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Research Paper

Subtyping of Type 1 Diabetes as Classified by Anti-GAD Antibody, IgE Levels, and *Tyrosine kinase 2* (*TYK2*) Promoter Variant in the Japanese

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ABSTRACT

Objective: Type 1 diabetes (T1D) is known to be caused by Th1 cell-dependent autoimmunity. Recently, we reported that *TYK2* promoter variant serves as a putative virus-induced diabetes susceptibility gene associated with deteriorated interferon-dependent antiviral response. *TYK2* is also related to HIES, that is, Th2 cell-dependent. Therefore, *TYK2* promoter variant may be also associated with the pathogenesis of T1D, modulating Th1/Th2 balance.

Research Design and Methods: We assessed the association between anti- GAD Ab, IgE levels, and *TYK2* promoter variant among 313 T1D patients, 184 T2D patients, and 264 YH controls in the Japanese.

Results: T1D patients had elevated IgE (median, 56.7 U/ml; $p < 0.0001$) compared with T2D patients (22.5 U/ml) and controls (43.3 U/ml). Contrary to our expectations, there was no correlation between *TYK2* promoter variant and IgE levels. We found that T1D could be subtyped as four groups based on anti-GAD Ab and IgE profile: Subtype 1, anti-GAD Ab positive and non-elevated IgE (47.0%); Subtype 2, anti-GAD Ab negative and non-elevated IgE (35.1%); Subtype 3, anti-GAD Ab positive and elevated IgE (10.9%); and Subtype 4, anti-GAD Ab negative and elevated IgE (7.0%). In Subtype 2, a significantly higher incidence was observed in T1D cases carrying the *TYK2* promoter variant (OR, 2.60; 95%CI, 1.03–6.97; $p = 0.032$), and also showing a flu-like syndrome at diabetes onset (OR, 2.34; 95%CI, 1.27–4.35; $p = 0.003$).

Interpretation: Anti-GAD Ab and IgE profiling helps classifying T1D into four groups that recognize variable pathogenic bases of T1D.

Abbreviations: T1D, Type 1 diabetes; Th1, type 1 T helper; *TYK2*, *Tyrosine kinase 2*; HIES, hyper-IgE syndrome; Th2, type 2 T helper; Anti-GAD Ab, anti-glutamic acid decarboxylase antibody; YH, young healthy controls; OR, odds ratio; CI, confidence interval.

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1. Introduction

T1D is caused by extensive destruction of insulin-producing pancreatic beta-cells leading to absolute insulin deficiency, and the incidence has been increasing worldwide at a rate of 3% every year (American Diabetes Association, 2014; Atkinson et al., 2014; *IDF Diabetes Atlas Seventh Edition 2015*, 2015; Scully, 2012). The American Diabetes Association has proposed two classifications of T1D, immune-mediated (Type 1A) and idiopathic (Type 1B) (American Diabetes Association, 2014). The immune-mediated form of T1D results from cellular mediated autoimmune destruction of pancreatic beta-cells, has strong associations with HLA, and is characterized by the production of several autoantibodies including anti-insulin antibody (IAA), anti- GAD Ab, islet antigen 2 antibody (IA-2 Ab), anti-zinc transporter antibody (ZnT8 Ab), and historic anti-islet cell antibody (ICA Ab) (American Diabetes Association, 2014). It has been well established that T1D is mainly a Th1 cell-dependent autoimmune associated disease (Haskins and Cooke, 2011), while this assignment of Th cells has been largely based on precarious conditions in experimental animals that did not correctly reflect the delicate balance or the relative contribution of each Th subset throughout the disease (Azar et al., 1999). It was reported that Th2 cells may play a progressive role by accelerating autoimmunity due to production of Th2 cytokines (Azar et al., 1999). In contrast, the idiopathic form of T1D is strongly inherited, but there is no evidence of autoimmunity or HLA association (American Diabetes Association, 2014). Fulminant T1D in which a non-autoimmune process may associate with the onset was also reported as an important subtype in East Asia (Imagawa and Hanafusa, 2011). These observations imply that T1D patients possibly possess a delicate Th1/Th2 balance. Overall, it was suggested that T1D seems to include heterogeneous diseases whose pathogenic processes, immunologic basis, genetics, and phenotypic characteristics present marked variations (Atkinson et al., 2014; Kawasaki and Eguchi, 2004).

The importance of environmental factors for T1D onset has also been well documented (Atkinson et al., 2014; Coppieters et al., 2012; de Beeck and Eizirik, 2016). Viruses, as one of the environmental factors, particularly coxsackieviruses that belong to the genus enterovirus in the *Picornaviridae* family, have long been suspected to contribute to the T1D onset (Coppieters et al., 2012; de Beeck and Eizirik, 2016; Jun and Yoon, 2003). Multiple factors could interplay among enterovirus, immune system and host genes (Hober and Sauter, 2010), as enterovirus infection may lead to the activation of innate and adaptive immunity against pancreatic beta cells (Hober and Sauter, 2010). The mechanisms of beta-cell destruction by viruses have been reported: induced direct virolysis of beta-cells, local inflammatory responses, or virus infection triggering beta-cell specific autoimmunity, together leading to destruction of beta-cells (de Beeck and Eizirik, 2016; Jun and Yoon, 2003). The former situation seems to be the case of high dose encephalomyocarditis (EMC)-D virus (a picornavirus)-induced diabetes in inbred mice, which is an excellent animal model resembling fulminant T1D in humans (Shimada and Maruyama, 2004; Nagafuchi et al., 2013). Since intact innate anti-viral responses play a pivotal role in the protection against picornavirus infection (Takeuchi and Akira, 2009), it is suggested that innate immunity-associated genes are candidates for determining susceptibility to virus-induced diabetes (Kounoue et al., 2008; Nagafuchi et al., 2013). Consistently, some innate immunity associated genes have been reported as candidate genes for T1D. These include helicase C domain 1 (*IFIH1*) (or melanocyte differentiation antigen (*MDA*) 5), protein tyrosine phosphatase non-receptor type 2 (*PTPN2*), BTB domain and CNC homolog 2 (*BACH2*), and *TYK2* (de Beeck and Eizirik, 2016; Marroqui et al., 2015; Onengut-Gumuscu et al., 2015).

TYK2 is a member of the Janus kinase (JAK) family, and plays an important role in signals of type 1 IFN and IL-12 to resist against microbial infections (Leitner et al., 2015; Shimoda et al., 2000). Genetically-determined alternations of IFN responses, including *TYK2* gene, are detrimental in immune and inflammatory disease such as T1D (Leitner et al., 2015; Jean-Baptiste et al., 2017). Recently, based on the discovery of natural mutations of *TYK2* gene as murine encephalomyocarditis (EMC)-D virus-induced diabetes susceptibility gene causing deteriorated type 1 interferon (IFN) response (Izumi et al., 2015), we could show that "*TYK2* promoter variant" in Japanese subjects is associated with an increased risk of T1D (Nagafuchi et al., 2015). The prevalence rate of *TYK2* promoter variant is high in overall T1D (9.6%; OR, 2.4: $p = 0.012$), most highly in T1D associated with flu-like syndrome at diabetes onset (13.7%; OR, 3.6: $p = 0.005$), and anti-GAD Ab negative T1D (12.8%; OR, 3.3: $p = 0.0021$), compared with age- and sex-matched healthy controls (4.2%) (Nagafuchi et al., 2015). These results suggested that *TYK2* promoter variant may serve as a virus-induced T1D susceptibility gene, possibly due to reduced type 1 IFN response (Nagafuchi et al., 2015), but not Th1 cell-dependent autoimmunity. Consistently, it was reported that *Tyk2*-mediated signaling was not essential for the development of Th1 cell (Hashiguchi et al., 2014). *TYK2* promoter variant serves as a risk not only in T1D but also in T2D, suggesting that *TYK2* promoter variant is associated with an overall risk for diabetes (Nagafuchi et al., 2015). It has also been reported that *TYK2* gene is closely linked with HIES, that is, Th2 cell-dependent immune response-associated disease (Minegishi et al., 2006). *TYK2* deficiency is a type of primary immunodeficiency displaying the phenotype of the autosomal recessive HIES, and is likely to account for the phenotype of impaired Th1 differentiation and accelerated Th2 differentiation (Minegishi et al., 2006). *TYK2* gene is thus closely related to immunologic condition and therefore, the *TYK2* promoter variant may possibly be associated with the pathogenesis of T1D modulating Th2 cell-dependent immunologic responses.

In the present study, we focused on *TYK2* gene as a susceptibility gene for both T1D and HIES, and assessed the association among anti-GAD Ab, IgE levels, and *TYK2* promoter variant in diabetic patients. Here we report the immunological bases on which T1D forms may be classified into four subtypes among T1D by simple clinical markers.

2. Materials and Methods

2.1. Subjects

We studied 313 patients with T1D, 184 patients with T2D and 264 young non-diabetic subjects (YH) (these are not age-matched with the T1D and T2D cohorts - see also Table 1) in the western Japan region. These subjects partly provided the same samples which were studied in our previous article (Nagafuchi et al., 2015), and sample size of T1D has been shown to be appropriate for the T1D case control study. For comparison, estaimated suitable number of patients with T2D and Young Healthy Controls were chosen to be applicable for appropriate statistical analysis in case-control study by computer. The clinical profiles of patients studied are presented in Table 1. Among the 313 patients with T1D, 76 patients were associated with flu-like syndrome at the onset. Symptoms of flu-like syndrome include fever, chills, sore throat, muscle and joint aches, poor appetite, diarrhea, cough, and fatigue, suggestive of certain viral infections not limited to enterovirus infection. Those patients with clinical features such as tonsillitis, pneumonia, or urinary tract infection associated with neutrophilia, suggestive of bacterial origin, were not regarded as patients with flu-like syndrome and were excluded from the group. The study was conducted in accord with case-control studies of STROBE statement. Since it was reported that there was a peak of IgE levels in the group of 19 to 21 years old (De Amici and Ciprandi, 2013), we selected YH as a control group. Patients were designated as T1D if fasting C-peptide was <0.5 ng/ml with insulin-dependent condition (IDDM), or as T2D if fasting blood glucose levels were higher than 126 mg/dl and HbA1c levels exceeded 6.5% with non-insulin-dependent status (NIDDM). In Japanese T1D patients, positivity of anti-GAD Ab is reported to be 60–70% (Kawasaki and Eguchi, 2004). The individuals had no clinical sign of allergy. The study was conducted according to the guidelines for human study and was approved