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Interleukin-12p40 gene (IL-12B) polymorphism and Type 1 diabetes mellitus in Japanese: Possible role in subjects without having high-risk HLA haplotypes

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Abstract

The present study was undertaken to clarify a role of interleukin-12p40 gene (IL-12B) polymorphism, located on chromosome 5q33-34 (IDDM 18), in Japanese subjects with Type 1 diabetes mellitus (T1DM) and autoimmune thyroid diseases (AITD). In 179 subjects with T1DM, 166 with AITD (128 with Graves' disease and 38 with Hashimoto's thyroiditis) and 115 healthy control subjects, the IL-12B 3′UTR A-C polymorphism was determined by PCR-RFLP method. In T1DM subjects, the genotype was also analyzed in relation to human leukocyte antigen (HLA)-DRB1-DQB1 haplotype status. There was a weak difference in the distribution of the genotype frequency between T1DM and control subjects, and the C allele frequency was higher in T1DM subjects ($P < 0.05$). In 68 T1DM subjects without having high-risk HLA haplotypes to T1DM in this population, the genotype distribution and C allele frequency was significantly different from control subjects without high-risk HLA haplotypes ($P < 0.01$), and from T1DM subjects with high-risk HLA haplotypes ($n = 111$) ($P < 0.05$). There was no difference in the genotype and allele frequencies between AITD and control subjects. In conclusion, the IL-12B 3′UTR A-C polymorphism did not seem to play a major role on genetic susceptibility to T1DM and AITD in Japanese, although the polymorphism conferred susceptibility in T1DM subjects without having high-risk HLA haplotypes. The IL-12B 3′UTR A-C polymorphism would be considered as a supplementary risk factor to T1DM in conjunction with HLA haplotypes.

Keywords: Type 1 diabetes mellitus; Interleukin-12; HLA; Graves' disease

1. Introduction

Susceptibility to Type 1 diabetes mellitus (T1DM) is determined by both environmental and genetic factors. The genetic contribution to T1DM is proved to

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be polygenic, and it is supposed that a large number of susceptibility genes work in concert, interacting each other and with environmental factors [1]. The gene of human leukocyte antigen (HLA, IDDM 1), which encodes fine structures responsible for antigen presentation, is proven to be a major genetic factor of T1DM, which accounts for about 50% [2]. Insulin gene (IDDM 2) and cytotoxic T lymphocyte antigen 4 gene (CTLA-4, IDDM 12) are also established genetic determinants of T1DM [3]. A variety of new susceptible loci contributing to T1DM have been found by genome wide linkage analyses [1,2,4]. A new susceptibility locus, IDDM 18, has been identified on chromosome 5q33-34, where the gene encoding p40 subunit of interleukin-12 (IL-12) is located [5].

IL-12, composed of two subunits, p40 and p35, is a key cytokine driving differentiation of T lymphocyte into type 1 helper (Th1) cell and promotes cell-mediated immunity [6]. A pathogenic role of IL-12 has been shown in NOD mice and human T1DM [7–9]. IL-12 also seems to play some role in evolution of autoimmunity to the thyroid gland in BB rats [10].

As for IDDM 18, Morahan et al. demonstrated that a few polymorphisms of IL-12p40 (IL-12B) gene confer genetic susceptibility to T1DM in Australian and British diabetes families, especially in case of HLA-identical sibpairs [5]. Following the initial presentation, several papers examined an association of IL-12B alleles with T1DM, but the results were inconsistent [11–16]. Since most studies were conducted mainly in Caucasian subjects, it is important to examine an involvement of IL-12B polymorphism in other ethnic population. The present study was undertaken to clarify a role of IL-12B polymorphism in Japanese subjects with T1DM and autoimmune thyroid diseases (AITD). Possible interactions with HLA genotypes were also examined in subjects with T1DM.

2. Materials and methods

2.1. Subjects

The study population comprised a total of 460 unrelated Japanese subjects; namely 179 with T1DM (71 males and 108 females), 166 with AITD (46 and 120), and 115 healthy control subjects (45 and 70, aged 33 ± 13 years old, mean ± S.D.) without having any family history of T1DM and AITD.

The diagnosis of T1DM was based on the criteria of the Expert Committee of the American Diabetes Association [17]. The onset age of T1DM was 34 ± 16 years ranging from 1 to 75 years, and all subjects displayed insulin dependency at the time of this study with the duration of 10 ± 9 years and fasting serum C-peptide levels of 0.15 ± 0.15 nmol/l, respectively. Fifteen (8%) out of the 179 subjects displayed fulminant onset of diabetes as reported by Imagawa et al. [18], 108 subjects (60%) typical acute onset of diabetes which required insulin therapy within 1 year after the initial documentation of hyperglycemia, and the remaining 56 subjects (31%) slow onset of diabetes. Coexistence of AITD was found in 40 patients (22%), 16 subjects had Graves' disease and 24 Hashimoto's thyroiditis, respectively.

The overall prevalence of positive GAD and anti-insulinoma-associated protein 2 (IA-2) antibodies (measured by commercial RIA kits; Hoechst Japan or Cosmic Corporation, Tokyo, Japan) was 50% (90/179) and 30% (52/176), respectively, and at least either antibody was positive in 105 out of the 179 subjects (59%). At the time of antibodies' measurement, the duration of T1DM was less than 1 year in 50 patients, from 1 to 5 years in 30, from 6 to 10 years in 25, and greater than 10 years in 74, respectively. The prevalence of GAD and IA-2 antibodies was 52 and 41% in subjects with the antibodies' measurement performed within 1 year after the disease onset ($n = 50$), 70 and 43% in subjects with the duration from 1 to 5 years ($n = 30$), 40 and 24% in subjects with that from 6 to 10 years ($n = 25$), and 45 and 18% in subjects with that greater than 10 years ($n = 74$), respectively.

In T1DM and control subjects, HLA-DRB1-DQB1 genotype was determined by allele-specific polymerase chain reaction (PCR) kits (Dynal Allset or SSP Unitray, Dynal Biotech Ltd., UK) and/or PCR-restriction fragment length polymorphism (PCR-RFLP) kits (DR Lab, Saitama, Japan). In two control subjects, HLA genotype could not be determined because of lack of enough amount of genomic DNA.

Among 166 subjects with AITD, 128 (41 males and 87 females) were diagnosed as having Graves' disease, based on thyrotoxicosis, and positive anti-thyrotropin receptor antibody measured by commercial radioimmunoassay kits and/or increased uptake of ^{123}I in