Research following genome-wide association study focuses on the multifaceted nature of Src kinase-associated phosphoprotein 2 in type 1 diabetes

In type 1 diabetes patients, pancreatic β-cells are destroyed by autoimmunity, leading to absolute insulin deficiency. Antigen-presenting cells, such as macrophages and dendritic cells, cytotoxic T cells, and inflammatory cytokines, contribute to this destruction of the pancreatic β-cells through mechanisms that have not yet been fully elucidated.

Type 1 diabetes is associated with genetic factors, and genetic studies including genome-wide association studies (GWAS) have identified more than 60 risk loci. These loci contain a large number of candidate genes associated with pancreatic β-cells, inflammation, cytokines and apoptosis, and have been suggested to play an important role in the pathogenesis of type 1 diabetes. Therefore, investigating the functions of the identified candidate genes and the effects of their variants is important for further assessing pathological conditions.

Determining how the variants reported in GWAS affect gene expression and gene product function is challenging. To that end, integrated analyses with quantitative trait locus analysis, next-generation sequencing approaches, omics analysis and clinical parameters are required.

If each of the multiple gene polymorphisms shown by GWAS is associated with the onset, it can be said that the GWAS data are useful for stratifying and predicting the prognosis of type 1 diabetes, and recently these were analyzed. Among the candidate genes revealed by GWAS, Brorsson et al. focused on those expressed in islets and regulated by cytokines, and attempted to construct a genetic risk score for those. For this purpose, they identified 11 genes that are expressed in pancreatic β-cells and whose expression is affected by cytokine treatment. The greater the duplication of the 11 genes, the poorer the glycemic control and residual β-cell function. Src kinase-associated phosphoprotein 2 (SKAP2) is one of those 11 genes. Cytokine treatment was observed to reduce the expression of SKAP2 in pancreatic β-cells; furthermore, increased expression of SKAP2 in pancreatic β-cells reduced the apoptosis of pancreatic β-cells on cytokine treatment, suggesting that SKAP2 acts protectively in pancreatic β-cells.

Fløyel et al. developed this study by focusing on the disease-related single-nucleotide polymorphism, rs7804356, reported in GWAS; they found that the CC genotype of rs7804356 was significantly associated with poorer glycemic control and residual β-cell function. Because rs7804356 is located in the third intron of SKAP2, it does not affect the gene coding region. This fact requires investigation of the expression quantitative trait locus effect of rs7804356.

Interestingly, there was no difference between the SKAP2 expression in the islets of the two alleles; however, the T allele showed decreased SKAP2 expression in B- and T lymphocytes. This suggests the complexity of the association between SKAP2 and type 1 diabetes. First, higher expression of SKAP2 has a protective effect on pancreatic β-cells, whereas higher expression of SKAP2 in immune cells can lead to a more aggressive immune attack on pancreatic β-cells (Figure 1). Second, the expression of SKAP2 can vary among tissues.

In fact, they used the Genotype-Tissue Expression portal, which is a public resource, to examine the effects of rs7804356 on other tissues. They found that this single-nucleotide polymorphism exerts an expression quantitative trait locus effect on SKAP2 in whole blood, pancreas, lymphocytes and fibroblasts, as well as on various nearby homeobox A genes in the blood and fibroblasts. Taken together, these results suggest that the rs7804356 genotype is associated with altered expression of SKAP2 in a tissue-specific manner. Further research is required to clarify the association between SKAP2 and type 1 diabetes.

GWAS have detected frequent single-nucleotide polymorphisms; however, the influence (odds ratio) of each phenotype is often not high. Therefore, infrequent gene mutations that strongly lead to disease outbreaks can be a blind spot in GWAS.

Unlike GWAS, whole exome sequencing (WES) is a new method developed for comprehensively analyzing exons, which are regions of protein translation. Although exons are sized at only approximately 1–1.5% of the entire genome, they are important, because most hereditary diseases are caused by mutations in the exon region, and WES has the advantage of efficiently identifying disease-related genes. Using this method, Rutsch et al. identified a patient with
The proband was a 24-year-old woman who was diagnosed with Hashimoto's thyroiditis and type 1 diabetes at the age of 14 years; she had hemolytic anemia, severe food allergy, eczema and Raynaud's disease. Laboratory findings showed the following: heterozygous anti-glutamic acid decarboxylase, anti-thyroglobulin, anti-thyroid peroxidase, anti-nuclear and anti-thyroid peroxidase 1 antibodies. WES and Sanger sequencing of deoxyribonucleic acid samples from the patient, her parents and her brother showed that she had a de novo germline coding variant in SKAP2 (c.475G>A, p.Gly153Arg).

SKAP2 contains three domains: (i) an N-terminal four-helix bundle dimerization domain; (ii) a pleckstrin homology domain; and (iii) an SRC homology 3 domain. It forms a homodimer using the N-terminal four-helix bundle dimerization domain; it is located within the lipid-binding loop of the pleckstrin homology domain, suggesting that it results in functional changes.

SKAP2 is a substrate for the Src family kinases and is involved in cytoskeleton remodeling in cell adhesion through integrin; it is being studied primarily with regard to the fields of cancer and immunity. SKAP2 is reportedly involved in the formation, proliferation, invasion and metastasis of cancer, and in the migration and activation of macrophages. Therefore, they focused on macrophages, and evaluated the phenotype of primary macrophages carrying the SKAP2 p.Gly153Arg heterozygous allele, collected from the proband (patient) and her unaffected mother (control), and differentiated those monocytes into macrophages ex vivo.

Western blot analysis of the patient and control macrophages showed that the c.475G>A variant did not alter the protein expression of SKAP2. Physiologically, chemokine signals induce murine SKAP2 to migrate from the cytosol to the cell membrane and bind the Wiskott–Aldrich syndrome protein (WASp) to promote actin polymerization. As a result, the extracellular region of integrins on the surface of the macrophages are induced to adopt an open conformation that enhances the binding force of integrin ligands (inside-out signaling). In addition, this change increased the cellular response to extracellular integrin ligands (outside-in signaling; Figure 2). They examined these processes in both the patient and control macrophages. Interestingly, in control macrophages, these processes run for the first time by cytokine treatment, whereas in patient macrophages, these processes run without cytokine treatment; that is, from the resting state. Therefore, they concluded that SKAP2 c.475 G>A is a gain of function mutation, and that increased macrophage activity and migration promote type 1 diabetes, as well as autoimmune and inflammatory diseases.

In summary, for the first time, a rare monogenic disease caused by the SKAP2 c.475 G>A mutation has been reported using the novel WES. It was also suggested that SKAP2 c.475 G>A mutation increases macrophage activity and
promotes type 1 diabetes, as well as autoimmune and inflammatory diseases. This condition is very similar to autoimmune polyglandular syndrome caused by mutations in autoimmune regulator (AIRE) or the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome caused by mutations in Forkhead Box P3 (FOXP3) and is different from the typical type 1 diabetes. However, understanding rare variants is essential for elucidating the pathophysiology of type 1 diabetes, and provides important information on the relationship between SKAP2 and type 1 diabetes. Upon researching, we wanted to know about the effects of SKAP2 c.475 G>A on pancreatic β-cells. In our field, we strongly felt that we would like to study cysteine sulfenic acid decarboxylase/long noncoding-integrin subunit beta 7-1 (CSAD/lnc-ITGB7-1), a candidate gene recently reported in a GWAS on Japanese fulminant type 1 diabetes, with reference to their method. Finally, we hope that the research following GWAS builds big data on type 1 diabetes and leads to the realization of pancreatic β-cell protective treatment; that is, the introduction of complete remission, which has not yet been realized.

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