Functional characterization of a novel *KLF11* mutation identified in a family with autoantibody-negative type 1 diabetes

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Introduction

- KLF11 is a transcription factor that is ubiquitously expressed in human tissues, including islet cells and exocrine pancreas.
- Klf11 knock-out mice showed lower serum insulin levels than wildtype mice, indicating that decreased KLF11 expression level causes impaired glycaemic regulation[1)].
- To date, two *KLF11* mutations (p.A347S, p.T220M) have been identified in three families clinically diagnosed with type 2 diabetes[2)].

Our case

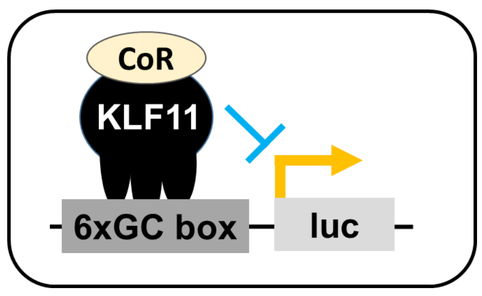
	Proband	Sister	Mother
Age at diagnosis (yr)	1	1	4
DKA at diagnosis	No	No	No
Age at last examination (yr)	15	10	44
BMI at last examination (kg/m^2, SDS)	22.2, 0.7	18.5, 0.6	19.0, -1.0
Fasting serum C-peptide at last examination (ng/mL)	0.4	0.4	0.6-1.2
Required insulin at last examination (units/kg/day)	1.0	1.0	0.6

They were diagnosed with autoantibody-negative type 1 diabetes (T1D).

Methods

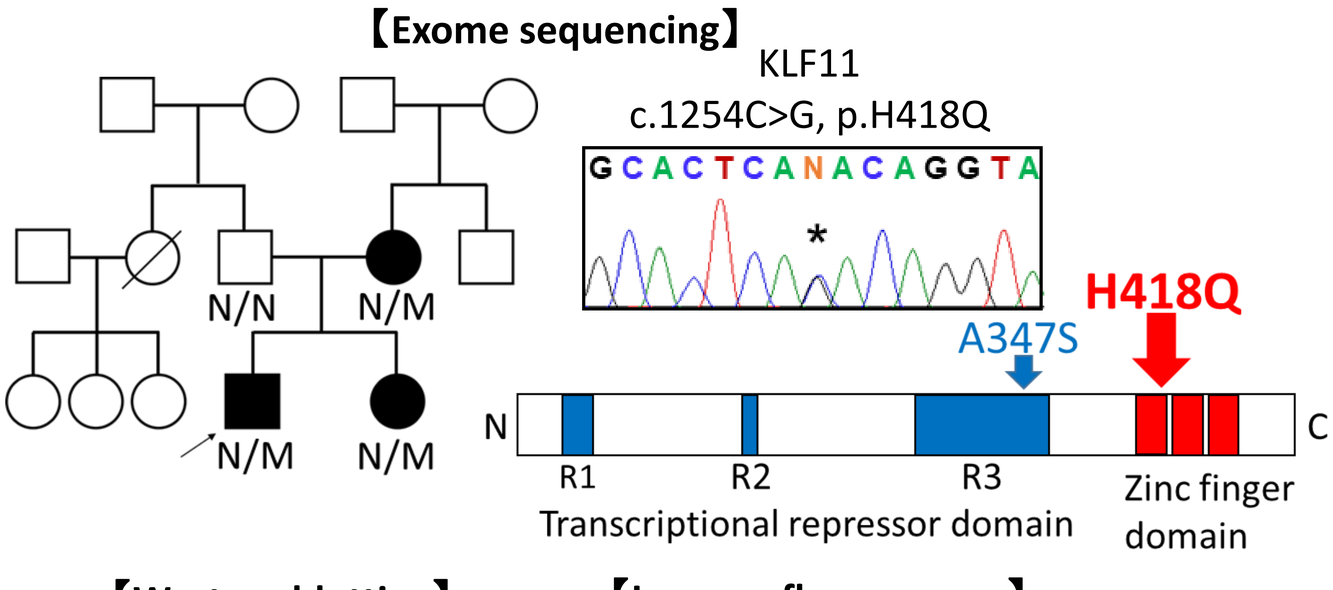
Mutation detection:
Exome sequencing and Sanger sequencing

Evaluation of the *KLF11* variants:
3D structure modeling
Western blotting
Immunofluorescence
Luciferase assay
- CHO cells
- Transient transfection
- 6xGC-luc* *six tandem repeats of a KLF11-binding site

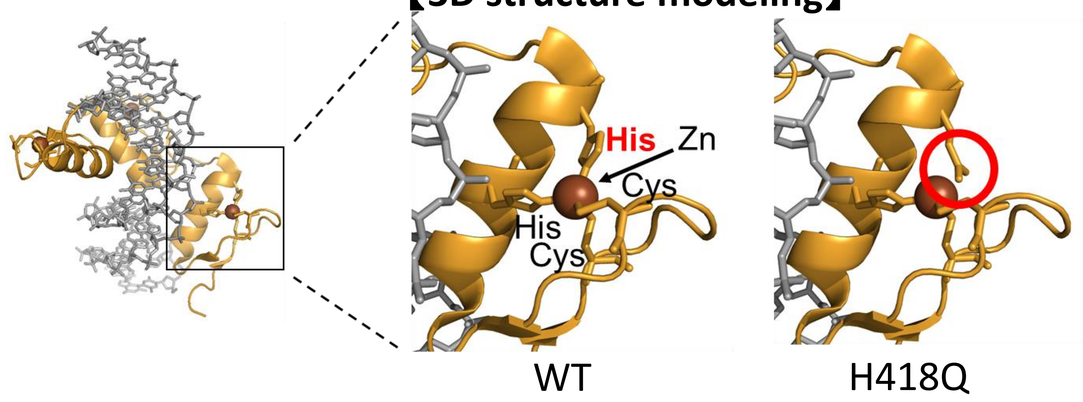


Results

【Exome sequencing】

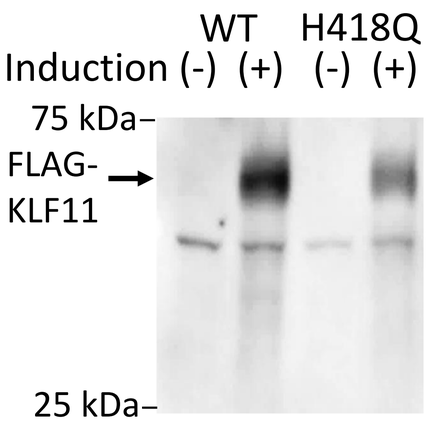


【3D structure modeling】

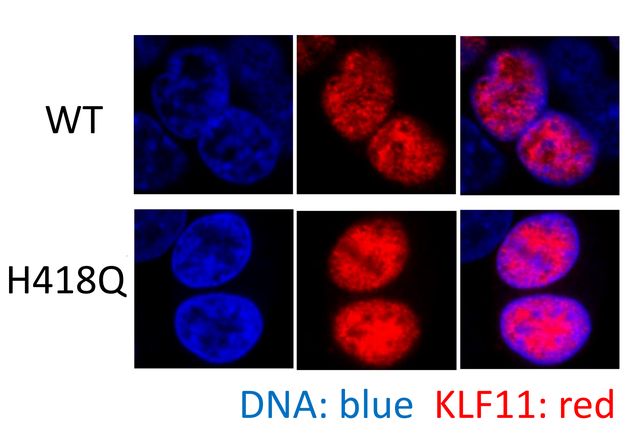


p.H418Q affects a functionally important histidine residue in the C_2H_2 zinc finger domain.

【Western blotting】

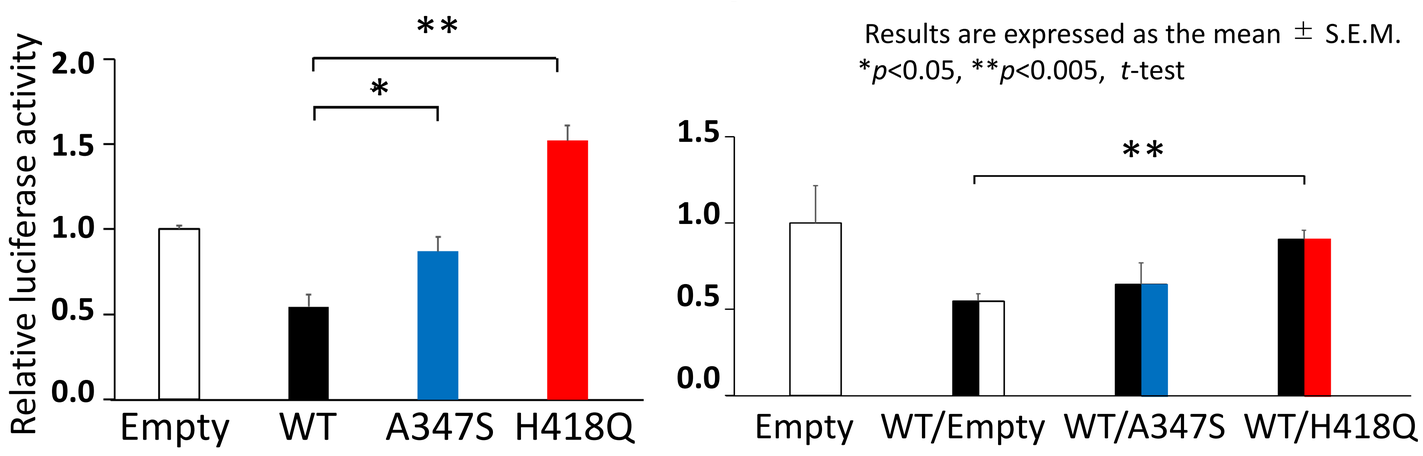


【Immunofluorescence】



The protein expression level and intracellular localization of H418Q-KLF11 were comparable with WT-KLF11.

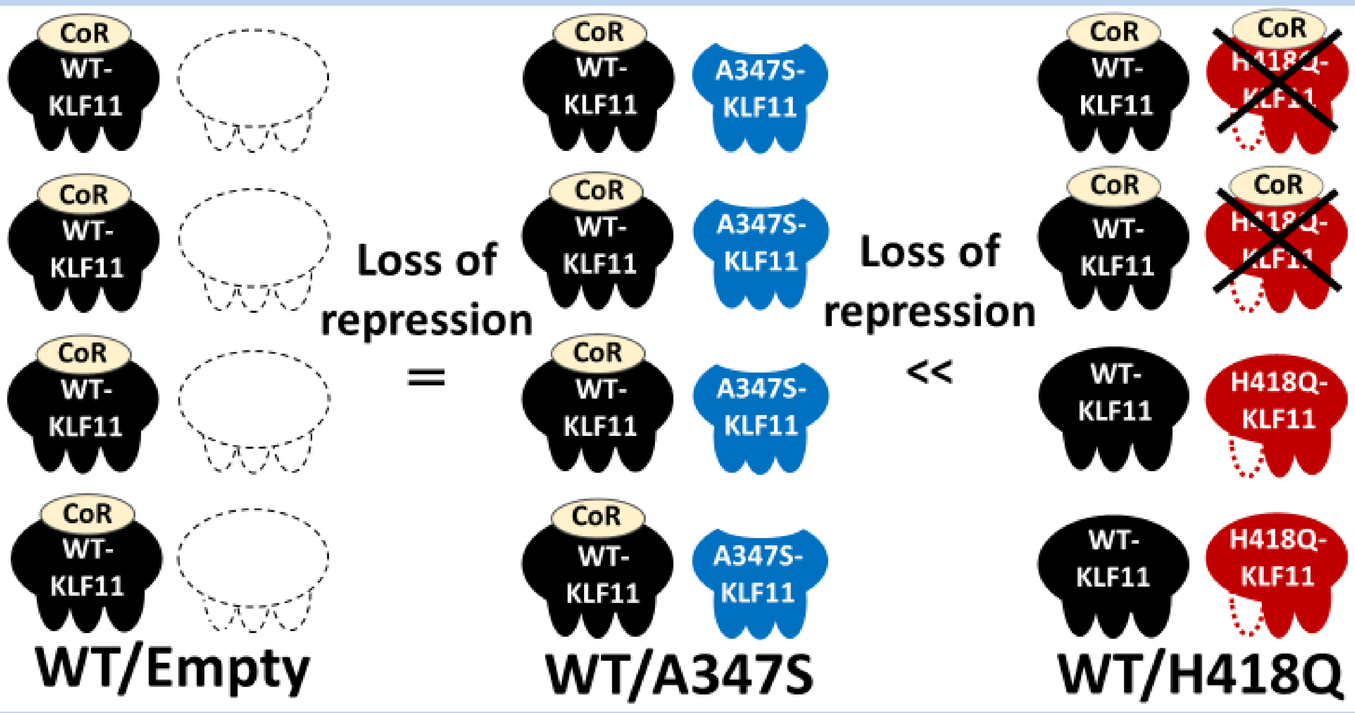
【Luciferase assay: 6×GC box】



H418Q-KLF11 and A347S-KLF11 demonstrated significantly decreased transcriptional repression activities. Co-expression of H418Q-KLF11 with WT-KLF11 caused significant loss of repression, indicating that H418Q-KLF11 had a dominant-negative effect.

Discussion

- For the first time, we identified the *KLF11* mutation-carrying family with antibody-negative "T1D".
- In our study, H418Q-KLF11 had a dominant-negative effect, which could possibly explain severer phenotypes observed in our patients than in previously reported patients.
- *KLF11* is known to cause transcriptional repression by direct interaction with the scaffold corepressor protein Sin3A[3,4)]. A347S-KLF11 is defective in corepressor binding, although do not interfere the binding between WT-KLF11 and corepressors.
- Contrastingly, the binding between WT-KLF11 and corepressors was interfered by H418Q-KLF11, probably through competitive bindings to the corepressors.



Conclusion

***KLF11* mutation with the dominant-negative effect is likely to be associated with the T1D-like phenotype.**

References: 1) Bonnefond A et al. J Biol Chem, 2011. 2) Neve B et al. Proc Natl Acad Sci USA, 2005. *3*) Lomberk G et al. Biochem J, 2005. 4) Fernandez-Zapico ME et al. EMBO J, 2003.

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