A novel clinical entity related to autoimmune polygladular syndrome (APS) termed “anti–PIT-1 antibody syndrome” has recently been reported [1]. PIT-1 plays a crucial role in regulating the expression of GH, PRL, and TSHβ in the anterior pituitary. Abnormalities in the PIT-1 gene result in short stature and congenital combined pituitary hormone deficiency (CPHD), which is characterized by specific GH, PRL, and TSH deficiencies [2, 3]. Anti–PIT-1 antibody syndrome is characterized by acquired GH, PRL, and TSH deficiencies associated with a presence of circulating anti–PIT-1 antibody along with various autoantibodies. A missense single-nucleotide polymorphism in the sialic acid acetyesterase (SIAE) gene is associated with anti–PIT-1 antibody syndrome.

Masaaki Yamamoto1), Genzo Iguchi1), Hironori Bando1), Hidenori Fukuoka1), Kentaro Suda1), Michiko Takahashi1), Hitoshi Nishizawa1), Ryusaku Matsumoto1), Katsuyoshi Tojo2), Atsuko Mokubo3), Tsutomu Ogata4) and Yutaka Takahashi1)

1) Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan
2) Division of Diabetes and Endocrinology, the Jikei University Kashiwa Hospital, Kashiwa, Japan
3) Mokubo Naika Clinic, Kawasaki, Japan
4) Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan

Abstract. A novel clinical entity related to autoimmune polygladular syndrome (APS) termed “anti–PIT-1 antibody syndrome” is characterized by a presence of circulating autoantibody against the pituitary-specific transcriptional factor-1 (PIT-1) with acquired specific defect in GH, PRL, and TSH. Although autoimmunity to PIT-1 has been suggested, the underlying mechanisms remain to be elucidated. Sialic acid acetyesterase (SIAE) plays a crucial role in regulating the threshold of autoantibody production of B-cells and the defective variants of SIAE are associated with an increased risk of various autoimmune diseases such as type 1 diabetes (T1DM). To explore the link between anti–PIT-1 antibody syndrome and SIAE, we analyzed SIAE gene in 3 patients with anti–PIT-1 antibody syndrome and 200 healthy control subjects, and compared the prevalence of single nucleotide polymorphisms. Intriguingly, we found A467V SIAE variants (c.1400C>T, rs7941523) in a heterozygous state in all the patients with anti-PIT-1 antibody syndrome, while we detected in 6 % of control subjects, in which the prevalence was significantly increased in the patients (P<0.0005). Considering the physiological function of SIAE and the clinical features of anti-PIT-1 antibody syndrome, present data imply a novel aspect of the pathogenesis in this disease.

Key words: Anti PIT-1 antibody syndrome, PIT-1, SNP, Autoimmune polygladular syndrome, SIAE

A NOVEL clinical entity related to APS termed “anti–PIT-1 antibody syndrome” has recently been reported. PIT-1 plays a crucial role in regulating the expression of GH, PRL, and TSHβ in the anterior pituitary. Abnormalities in the PIT-1 gene result in short stature and congenital combined pituitary hormone deficiency (CPHD), which is characterized by specific GH, PRL, and TSH deficiencies [2, 3]. Anti–PIT-1 antibody syndrome is characterized by acquired GH, PRL, and TSH deficiencies associated with a presence of circulating anti–PIT-1 antibody along with various autoantibodies. APS is defined by the occurrence of 2 or more autoimmune-based organopathy including endocrine tissue and is generally classified into 3 groups [4]. APS-1 is a rare monogenic disorder caused by mutations in the autoimmune regulator (AIRE) gene [5, 6]. The more common syndrome, APS-2, is less well defined, and includes overlapping groups of disorders. It is strongly associated with polymorphic genes of the human leukocyte antigen (HLA). In immune dysfunction, polyendocrinopathy, and X-linked (IPEX) syndrome, the patients harbor mutations in the forkhead box P3 (Foxp3) gene that lead to severe autoimmunity and infiltrate of lymphocytes and plasma cells in multiple organs, which is compatible with APS. These features suggest an impairment of PIT-1–specific lineage cells in the pituitary by autoimmunity, although the precise mechanisms remain unknown.

APS is defined by the occurrence of 2 or more autoimmune-based organopathy including endocrine tissue and is generally classified into 3 groups [4]. APS-1 is a rare monogenic disorder caused by mutations in the autoimmune regulator (AIRE) gene [5, 6]. The more common syndrome, APS-2, is less well defined, and includes overlapping groups of disorders. It is strongly associated with polymorphic genes of the human leukocyte antigen (HLA). In immune dysfunction, polyendocrinopathy, and X-linked (IPEX) syndrome, the patients harbor mutations in the forkhead box P3 (Foxp3) gene that lead to severe autoimmunity

©The Japan Endocrine Society
and immune deficiency [7]. Of interest, family members of APS-2 patients are frequently diagnosed with autoimmune disorders, although those are not necessarily the same combination of autoimmune diseases as the patients, strongly suggesting a presence of genetic pathogenesis for the immune intolerance [8]. In anti-PIT-1 antibody syndrome, no common HLA haplotype was observed in the patients [1], suggesting that non-HLA locus may be related as a susceptibility gene.

Highly powered genetic studies have found and confirmed many genes outside the established role of the HLA locus with APS, such as PTPN-22 and CTLA-4, and indicate an essential role of immune response pathways in these diseases [9]. Recently, it has been reported that a panel of rare and functionally defective genetic variants in the sialic acid acetylesterase (SIAE) are strongly associated with many autoimmune conditions including Crohn’s disease, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and type 1 diabetes (T1D) [10]. Although there was a limitation in the number of patients, we explored whether these rare SIAE variants are associated with anti–PIT-1 antibody syndrome.

**Subjects and Methods**

This study was approved by the Kobe University Graduate School of Medicine Ethics Committee and written informed consent was obtained from the patients and control subjects. We recruited 3 patients with anti-PIT-1 antibody syndrome and 200 healthy control subjects. The details of the patients were described in the previous report [1]. Briefly, 3 middle-aged or elderly male patients with acquired and specific GH, PRL, and TSH deficiency manifested various endocrine organ impairment with several autoantibodies. Circulating anti-PIT-1 antibody was detected in all these patients. Genomic DNA was isolated from peripheral-blood leukocytes, the SIAE gene was amplified by the polymerase chain reaction (PCR), and sequenced as previously described [10]. The statistical analysis was performed using Fisher’s exact test.

**Results**

We sequenced all the coding and exon-intron boundary region and compared 4 non-synonymous common variants in SIAE gene [11] between the patients with anti-PIT-1 antibody syndrome [1] and healthy control subjects. We found 2 variants, K71R (c.190G>A, rs12282107) and A467V (c.1400C>T, rs7941523) in a heterozygous state in these patients (Fig. 1). Although K71R was detected only in case 1 and 2, A467V was detected in all 3 cases. The analysis in 200 control sub-

![Fig. 1 Sequencing analysis of SIAE gene in patients with anti-PIT-1 antibody syndrome and control subjects. The results of K71R and A467V are shown.](image-url)
jests revealed that the prevalence of K71R and A467V was 11% and 6%, respectively (Table 1). Intriguingly, the prevalence of A467V was significantly increased in the patients with anti-PIT-1 antibody syndrome compared with control subjects. In contrast, the prevalence of other variants was not different between these 2 groups (Table 1). Haplotype analysis of these variants did not show any differences between these 2 groups (data not shown).

**Discussion**

We here demonstrate that heterozygous state of A467V SIAE variant was significantly associated with anti-PIT-1 antibody syndrome. It has been shown that catalytically defective heterozygous rare variants of SIAE were shown to be linked to autoimmune disorders [10, 12]. These SIAE variants are associated with high odds ratios (OR) in a number of autoimmune disorders, including rheumatoid arthritis (OR 8.3), T1DM (OR 7.9), and all autoimmune disorders (OR 8.6) [10]. Given that SIAE is a negative regulator of B lymphocyte signaling by acting at inhibitory receptors, a deletion of Siae gene in mice results in a defect in B-cell tolerance as evidenced by the spontaneous development of autoantibodies [13], and the risk of primary biliary cirrhosis was associated with the functionally defective SIAE variants [12], it has been suggested that SIAE plays an important role in the development of autoimmunity. SIAE has reportedly contributed to a signaling mechanism that helps set a threshold for the B-cell activation [14], suggesting that a reduction in the activity of SIAE may increase the risk for the production of autoantibodies.

We found that the prevalence of A467V SIAE variant was 6% in Japanese healthy control subjects and this indicates that it is a relatively common variant. It is compatible with the results that the prevalence of this variant in European ancestry was 2.3% and that in African-Americans and European-Americans was 4.3% [11]. It remains unclear that this heterozygous state of A467V variant plays a causal role in the development of anti-PIT-1 antibody syndrome. Recently, Chellappa et al. has reported that A467V SIAE variant was not catalytically defective in esterase assay in vitro as well as the common variant K71R [11]. These data rather suggest that not the A467V SIAE variant itself but the neighbouring SNPs associated with A467V SIAE variant that affect the function or expression of SIAE protein may causally be related with the pathogenesis. Also, while it explains the susceptibility for the autoimmunity, it cannot explain the specific immune intolerance for PIT-1, suggesting a presence of multiple hits for developing this condition.

Because of the limited number of the patients of this syndrome, co-incidence cannot be ruled out and further study is necessary to clarify the significance of the variants. Nevertheless, considering the physiological function of SIAE for regulating the threshold of the B-cell activation and the clinical features of anti-PIT-1 antibody syndrome, the present data imply a novel aspect of the pathogenesis in anti-PIT-1 antibody syndrome.

**Acknowledgements**

The authors are grateful to C. Ogata and K. Imura for their excellent technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology 23659477, 23591354, and 22591012, Grants-in-Aid for Scientific Research (research on hypothalamic-hypophyseal disorders) from the Ministry of Health, Labor, and Welfare, Japan, Daiichi-Sankyo Foundation of Life Science, and Naito Foundation.

**Conflict of Interest**

The authors have nothing to disclose.
References


