established that genes in the HLA region contribute to predisposition to typical type 1 diabetes. However, previous studies failed to find an influence of HLA on WS [4, 5]. A second distinction is the apparent absence of an autoimmune process in WS-associated diabetes [6]. The lack of islet cell antibodies has been reported for most cases and GAD antibodies were negative in all studied respectively [2, 7].

The diabetes mellitus associated with WS is clearly related to loss of β cells in the pancreas [8, 9]. In one series of an autopsy study, loss of β cells or atrophy of the islets was noted in 9 of 11 of WS patients [8]. The exocrine portion of the pancreas was reported to be normal with the exception of focal areas of fibrosis [9]. Immunohistochemical studies of the pancreas reveal normal staining for glucagon, somatostatin, and pancreatic polypeptide but

the virtual absence of cells staining for insulin. This indicates a selective β cell loss with preservation of α and δ cells in the islets [9] and allows for the conclusion that diabetes in WS is caused not by a functional defect in the β cells, but by actual β cell depletion.

In our case, HLA typing of DR was DR-4 and DR-8 which is an increased risk for Japanese juvenile-onset type 1 diabetes [10]. In addition, serological examination showed positive GAD and IA-2 antibodies. These results suggest that the diabetes in our patient was caused by an immune-mediated destruction of the insulin-producing β cells of the pancreas in addition to a selective β cell loss not been described previously in this syndrome.

Many mutations, along the entire gene, with homozygous and compound heterozygous mutations, have been described since the identification of *WFS1* as the cause of WS. The patient had a novel homozygous insertion mutation causing a frameshift at codon 371 leading to premature termination at codon 443, resulting in a complete absence of the carboxy tail of the *WFS1* protein. Although function of *WFS1* gene has not been fully elucidated, a role in membrane trafficking, protein processing, or calcium homeostasis in the endoplasmic reticulum has been postulated [11].

Furthermore, it is speculated that the carboxy tail is interacting with other, unknown proteins [11]. Expression studies of mutant proteins are necessary to determine which parts of the protein are essential for biological function.

In summary, we have reported a case of WS patient carrying a novel mutation in *WFS1* gene with positive GAD and IA-2 antibodies. In the present case, the diabetes might be caused by an immune-mediated destruction and a selective loss of the insulin-producing β cells of the pancreas. In addition, further molecular analysis is necessary to uncover the pathogenesis of this syndrome.

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Table 1 - Diabetes related findings

Fasting plasma glucose (mg/dl)	109
Glycohemoglobin A1c (HbA1c) (%)	9.2
Urinary output of C peptide reactivity (CPR) (mg/day)	<0.7
Plasma CPR to glucagon (0-6 min) (ng/ml)	<0.05 - <0.05
Urinary microalbumin (mg/day)	3.2
Glutamic acid decarboxylase (GAD) antibody	(+)
Insulinoma-associated antigen-2 (IA-2) antibody	(+)
Autoantibodies to iscet cells (ICA)	(-)
Human leucocyte antigen (HLA)	DR 4, 8
Mitochondrial tRNA Leu (3243) mutation	(-)