

# A case of early-onset diabetes with impaired insulin secretion carrying a PAX6 gene Gln135\* mutation

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## Summary

A paired homeodomain transcription factor, PAX6 (paired-box 6), is essential for the development and differentiation of pancreatic endocrine cells as well as ocular cells. Despite the impairment of insulin secretion observed in PAX6-deficient mice, evidence implicating causal association between PAX6 gene mutations and monogenic forms of human diabetes is limited. We herein describe a 33-year-old Japanese woman with congenital aniridia who was referred to our hospital because of her uncontrolled diabetes with elevated hemoglobin A1c (13.1%) and blood glucose (32.5 mmol/L) levels. Our biochemical analysis revealed that her insulin secretory capacity was modestly impaired as represented by decreased 24-h urinary C-peptide levels (38.0 µg/day), primarily explaining her diabetes. Intriguingly, there was a trend toward a reduction in her serum glucagon levels as well. Based on the well-recognized association of PAX6 gene mutations with congenital aniridia, we screened the whole PAX6 coding sequence, leading to an identification of a heterozygous Gln135\* mutation. We tested our idea that this mutation may at least in part explain the impaired insulin secretion observed in this patient. In cultured pancreatic β-cells, exogenous expression of the PAX6 Gln135\* mutant produced a truncated protein that lacked the transcriptional activity to induce insulin gene expression. Our observation together with preceding reports support the recent attempt to include PAX6 in the growing list of genes causally responsible for monogenic diabetes. In addition, since most cases of congenital aniridia carry PAX6 mutations, we may need to pay more attention to blood glucose levels in these patients.

## Learning points:

- PAX6 Gln135\* mutation may be causally associated not only with congenital aniridia but also with diabetes.
- Blood glucose levels may deserve more attention in cases of congenital aniridia with PAX6 mutations.
- Our case supports the recent attempt to include PAX6 in the list of MODY genes, and Gln135\* may be pathogenic.

## Background

The development and differentiation of pancreatic endocrine cell lineages are tightly controlled by transcriptional networks, and paired homeodomain transcription factor paired-box 6 (PAX6) has been reported

to be indispensable for pancreatic β-cells. The mice with global PAX6 deletion manifest post-natal lethality with an absence of glucagon-producing α-cells as well as markedly decreased insulin-producing β-cells (1, 2). In agreement

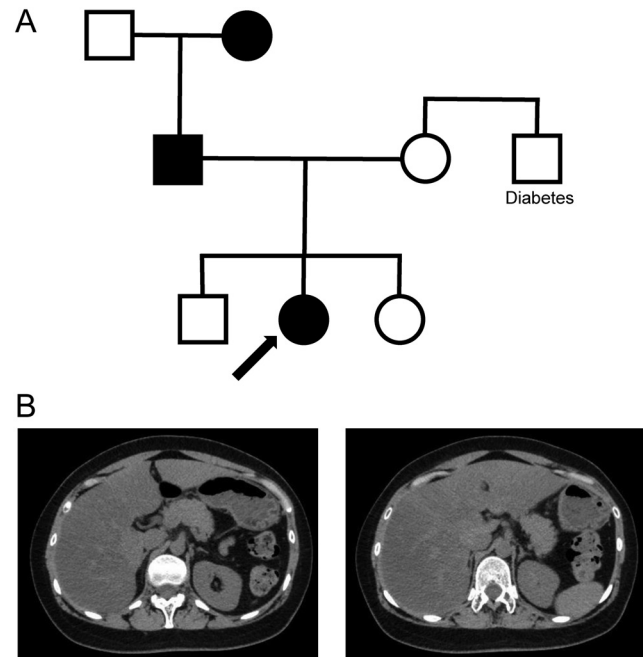
with these rodent studies, human diabetes associated with PAX6 gene mutations has been reported (3, 4, 5) although PAX6 has been under-recognized as a gene associated with monogenic diabetes.

PAX6 gene mutations have been rather widely accepted to be causal for congenital aniridia, a rare disorder characterized by aplasia of iris (6). PAX6 is also critically involved in neurogenesis, and the association of PAX6 mutations with cognitive and behavioral abnormalities has been implicated (7). The understanding of this broad spectrum of manifestations can be facilitated by considering the functional domains of PAX6. The paired domain and homeodomain, two DNA-binding domains with different DNA-recognition motifs, are separated by a glycine-rich linker. The transactivation domain is located at its C-terminal.

## Case presentation

A 33-year-old Japanese woman with congenital aniridia was referred to our hospital because of her uncontrolled diabetes with elevated hemoglobin A1c (13.1%) and blood glucose (32.5 mmol/L) levels. She has developed glaucoma and cataract as well as social anxiety disorder during her life. Her elevated blood glucose level was initially detected by an annual screening health checkup at 31 years old (9.9 mmol/L, HbA1c 13.1%). She had a family history of diabetes concurrent with aniridia with dominant inheritance (Fig. 1A).

On admission, her BMI was 22.6 kg/m<sup>2</sup> (height: 149.5 cm, weight: 50.5 kg). Reflecting the relatively recent onset, we could not detect any signs of diabetic retinopathy and nephropathy (5.10 mg/day of microalbuminuria). Her fasting and postprandial serum C-peptide levels and blood glucose levels were 1.33 ng/mL and 11.6 mmol/L and 3.12 ng/mL and 16.8 mmol/L, respectively, while her urinary excretion of C-peptide was 38.0 µg/day, indicating modestly impaired insulin secretion. Notably, her serum glucagon level was 7.3 pg/mL (normal range: 5.4–55.0) exhibiting a decreasing trend (under condition with 7.7 mmol/L of blood glucose and 0.90 ng/mL of serum C-peptide). Both of the autoantibodies, glutamate decarboxylase (GAD) and insulinoma-associated protein-2 (IA-2), were negative, and her pancreas appeared morphologically normal on the CT scans (Fig. 1B). Since some neuronal defects have been reported in cases with PAX6 mutations (7, 8), we measured her endocrine hormonal levels which were all within the normal ranges. We obtained well-controlled blood glucose levels with 22 U of the total daily dose of insulin which

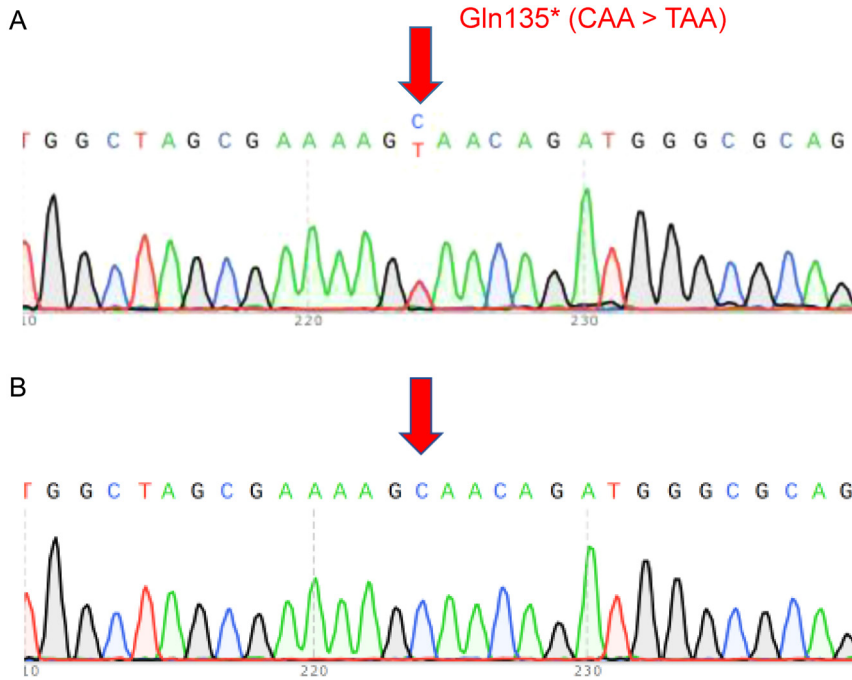


**Figure 1**  
(A) The family tree of this patient. Filled symbols indicates individuals manifesting diabetes concurrent with aniridia. An individual with type 2 diabetes without aniridia is also indicated. (B) The images of abdominal CT scans of the patient.

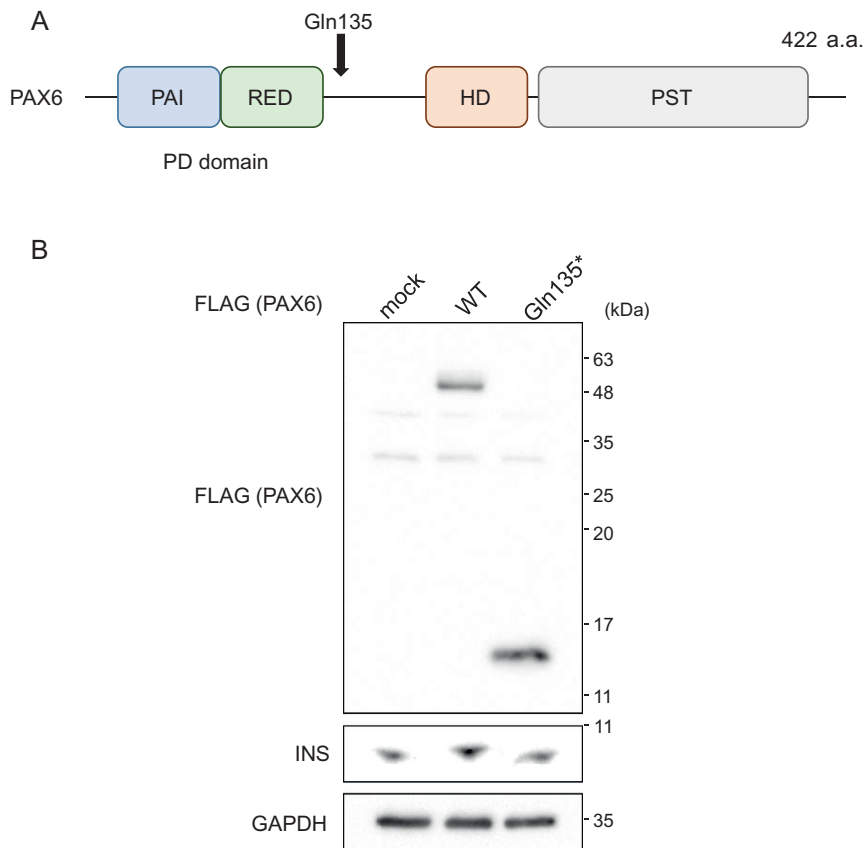
was also consistent with her modestly impaired insulin secretion.

## Investigation

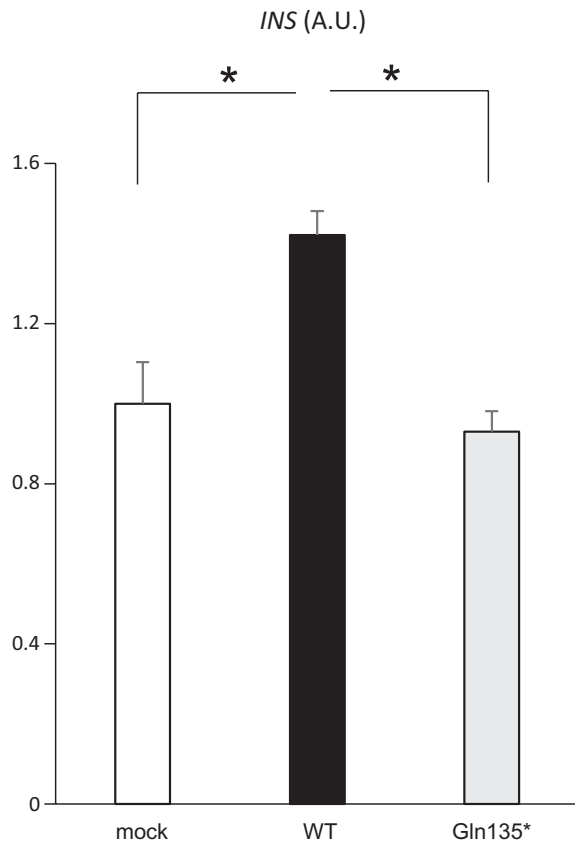
These observations prompted us to examine the sequence integrity of the PAX6 gene, leading to an identification of a heterozygous Gln135\* mutation (rs1131692304) (Fig. 2). The mutant PAX6 was predicted to produce a truncated protein lacking the HD domain as well as C-terminal domain critical for its transactivation (Fig. 3A). To ensure this prediction, we generated expression plasmids encoding WT human PAX6 as well as the Gln135\* mutant PAX6 with N-terminal FLAG-tag and exogenously expressed them in HIT-T15 β-cells. As predicted, the WT PAX6 and the mutant PAX6 migrated around ~50 kDa and ~15 kDa on SDS-PAGE, respectively (Fig. 3B). Although the mutant PAX6 may retain a residual DNA-binding capacity, it did not increase insulin gene expression, unlike WT PAX6 at mRNA (Fig. 4) and protein levels (Fig. 3B). We were not able to examine the integrity of other genes associated with MODY due to the restriction of the informed consent that allows us to specifically examine the PAX6 gene. To fill the gap, we performed an additional experiment where we suppressed the endogenous PAX6 gene expression to create



**Figure 2**  
DNA sequencing chromatograms corresponding to the Sanger sequencing of the rs1131692304 SNV region in the patient (A) and a healthy volunteer (B).



**Figure 3**  
(A) Schematic description of functional domains of PAX6 gene product. The paired domain (PD) consisted of two sub-domains, PAI and RED. HD, homeodomain; PST, proline-serine-threonine. (B) Western blot analysis. Either FLAG-tagged WT PAX6 or FLAG-Gln135\* mutant PAX6 along with empty vector was transfected into HIT-T15 pancreatic  $\beta$ -cells. The cell lysates were separated by SDS-PAGE and the transferred membranes were incubated with anti-FLAG, anti-insulin and anti-GAPDH antibody. Size markers are shown to the right of the blots.



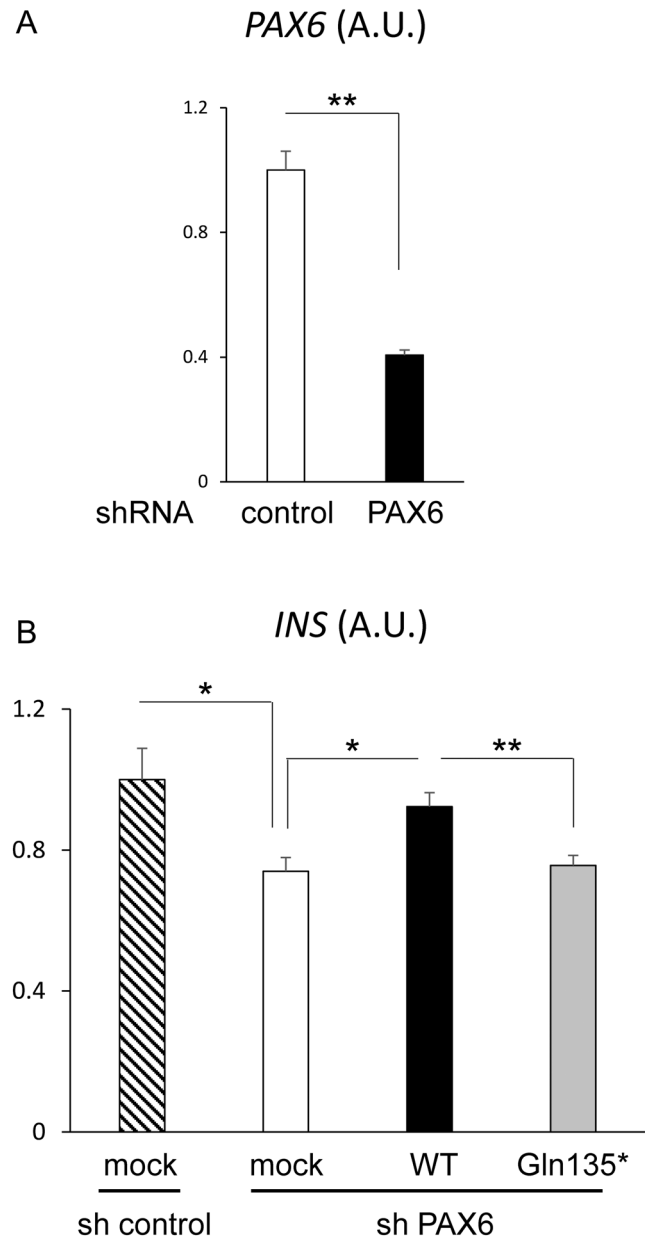
**Figure 4**  
Insulin gene expression (INS) induced by exogenous expression of PAX6. Either FLAG-tagged WT PAX6 or FLAG-Gln135\* mutant PAX6 along with empty vector was transfected into HIT-T15 pancreatic  $\beta$ -cells. Insulin gene expression was normalized by  $\beta$ -actin expression ( $n = 4-5$ ). \* denotes  $P < 0.05$ .

a haploinsufficient state (Fig. 5A). Thereafter, we restored WT human PAX6 or the Gln135\* mutant PAX6 (Fig. 5B). The suppression of the PAX6 gene reduced the insulin gene expression that was restored by re-expression of WT human PAX6, not by that of mutant PAX6 (Fig. 5B).

## Discussion

We described a patient who manifested congenital aniridia, social anxiety disorder and pancreatic endocrine deficits with a PAX6 mutation. Intriguingly, not only  $\beta$ -cell function but also  $\alpha$ -cell function may be influenced in this case. Since insulin suppresses glucagon secretion, lower glucagon levels in the presence of a decrease in insulin secretion may indicate impaired  $\alpha$ -cell function.

The limitation in this study is that we did not examine the integrity of other genes than PAX6 due to the restriction of informed consent. However, our *in vitro* study suggests



**Figure 5**  
(A) The knockdown efficiency of shRNA targeting PAX6. (B) Insulin gene expression in the reconstitution experiment. The endogenous PAX6 was suppressed and thereafter human WT PAX6 or the Gln135\* PAX6 was re-expressed ( $n = 4$ ). \* and \*\* denote  $P < 0.05$  and  $< 0.01$ , respectively.

that the Gln135\* mutation would contribute to insulin gene expression at least to some extent. Another limitation is that we could not genetically demonstrate the heritability of this mutation. Her father who manifested aniridia and diabetes was untraceable because of a separation right after her birth.

While the identified Gln135\* mutation has been reported to be associated with congenital aniridia (7), it



has not been reported as a mutation for diabetes. Our case, similar with literature (3, 4, 5), supports the emerging idea that PAX6 may be added to the pre-existing collection of genes for monogenic diabetes. Historically, ocular manifestations have been emphasized with little attention to endocrine functions in patients with PAX6 mutations (9). This may be a reflection of a lower penetrance of diabetes like MODY6 (10). Our case implicates a potential pitfall in the follow-up of patients with congenital aniridia.

#### Declaration of interest

The authors declare there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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#### Patient consent

Written informed consent for publication of the clinical details was obtained from the patient, and this study was approved by the University of Tsukuba Hospital Ethics Committee (protocol no. R01-367).

#### Author contribution statement

M S, M Y and Y M were in charge of the medical care for the patient. M S and M Y extracted genomic DNA from patient's peripheral blood and analyzed the DNA sequence of PAX6 gene. M S performed the *in vitro* experiment using HIT-T15 cells. M O, R N, N O, N M, D Y, S M, T M, Y S, Y O, H I, H S1 (Hiroaki Suzuki) and H S2 (Hitoshi Shimano) supported the medical care and manuscript preparation. M S wrote the manuscript. H S2 supervised this project. All authors read and approved the final manuscript.

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