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New HLA DRB1 and DQB1 Haplotypes in a Pedigree of Familial Graves' Disease in Japan

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Abstract. The present study demonstrated genetic analysis of human leukocyte antigen (HLA) in a familial Graves' disease linked to autoimmune mechanism. The proband was a 17 year-old female. At 15 years, Graves' disease was diagnosed with serum TSH was <0.015 IU/ml; free T_3, 13.6 pg/ml; free T_4, 4.51 ng/dl; and TSH receptor antibody (TRAb), 94.1%. She had two brothers (19 and 13 years-old), who manifested Graves' disease at 18 and 13 years, respectively. They also had elevated TRAb as high as 48.4 and 49.1%, respectively. There was a strong family history of Graves' disease in their maternal pedigree. Namely, their two aunts and a cousin had Graves' disease, and their onset ages of Graves' disease were also during their teen-age years. However, there was no patient with Graves' disease in the paternal pedigree. We checked HLA-DRB and -DQB haplotype in the members of maternal pedigree and proband's father. The members of maternal pedigree including both affected and unaffected Graves' disease had haplotypes of DRB1*150101 and DQB1*0602, except for the cousin who had DRB1*140301 and DQB1*030101. The haplotypes of DRB1*150101 and DQB1*0602 were different from susceptible HLA types in Japanese childhood onset Graves' disease. However, two cases of Graves' disease also had HLA types of DRB1*40501 and DQB1*0401, in addition to the haplotypes of DRB1*150101 and DQB1*0602. There was no other autoimmune disease including type 1 diabetes mellitus in their family. The present findings indicated that familial Graves' disease was found mainly in the maternal females and become overt during their teen-age years. They had new HLA haplotypes distinct from those susceptibly in Japanese Graves' patients. Further study will be necessary to analyze the mutant locus of DNA to elucidate pathogenesis of familial Graves' disease.

Key words: Graves' disease, Familial onset, HLA, Hyperthyroidism, Childhood-onset

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GRAVES' disease is an autoimmune disease defined by the presence of TSH receptor antibodies which stimulate thyroid gland to increase synthesis and release of thyroid hormones [1, 2]. Both genetic and acquired factors are believed to be involved in the pathogenesis of Graves' disease [3, 4]. The genetic factors include polymorphism of human leukocyte antigen (HLA), cytotoxic T cell antigen-4 (CTLA-4) and unknown genes in other chromosomes [5–34]. In the literature HLA typing has a strong association with the development of Graves' disease with childhood onset, but CTLA-4 polymorphism does not [13]. In addition, HLA polymorphism associated with Graves' disease is different between childhood and adult onset [5–13, 24–33].

Familial Graves' disease is defined as a patient who has at least one Graves' disease patient within first-degree relatives. The national survey of Japanese familial Graves' disease revealed that the 2.1–3.1% of hyperthyroidism appeared to be familial onset of Graves' disease, and that the relative risk of familial Graves' disease was estimated to be 19–41 times high as compared to that of non-familial one [35]. There are several reports showing HLA and CTLA-4 polymorphism in a group of familial Graves' disease [6, 7, 11, 17, 27]. However, it is quite rare to obtain the

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genetic analysis of pedigree with familial Graves' disease. In the present study we determined the HLA haplotypes in a rare pedigree of familial Graves' disease with childhood onset.

Case Report

A 17 year-old woman visited Jichi Medical University Omiya Medical Center in October 2001 because of palpitation, hyperhydrosis and irritable state. She was diagnosed as having Graves' disease, with serum TSH less than 0.015 IU/ml; free T_3, 13.6 pg/ml, free T_4, 4.51 ng/dl and TSH receptor antibody (TRAb), 94.1%. She was started on treatment with methimazole 30 mg/day. Her thyroid function was normalized in January 2002, and has been kept in the normal range with 10 mg/day methimazole. She has two brothers of 19 and 13 years-old. Two years after she was diagnosed, her elder brother complained of similar symptoms and visited our medical center at 18 years. He was also diagnosed as Graves' disease, with serum TSH less than 0.015 IU/ml; free T_3, 22.8 pg/ml, free T_4, 6.45 ng/dl and TRAb, 48.4%. A year later her younger brother was also diagnosed as Graves' disease at 13 years. Serum TSH was less than 0.015 IU/ml; free T_3, 17.5 pg/ml, free T_4, 4.77 ng/dl and TRAb, 49.1%. These two bothers have been treated with methimazole, and kept in euthyroid state. During the therapeutic periods the dose of methimazole has been gradually reduced from 30 to 10 mg/day. We asked their mother about Graves' disease in her family and relatives, and learned that 3 other persons have or had Graves' disease. As shown in Figure 1, two aunts and a cousin of the proband had Graves' disease. Their onset of Graves' disease was also at teen-age. However, there is no patient with Graves' disease in the paternal pedigree. We obtained informed consent from all the subjects for determining their HLA haplotypes. The present study was approved by the ethical committee of Jichi Medical University for human studies. We obtained informed consent from all the subjects who joined the present study.

Table 1 shows the characteristics of thyroid function in the present family. Graves' disease was all childhood onset in the six patients. Three patients (Nos. 7, 8 and 9) had high titers of TRAb. Detailed data were not available in the patients Nos. 3, 4 and 5, because their onsets were 5–31 years ago, and the hospitals they were treated at were located in Iwate Prefecture, in northeastern Japan. Four patients have been treated with methimazole, and subtotal thyroidectomy was performed in two patients. All the patients are now kept in euthyroid state. In other persons their thyroid function was in normal range and TRAb was negative.

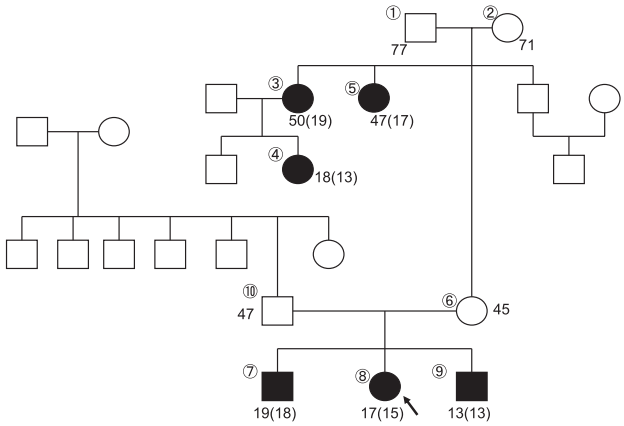

Fig. 1. The present pedigree of familial Graves' disease. The numbers in the brackets show the onset age of Graves' disease.

Table 2 shows HLA-DRB1 and -DQB1 haplotypes in the subjects of the present study. The haplotypes of DRB1*150101 and DQB1*0602 were highly frequent in the members of maternal pedigree including both affected and unaffected Graves' disease, except for the cousin of the proband (No. 4) who had DRB1*140301 and DQB1*030101. We could not find any difference in clinical profile between the subjects with DRB1*150101 and DQB1*0602 and the subject No. 4 without these haplotypes. Also, DRB1*140101 was obtained in this maternal pedigree, but they were less frequent in the members of maternal pedigree affected with Graves' disease. However, two cases of Graves' disease also had HLA haplotypes of DRB1*40501 and DQB1*0401, which were susceptible for Japanese childhood onset Graves' disease, in addition to the haplotypes of DRB1*150101 and DQB1*0602. The father of the proband (No. 10) had the HLA-DRB1*150201 and *40501 and HLA-DQB1*0401 and *0601.

Discussion

Graves' disease is an autoimmune disease, in which TRAb exaggerates thyroid hormone synthesis mediated via TSH receptors. Both genetic and environmental

Table 1. Characteristics of thyroid function in the family

No.	Age	Sex	Autoimmune diseases	Onset	Thyroid function at the onset				Therapy
					TSH (IU/ml)	FT_3 (pg/ml)	FT_4 (ng/dl)	TRAb (%)	
1	77	M	No	—	1.09	2.19	1.06	0.6	—
2	71	F	No	—	2.32	3.14	1.14	0	—
3	50	F	GD	19	*				Thyroidectomy
4	18	F	GD	13	*				Methimazole
5	47	F	GD	17	*				Thyroidectomy
6	45	F	No	—	3.66	2.36	1.08	0.6	—
7	19	M	GD	18	<0.015	22.8	6.45	48.4	Methimazole
8	17	F	GD	15	<0.015	13.6	4.51	94.1	Methimazole
9	13	M	GD	13	<0.015	17.5	4.77	49.1	Methimazole
10	47	M	No	—	4.99	2.81	1.08	0	—

* The data were not available because the onset were 5–31 years ago, and the hospitals were located in Iwate prefecture, northeastern Japan. GD: Graves' disease

Table 2. HLA haplotypes in the subjects of the present family

		DRB1				DQB1			
		140101	150101	40501	others	0401	0602	50301	others
1		+	+				+	+	
2			+		140301		+		30101
3	GD	+	+				+	+	
4	GD	+			140301			+	30101
5	GD (not examined)								
6		+	+				+	+	
7	GD		+	+		+	+		
8	GD		+		150201		+		0601
9	GD		+	+		+	+		
10				+	150201	+			0601

factors may affect the development of Graves' disease. Such an autoimmune mechanism could be associated with HLA on chromosome 6p [5–13, 24–33] and CTLA-4 on chromosome 2q33 [14–18]. There are several different results regarding HLA haplotypes associated with Graves' disease in Japan [13, 28, 29, 32, 33]. Positive associations were obtained with the haplotypes of DRB1*0803, DQB1*1403, DQA1*0103 alleles [32], DRB1*0803, DQA1*0103, DQB1*0601 alleles [32], DRB1*1403, DQA1*0501, DQB1*0301 alleles [32], DRB1*0405, DQB1*0401 alleles [13], and DPB1*0501 alleles [28, 33]. Also, the positive association of different haplotypes with Graves' disease was reported in other ethnic population; that is, DQB1*0301, DR 11 and DR 3 in male Caucasian patients [30], DRB3*0202, DQA1*0501 in adult African Americans [31], DRB1*0301, DRB1*0201, DRB3*0101 in juvenile Danish patients [25, 26], and DQB1*0303 in child Chinese patients [24].

The frequency of hyperthyroidism among familial Graves' disease is 2.1-3.1% in Japan [35]. There were no differences in age, sex, clinical feature and laboratory findings between familial and non-familial Graves' disease. There are a few reports regarding analysis of HLA haplotypes in familial Graves' disease [6, 7, 11, 27]. In the present study the haplotypes of DRB1*150101 and DQB1*0602 were highly frequent in the members of maternal pedigree including affected and unaffected Graves' disease, except for the cousin. These haplotypes were not found in the patients with sporadic Graves' disease in Japanese and other ethnic population [7, 9, 13, 24, 28]. Because the HLA haplotypes specifically conferred an increased risk for Graves' disease in sporadic form, it is of value to find the differences in HLA typing between familial and sporadic forms of Graves' disease. Because the cases

of Nos. 2 and 6 who had the HLA haplotypes of DRB1*150101 and DQB1*0602 did not have Graves' disease, the present haplotypes may not be a highly penetrating gene. The previously reported HLA haplotypes of DRB1*40501 and DQB1*0401 were found in two cases of Graves' disease in this maternal pedigree, who also had DRB1*150101 and DQB1*0602. The father had DRB1*40501 and DQB1*0401, but he did not have Graves' disease. These findings indicate that the HLA haplotypes of DRB1*40501 and DQB1*0401 may not associate with the onset of familial Graves' disease in the present pedigree, and that additional haplotypes of HLA may contribute to the development of Graves' disease in childhood-onset familial form.

We may further consider that gene mutations are related to the pathogenesis of autoimmune disorder in Graves' disease. In this point of view, it is known that autoimmune regulator gene on chromosome 21q 22.3 is responsible for autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome (APECED) [36]. APECED is the first autoimmune disease which is clarified by single gene mutation in the recessive inheritance. However, there is not any report regarding single gene mutation in Graves' disease yet. Further study will be necessary to elucidate the possible genetic factor involved in the pathogenesis of familial Graves' disease, including unknown HLA haplotypes, CTLA-4 polymorphism and APECED-related genes.

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