

## Familial Graves' Disease Associated with Type 1 Diabetes

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### Abstract

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A 59-year-old woman was diagnosed as having Graves' disease and type 1 diabetes. DNA molecular HLA typing detected DRB1\*0405 and DQB1\*0401, as well as the haplotypes of DRB1\*0901-DQB1\*0303. We also performed polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to identify allele variations of other loci. The patient's son also manifested Graves' disease and type 1 diabetes, with both cases having strikingly homologous clinical features. Familial clustering of Graves' disease and type 1 diabetes, and their tendency to occur together along with a similar clinical course suggest that their etiology may involve shared genetic factors.

**Key words:** Graves' disease, type 1 diabetes, familial onset

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### Introduction

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Graves' disease and type 1 diabetes are autoimmune disorders under complex genetic control that is believed to develop through a process mediated by organ-specific T-cells. There is a well-known strong association between Graves' disease and type 1 diabetes, with these disorders clustering within the same individual as well as within the same family indicating common genetic factors in their pathogenesis (1, 2). Many autoimmune disorders, including Graves' disease and type 1 diabetes are HLA-associated. While the HLA locus has been demonstrated to strongly contribute to susceptibility, recent studies have also revealed that some other loci might harbour genes susceptible to these disorders (3-7). Here, we report two cases of familial Graves' disease associated with type 1 diabetes, with strikingly homologous clinical features. HLA typing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were performed to further investigate the relationships between genetic characteristics and phenotypic features of this family.

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### Case Report

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#### Case 1

In 1996, a 48-year-old woman was admitted to our hospital, complaining of palpitations and irritability. Electrocardiogram showed atrial fibrillation. She was diagnosed as having Graves' disease, with serum TSH 0.05  $\mu$ IU/mL, free T3 25.0 pg/mL, free T4 8.0 ng/dL and TSH receptor antibody (TRAb) 55.3%. She was initially treated with methimazole 30 mg daily, with the dose gradually reducing during therapeutic periods from 30 to 2.5 mg daily. However, bimonthly monitoring of thyroid function revealed that serum levels of free T3 and free T4 had increased to 10.99 pg/mL and 5.28 ng/dL, accompanied with suppressed TSH (< 0.01  $\mu$ IU/mL) in November 2005. When the dose of methimazole was increased to 10 mg daily, she regained a euthyroid state. Her thyroid function was kept within the normal range with methimazole 5 mg daily. One year later, she was admitted for polyuria and weight loss up to 5 kg within four weeks.

On physical examination, her blood pressure was 110/46 mmHg, body temperature 36.8°C, and pulse rate 78/min. Her weight was 48 kg and height was 159 cm (body mass index, 18.0 kg/m<sup>2</sup>). Exophthalmos was not present. Thyroid gland was not palpable. The results of all other physical ex-

**Table 1. Endocrine Data on Admission**

Fasting plasma glucose	260 mg/dL
HbA1c	13.9%
β-hydroxybutyrate	1421 μmol/L (<85 μmol/L)
Urinary C-peptide immunoreactivity	14.1 μg/day (29.2-167 μg/day)
Anti-glutamic acid decarboxylase (GAD) antibody	740.0 U/mL (<1.5 U/mL)
Anti-insulinoma-associated protein 2 (IA-2) antibody	37 U/mL (<0.4 U/mL)
TSH	1.75 μIU/mL (0.34-4.04 μIU/mL)
Free T3	1.70 pg/mL (2.36-5.00 pg/mL)
Free T4	1.14 ng/dL (0.88-1.67 ng/dL)
TSH receptor antibody (TRAb)	4.9% (<15%)
Thyroid stimulating antibody (TSAb)	106% (<180%)
Anti-thyroglobulin antibody	1.6 U/mL (<0.3 U/mL)
Anti-thyroid peroxidase antibody	139.0 U/mL (<0.3 U/mL)
ACTH	27.5 pg/mL (7.4-55.7 pg/mL)
Cortisol	7.0 μg/dL (4.0-18.3 μg/dL)
Anti-adrenocortical antibody	negative

aminations were unremarkable.

Table 1 illustrates the results of laboratory data showing that serum levels of free T3, free T4 and TSH were in the normal range. At that time, her urinary ketone bodies were strongly positive, and HbA1c was 13.9%. The data demonstrated diabetic ketosis.

She was treated with intensive insulin therapy. The following day, all biochemical indicators recovered to the normal range and her fasting plasma glucose was 148 mg/dL. Urinary C-peptide was 14.1 μg/day, indicating insulin secretion deficiency. Diabetes-related autoantibodies including anti-glutamic acid decarboxylase (GAD) and anti-insulinoma-associated protein 2 (IA-2) antibodies were positive. Based on these findings, she was diagnosed as having type 1 diabetes. Other autoantibodies including anti-nuclear, anti-intrinsic factor, anti-adrenocortical, and anti-mitochondrial antibodies were negative. Diabetic retinopathy was not seen in ocular fundus as assessed by an ophthalmologist. Neurological tests showed that her sensory and motor nerves were intact. Microalbuminuria was not detected. She was discharged from the hospital after 14-days, receiving methimazole (5 mg daily) and intensive insulin therapy (4 units intermediate-acting insulin nocturnally and 8-10 units rapid-acting insulin premeals). After three months of treatment, HbA1c decreased to 8.0%. Her thyroid function remained in the normal range with methimazole 5 mg daily.

DNA molecular HLA typing detected DRB1\*0405 and DQB1\*0401, as well as the haplotypes of DRB1\*0901-DQB1\*0303. Written informed consent was obtained from her at Jikei University School of Medicine. PCR-RFLP was performed to identify allele variations of susceptibility genes

for type 1 diabetes and Graves' disease. Cytotoxic T-lymphocyte-associated protein 4 gene (*CTLA4*) polymorphism +49A>G (rs231775), small ubiquitin-like modifier 4 gene (*SUMO4*) polymorphism +163A>G (rs237025) and protein tyrosine phosphatase non-receptor type 22 gene (*PTPN22*) polymorphism -1123 G>C (rs2488457) were identified in the heterozygous state.

## Case 2

The 33-year-old son of case 1, who had a 13 year history of Graves' disease had been treated at many other clinics, but the details were unclear. He worked as a mechanic, but his chief physician was changed whenever his workplace changed. We interviewed his mother and his current chief physician about the following clinical history.

He complained of palpitations and visited a hospital nearby when he was 20 years old, where he was diagnosed as having Graves' disease and treated with methimazole. Three years later, he was hospitalized with diabetic ketosis. Anti-GAD antibody was positive (500 U/mL). He was diagnosed as having type 1 diabetes. It was not clear whether his thyroid function had worsened before he developed type 1 diabetes in the same way as his mother. Intensive insulin therapy was required constantly throughout the hospitalization. Afterwards, he was discharged from the hospital with intensive insulin therapy (18-20 units intermediate-acting insulin nocturnally and 18-20 units rapid-acting insulin premeals) and methimazole 20 mg daily. His HbA1c ranged between 7.0 and 7.5%.

## Discussion

This family involved two complicated cases, each with two different autoimmune disorders presenting with a similar clinical course. Both cases developed type 1 diabetes after they had developed Graves' disease, and were both treated with methimazole and intensive insulin therapy. In case 1, thyroid function worsened before developing type 1 diabetes. Thyrotoxicosis worsens metabolic control of diabetes and increases its lability, often with a need for increased insulin dosage. In case 2, it was not clear whether thyroid function worsened before the development of type 1 diabetes.

Graves' disease and type 1 diabetes are multifactorial autoimmune disorders, associated with the HLA locus. Although the HLA locus is highly polymorphic and linked with a variety of autoimmune disorders, apparent differences in disease-associated alleles and haplotypes are observed between Japanese and Caucasian populations. In case 1, DNA molecular HLA typing detected DRB1\*0405 and DQB1\*0401, which demonstrated susceptibility for autoimmune polyglandular syndrome (APS) patients with both Graves' disease and type 1 diabetes in Japanese populations (8, 9), as well as the haplotypes of DRB1\*0901-DQB1\*0303.

In recent years, considerable effort has gone towards defining susceptibility genes for autoimmune disorders. In ad-

dition to the strong contribution to susceptibility made by the HLA locus, Graves' disease and type 1 diabetes have also been reported to be associated with different loci that might harbour genes susceptible to these disorders (3-7). On the basis of these reports and their biological relevance, we performed PCR-RFLP to identify allele variations of *CTLA4*, *SUMO4* and *PTPN22*. *CTLA4* polymorphism +49A>G (rs231775), *SUMO4* polymorphism +163A>G (rs237025) and *PTPN22* polymorphism -1123 G>C (rs2488457) were identified in case 1.

In addition to the HLA locus, *CTLA4* has been implicated as a general susceptibility gene for autoimmune disorders including autoimmune thyroid disease (AITD) (10). *CTLA4*, located on chromosome 2q33 encodes an important regulatory molecule involved in the immune system. An increased risk for developing both AITD and type 1 diabetes has been linked to *CTLA4* in multiple ethnic groups (4, 5, 11, 12). Among Japanese, *CTLA4* is associated with type 1 diabetes only in a subset of patients with type 1 diabetes complicated with AITD (13). The +49A>G polymorphism in exon 1 (rs231775) has been implicated in several autoimmune disorders. This polymorphism causes an amino acid exchange (threonine to alanine). Several autoimmune disorders, including AITD and type 1 diabetes, have been shown to be associated with the G-allele (14, 15).

In addition to +49A>G polymorphism, a recent study showed that +6230 G>A polymorphism (rs3087243) is also associated with type 1 diabetes complicated with AITD in Japanese populations (13). The +6230 G>A polymorphism is in linkage disequilibrium with the +49A>G polymorphism. The +6230 G>A polymorphism in 3' untranslated region of *CTLA4* was reported to affect the variation in *CTLA4* splicing (5). Although we have not performed genotyping in the present study, this polymorphism is also important in order to elucidate the effect of *CTLA4* on the development of type 1 diabetes and AITD.

*SUMO4*, located in *IDDM5* interval on chromosome 6q25, is a newly identified posttranscriptional modifier. *SUMO4* can modify the immune response through suppression of

nuclear factor kappa B (NF- $\kappa$ B). *SUMO4* polymorphism +163A>G (rs237025) results in an amino acid methionine to valine substitution at codon 55 (M55V). This M55V substitution was reported to result in higher levels of activated NF- $\kappa$ B (6). Recent studies have revealed an association between *SUMO4* and type 1 diabetes (6, 16-18) but no association with Graves' disease (19) in Caucasian populations. In contrast, several studies have found a strong association with type 1 diabetes in Asian populations including Japanese (20), and with AITD in Japanese groups (21). These reports indicate that discrepancies may be caused by genetic heterogeneity among diverse ethnic groups.

*PTPN22*, located on chromosome 1p13 (22) encodes a lymphoid-specific phosphatase (Lyp). Lyp is an intracellular protein tyrosine phosphatase that is important in the negative control of T-cell activation via association with several accessory molecules, including Grb2 (23) and Csk (24). Although it is not known how -1123 G>C promoter polymorphism acts to influence the expression of Lyp, this polymorphism is associated with acute-onset, but not with slow-onset type 1 diabetes in Japanese populations (25). The genotype frequencies and allele frequencies of -1123 G>C polymorphisms did not differ between Graves' disease and controls in Japanese populations (26).

In summary, we report two cases of familial Graves' disease associated with type 1 diabetes. The mother's case carried known polymorphisms in genes encoding *CTLA4*, *SUMO4* and *PTPN22*. Our results suggest that such polymorphisms may have played a major role in the pathogenesis of this case. We plan to further investigate case 2 in the future. Further studies on a large number of patients with Graves' disease and type 1 diabetes are clearly required to determine the incidence and pathogenesis of such polymorphisms.

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