Review Article

Current and Future Clinical Applications of Zinc Transporter-8 in Type 1 Diabetes Mellitus

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Abstract

Objective: To evaluate the utility of zinc transporter-8 (ZnT8) in the improvement of type 1 diabetes mellitus (T1DM) diagnosis and prediction, and to explore whether ZnT8 is a potential therapeutic target in T1DM.

Data Sources: A search was conducted within the medical database PubMed for relevant articles published from 2001 to 2015. The search terms are as follows: "ZnT8," "type 1 diabetes," "latent autoimmune diabetes in adults," "type 2 diabetes," "islet autoantibodies," "zinc supplement," "T cells," "β cell," "immune therapy." We also searched the reference lists of selected articles.

Study Selection: English-language original articles and critical reviews concerning ZnT8 and the clinical applications of islet autoantibodies in diabetes were reviewed.

Results: The basic function of ZnT8 is maintaining intracellular zinc homeostasis, which modulates the process of insulin biosynthesis, storage, and secretion. Autoantibodies against ZnT8 (ZnT8A) and ZnT8-specific T cells are the reliable biomarkers for the identification, stratification, and characterization of T1DM. Additionally, the results from the animal models and clinical trials have shown that ZnT8 is a diabetogenic antigen, suggesting the possibility of ZnT8-specific immunotherapy as an alternative for T1DM therapy.

Conclusions: ZnT8 is a novel islet autoantigen with a widely potential for clinical applications in T1DM. However, before the large-scale clinical applications, there are still many problems to be solved.

Key words: Autoantibodies; Autoimmunity; T Cells; Type 1 Diabetes Mellitus; Zinc Transporter-8

Introduction

Diabetes is one of the most challenging chronic and heterogeneous diseases all over the world,[1] affecting more than 10% of Chinese adults.[2,3] Type 1 diabetes mellitus (T1DM), although less common than type 2 diabetes mellitus (T2DM), is the major autoimmune diabetes in humans.[1] Approximately 6% adult T2DM subjects are afflicted with latent autoimmune diabetes in adults (LADA), a special subgroup of T1DM.[4] The treatment or preventive strategies for patients vary widely depending on the accurate determination of the specific type of diabetes. For example, a person with T1DM, but not T2DM, needs to follow a life-long insulin therapy. Therefore, early, quick, and accurate diagnosis of T1DM is crucial for treatment decisions and avoiding complications.

In general, the main diagnostic criteria of T1DM are based on the clinical manifestations and the conventional metabolic biomarkers, such as β cell function. Because T1DM represents a tissue-specific autoimmune disease, the markers of autoimmunity are also applicable in T1DM. Indeed, the histopathologic results from pancreatic biopsies of patients with recent-onset T1DM (<1 year) have shown the infiltration of immune cells.[5] The following studies confirmed the presence of a predominantly T lymphocytic infiltration, especially islet-specific $CD8^+$ T cells.[5,6] Apart from the islet tissue, islet-reactive T cells are circulating in

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the blood of T1DM patients and exhibit a diverse immune phenotype.[5,7-10] Additionally, autoantibodies against islet autoantigens (AAbs) are also well-accepted immunologic markers for T1DM.[7]

For decades, a number of islet autoantigens have been identified. The major autoantigens are insulin, glutamic acid decarboxylase 65 (GAD65), tyrosine phosphatase-related molecules-2 (IA-2), and the zinc transporter-8 (ZnT8).[7,11] Researches have demonstrated that these autoantigens are involved in the secretory pathway,[7,12] and are common targets of autoantibodies and T cells.[7,13-16] However, the proinsulin/insulin molecules (especially B9-B23 peptide), but not others, are considered as a primary target of the autoimmunity.[7,17] Moreover, these autoantigens show diverse functions in the secretory pathway of insulin, as well as the metabolism regulation and the survival of β cells.[5] These results indicate that the biochemical nature and immune features of islet autoantigens may be different with each other.

As a novel islet autoantigen,[11] the broad immune features of ZnT8 will be discussed in this review to evaluate the utility of ZnT8 in improvement of T1DM diagnosis and prediction, and to explore whether ZnT8 is a potential therapeutic target.

The Structure, Expression, and Biochemical Function of Zinc Transporter-8

ZnT8 is a 369 amino acid trans-membrane protein, encoded by *SLC30A8* at the chromosome 8q14.11. It contains six trans-membrane domains, cytoplasmic amino- and carboxy-terminal tails [Figure 1a]. Due to two single-nucleotide polymorphisms (SNPs) within *SLC30A8*, rs13266634 and rs16889462, the amino acid at position 325 of ZnT8 could be arginine (R), tryptophan (W), or glutamine (Q). Previous researches have shown that rs13266634 was associated with T2DM,[18] but not with T1DM,[19,20] suggesting that it can stratify T1DM risk in children with autoantibody against ZnT8 (ZnT8A) positive.[21] Interestingly, the 12 rare mutations in *SLC30A8* (p.Arg138*, p.Lys34Serfs*50, c.71+2T>A, p.Met50Ile, c.271+G>A, c.419-1G>C, p.Trp152*, p.Gln174*, c.572+1G>A, p.Tyr284*, p.Ile291Phefs*2, and p.Ser327Thrs*55) could reduce the risk of T2DM by 65%.[22] These indicate that the genotypes of *SLC30A8* may be the common genetic triggers of T1DM and T2DM.

ZnT8 is more specifically expressed in insulin-containing secretory granules than GAD65 and IA-2.[11] However, little is known about factors that regulate ZnT8 expression [Figure 1b]. Pound and coworkers observed that pancreatic and duodenal homeobox 1 (Pdx1) is the main transcription factor regulating ZnT8 expression.[23] Several studies have shown that ZnT8 expression was down-regulated by cytokines,[24,25] hyperglycemia, and Zn^{2+} depletion.[26] Intriguingly, such factors could also induce β cell apoptosis or necrosis.[24,26] This might increase the opportunity of ZnT8 exposure in the islets, raise the risk of islet autoimmunity in genetically susceptible subjects, and could even trigger or exacerbate T1DM.[7]

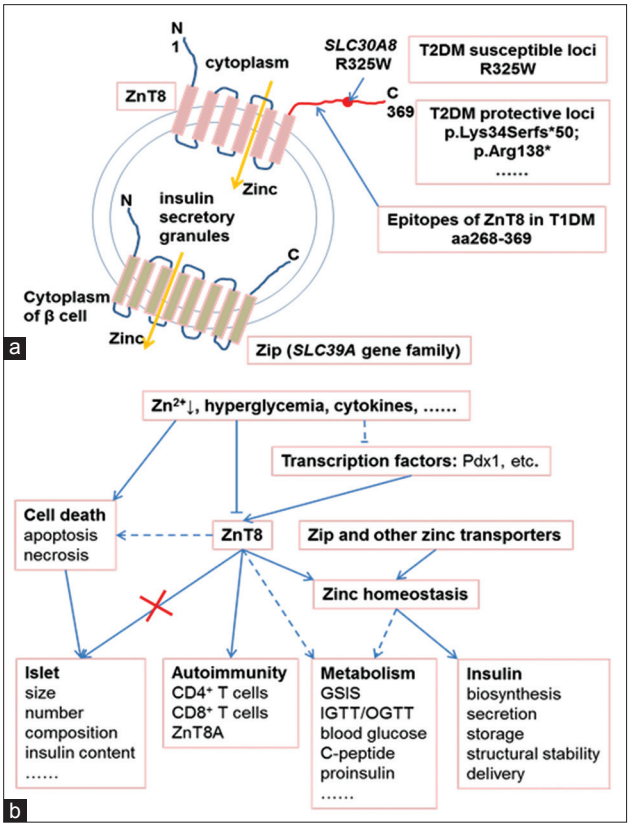

Figure 1: The expression and structure of ZnT8 (a) are adapted from Kawasaki 2012. The biochemical function and regulation of ZnT8 are shown (b). The solid lines represent the explicit function, and the dashed lines represent the unknown or ambiguity function. The Red Cross presents no effect. GSIS: Glucose-stimulated insulin secretion; IGTT: Intravenous glucose tolerance test; OGTT: Oral glucose tolerance test; Pdx1: pancreatic and duodenal homeobox 1; ZnT8: Zinc transporter-8.

The functions of ZnT8 were reviewed recently[27,28] [Figure 1b]. In brief, together with other zinc transporters,[28] the basic function of ZnT8 is maintaining intracellular zinc homeostasis, which is essential for the structural stability of insulin and the process of insulin storage and secretion. However, ZnT8 does not affect islet insulin content, islet size, and cell composition.[28] In addition, the effects of ZnT8 on glucose-stimulated insulin secretion (GSIS), insulin sensitivity, and glucose tolerance tests are conflicting. For example, some people reported ZnT8 could impair GSIS, but others observed enhanced or unchanged GSIS.[28] The role of ZnT8 on the survival of β cells is also indeterminate.[27] The possible explanation for these discordant results might be the interaction between ZnT8 and other influential factors, such as environmental factors, genetic background, gender and ages, and the subcellular localization of ZnT8.[28]

The Epitopes of Zinc Transporter-8 as an Autoantigen

When detecting the autoantibodies in T1DM patients' serum using the different fragments of human ZnT8, only C-terminus fragment produced the highest sensitivity and specificity (50.4% and 98%).[11] The autoantibody against

ZnT8-COOH (ZnT8A-COOH) was detected in 18.6% patients with T1DM, while ZnT8A-NH2 was rare.[29] Other studies demonstrated that the dominant epitope(s) of ZnT8 may be located at aa268-369 of ZnT8,[27,30,31] which is a conformational rather than linear epitope.[32] By using site-directed mutagenesis in C-terminus of ZnT8, researchers revealed that ZnT8 epitopes are critically dependent on the polymorphism at aa325.[33] It has been shown that among new-onset T1DM patients, the prevalence of ZnT8A-325R is approximately 50%, which is slightly more than ZnT8A-325W.[32,34,35] The positive rate of ZnT8A-325Q in T1DM is nearly 30%.[32,35] However, receiver operating characteristic curve of ZnT8A-325Q indicates that it could not significantly distinguish T1DM patients from healthy controls, which means ZnT8-325Q is not a valuable epitope for disease diagnosis.[35] Therefore, the polymorphism at aa325 of ZnT8 C-terminal, especially ZnT8-325R and/or ZnT8-325W, confers the key antigenicity and epitope specificity of ZnT8A. Additionally, linear epitope $R_{325}R_{332}E_{333}K_{336}K_{340}$ contributes another region of antigenicity.[31]

In view of the role of T cells in T1DM, the key epitopes of ZnT8-reactive T cells were identified. Chang and Unanue first identified ZnT8 as a protein bound to HLA-DQ8 molecules, especially aa166-179 of ZnT8.[36] In NOD mice, $ZnT8_{330–344}$ and $ZnT8_{345–359}$ were presented after protein immunization by islet antigen presenting cells, but only the latter was diabetogenic epitope.[37] Dang *et al.* found that $ZnT8_{8–22}$, $ZnT8_{15–29}$, $ZnT8_{120–134}$, $ZnT8_{134–148}$, $ZnT8_{260–274}$, $ZnT8_{267–281}$, and $ZnT8_{295–309}$ can be bound to HLA-DR4, while $ZnT8_{155–169}$ and $ZnT8_{323–337}$ are HLA-DR3 restricted epitopes in humans.[14] Although the $ZnT8_{186–194}$ is the predominant epitope for $CD8^+$ T cells in T1DM,[38] $ZnT8_{153–161}$, $ZnT8_{107–115}$, $ZnT8_{115–123}$, and $ZnT8_{145–153}$ are also identified as HLA-A*0201-restricted $CD8^+$ T cells epitopes in T1DM,[38-40] while $ZnT8_{253–261}$ was more frequently recognized in T2DM than T1DM.[38] Interestingly, most T cells epitopes against ZnT8 were mapped in the transmembrane region or the C-terminal, and none overlapped with the polymorphic region at aa325 of ZnT8.

Cellular Immunity of Zinc Transporter-8 and Clinical Application

It has been shown that T-cell responses to islet proteins can distinguish a subgroup of LADA patients from AAbs-negative phenotypic T2DM,[41] suggesting the utility of T cells in etiological classification at diagnosis. So far, T-cell responses against islet autoantigens have been identified in the peripheral blood of patients with T1DM,[16] including ZnT8.[13-15] $ZnT8_{345–359}$-specific T cells can accelerate diabetes in irradiated recipient mice (NOD or NOD.Rag1$^{-/-}$).[37] In humans, ZnT8-sepcific T-cell response was observed in the majority of T1DM patients (>68%).[14,15] In contrast, the frequencies of ZnT8-specific T cells are lower in the healthy population and T2DM patients (<8% and 30%, respectively).[13,14,38] Interestingly, as discussed above, the predominant $CD8^+$ T-cell epitopes of ZnT8 is different between T1DM and T2DM ($ZnT8_{186–194}$ and $ZnT8_{253–261}$, respectively).[38] ZnT8-specific $CD4^+$ T cells were tended to convert into Th1 cells in T1DM, but into Th2 and interleukin-10 producing cells in healthy adults.[13] These results indicate that the prevalence and predominant epitope of ZnT8-specific T-cell response and cytokine release could be used to distinguish T1DM from T2DM and healthy people. However, before the clinical application of ZnT8-specific T cells, the major challenge is the standardized T cells assays, which is one of the main goals of the T-cell workshop initiated by the Immunology of Diabetes Society.[42]

Clinical Applications of ZnT8A

The features of ZnT8A

As discussed above, autoantibodies against ZnT8-325W and/or ZnT8-325R (ZnT8A-325R and/or ZnT8A-325W) contribute to the most ZnT8A positivity in T1DM patients.[32,34,43] The prevalence and titer of ZnT8A-325R were significantly associated with T1DM patients carrying a C allele (CC + CT).[21,32,34] ZnT8A-325W was more prevalent in patients with TT and CT genotypes, and the titer was higher in patients carrying a T allele.[34] Meanwhile, an unusual ZnT8A type, ZnT8A-325Q, showed no relationship with the rs13266634.[32] The possible reason is that glutamine (Q) at position 325 of ZnT8 is determined by rs16889462, but not rs13266634. Additionally, the specificity of ZnT8A had little or no association with the adjacent rs2466295 and rs6469675 SNPs.[32] These studies confirm that *SLC30A8* rs13266634 is a critical determinant for ZnT8A specificity.

The dynamics of ZnT8A has also been reported by some studies. They found that ZnT8A typically appeared by 3 years of age in children with high risk for T1DM, and no child seroconverted to ZnT8A positivity at age 3 or 6 months.[44,45] In addition, ZnT8A titers were usually low before 2 years of age but increased gradually in the following 2 years.[11] Interestingly, ZnT8A-COOH developed at a median age of 3.2 years, which was earlier than ZnT8A-NH_2 (6.3 years).[21] After T1DM onset, the progressive decline curves of ZnT8A were approximated accordance with first-order exponential decay kinetics,[46-48] with a half-life ranging from 26 to 530 weeks.[47] Nevertheless, ZnT8A could be detected within 25 years after T1DM onset (6.7%), and even in patients with T1DM for more than 50 years.[47,49]

ZnT8A is a necessary complement for the classification and diagnosis of type 1 diabetes mellitus

The distributions of ZnT8A depend on the types of diabetes and ethnics[11,34,43,50-54] [Table 1]. As reported by Wenzlau *et al*. the prevalence of ZnT8A was 60–80% in new-onset T1DM, <3% in T2DM and <2% in normal population in Caucasian.[11] Our researches suggested that ZnT8A positivity in T1DM[51] and phenotypic T2DM patients (including LADA)[54] were lower in Chinese (24.1% and 1.99%, respectively), and it was 17.4% in fulminant T1DM.[55] In Japanese, the positive percentage of ZnT8A in childhood-onset T1DM, adult-onset T1DM, and fulminant T1DM were 58%, 34%, and 0%, respectively.[52,53]

Table 1: The distributions of ZnT8A in different types of diabetes subjects

Ethnicity	Childhood-onset T1DM (%)	Adult-onset T1DM (%)	Fulminant T1DM (%)	LADA* (%)	T2DM† (%)	FDR (%)	Reference(s)
USA	60.00–80.00‡	–	–	–	<3.00	0.90	[11,58]
Japan	58.00	34.00	0.00	–	–	–	[52,53]
China	24.10‡	–	17.40	10.70	0.40	–	[51,54,55]
Finland	63.00	18.70	–	34.30	–	–	[56]
Norway	8.70	22.00	–	6.20	–	–	[57]
Sweden	80.00	66.00	–	42.00	–	–	[46]
Argentinian	–	–	–	23.50	8.90	–	[43]
Belgium	58.00‡	–	–	–	–	1.46	[59,60]

*T2DM with GADA$^+$; †T2DM with GADA$^-$; ‡T1DM regardless of age. FDR: First-degree relative; LADA: Latent autoimmune diabetes in adults; T2DM: Type 2 diabetes mellitus; T1DM: Type 1 diabetes mellitus; GADA: Autoantibodies against to GAD65.

However, the results are variable in Europe. For instance, ZnT8A was more prevalent in LADA than adult-onset T1DM in Finland (34.3% vs. 18.7%),[56] which was opposite in Norway (6.2% vs. 22%).[57] It is recommended that all patients with diabetes should detect the ZnT8A regardless of the ethnicity.

It has been shown that at least 93% of the T1DM patients were autoantibody positive, based on autoantibodies against to GAD65 (GADA), IA-2 (IA-2A), insulin (IAA), and ZnT8A.[61] ZnT8A was found in nearly 30% other islet autoantibodies negative patients.[11,50] According to our previous data, the diagnostic sensitivity of T1DM and LADA was 65.5% and 8.62%, respectively, based on GADA, IA-2A, and ZnT8A.[51,54] With the addition of ZnT8A to ICA, GADA, and IA-2A increased the diagnostic sensitivity from 79.3% to 83.1% in newly diagnosed diabetic patients.[62] Furthermore, ZnT8A is a valuable marker to differentiate clinical phenotypes in a proportion of patients with LADA,[29] especially based on the detection of GADA and ZnT8A.[43]

ZnT8A is the most sensitive AAbs for predicting type 1 diabetes mellitus

It has been shown that AAbs, especially multiple AAbs positive, can help to screen people with high risk of T1DM.[12] In fact, among the first-degree relative (FDR) of T1DM, 6.1% person is persistently IAA$^+$, GADA$^+$, IA-2A$^+$, and/or ZnT8A$^+$, but only 34% of those persistently AAbs-positive FDR had developed T1DM within a median 63 months follow-up period.[63] In FDR positive for IAA, GADA, or IA-2A, the 4- and 5-year risk of T1DM were 7% and 17%, respectively.[58,59] When ZnT8A was added, the 4- and 5-year cumulative risk increased to 31% and 47%, respectively[58,59] [Figure 2]. However, for ZnT8A, the 5-year diabetes cumulative risk in the 58 ZnT8A-COOH-positive children was 48%, but ZnT8A-NH2 cannot stratify the T1DM risk.[21] Notably, screening for IA-2A and ZnT8A is a more cost-effective strategy to identify progressors to T1DM.[63] It allowed identifying up to 78% of FDR developing diabetes within 5-year, especially under the age of 40.[59,60,63] Additionally, the presence of CC or TT genotype of *SLC30A8* rs13266634 SNP could also increase the risk of T1DM in ZnT8A-COOH-positive children.[21,64] These mean ZnT8A, either alone or in combination with IA-2A, is a highly sensitive biomarker for the prediction of T1DM. Therefore, it is recommended that all of the FDR of T1DM should screen for ZnT8A and IA-2A. If needed, genotypes of *SLC30A8* and other islet autoantibodies, such as GADA and IAA, should be detected as well.

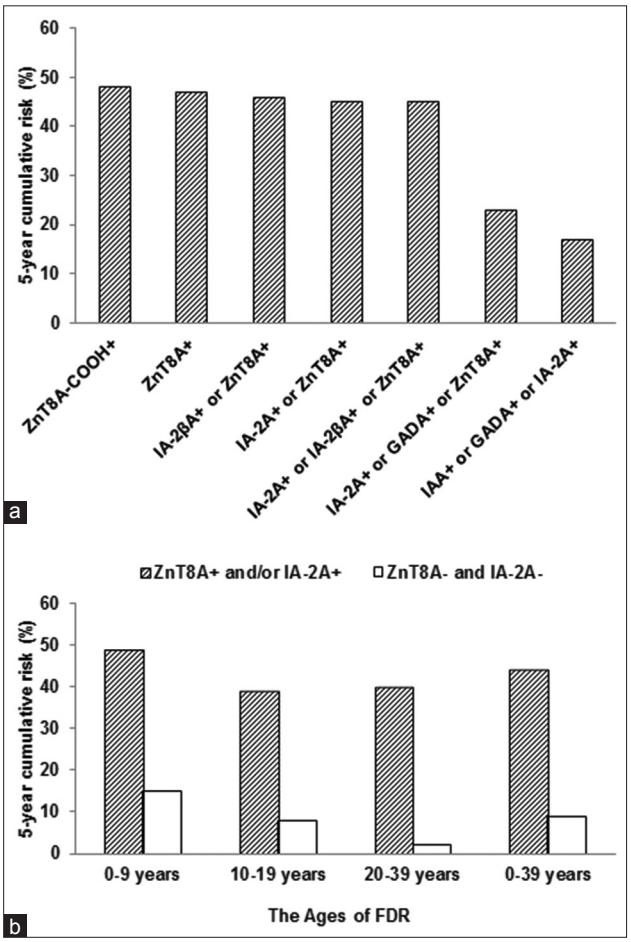

Figure 2: The 5-year cumulative risk of diabetes in relatives of T1DM patients according to the AAbs patterns (a) and age (b). FDR: First-degree relatives; GADA: Autoantibodies against glutamic acid decarboxylase 65; IA-2A: Autoantibodies against insulinoma antigen 2; IA-2βA: Autoantibodies against insulinoma antigen 2β; IAA: Autoantibodies against insulin; T1DM: Type 1 diabetes mellitus.

The associations between ZnT8A and β cells function

It has been shown that the high level of ICA, GADA, and/or IA2A at diagnosis in adult-onset T1DM predicts a future complete β cells failure.[65,66] Meanwhile, the persistent autoantibody negative T1DM patients have

slower β cell destruction.[67] Actually, the multiple islet autoantibodies (IAA, IA-2A, or ZnT8A) are considered as an independent risk factor for insulin requirement and appear to be better than GADA titer and GAD65-specific epitopes.[68] In contrast, for ZnT8A, initial higher titer (especially the level of ZnT8A-325R) was associated with higher concentrations of stimulated C-peptide, which means the better β cells function, during the 12 months follow-up in T1D patients.[69] Some studies also found a concurrent decline in ZnT8A titers and stimulated C-peptide.[47,70] A possible explanation for this discrepancy could be that a better β cell function might cause more ZnT8 antigen to be exposed.[69] In addition to the titer of autoantibodies, other biomarkers can be used for the prediction of β cells function. For example, decreases in IA-2A and ZnT8A-325W levels, but not ZnT8A-325R, can predict a lower residual β cell function at 3–6 years after diagnosis.[71] Furthermore, the C allele of *SLC30A8* rs13266634 SNP was associated with residual β cells function during the first year after diagnosis.[69,70] This SNP could account for 3.8% of the variance in the doses of insulin treatment.[72] Taken together, the biomarkers for the prediction of β cell function decline include the lower level of ZnT8A-325R, decreased ZnT8A-325W and IA-2A and the TT genotype of *SLC30A8* rs13266634 SNP. These also suggest that it is better to detect ZnT8A-325W and ZnT8A-325R in all diabetic patients, especially in LADA patients.

Is ZnT8A suitable for therapeutic monitoring?

So far, a number of biomarkers, such as metabolic biomarkers, AAbs and T cells, are used for therapeutic monitoring in T1DM.[9] Because of the weak diabetogenic role of ZnT8[37] and the methodological limitations,[9] ZnT8-specific T cells may not be suitable for routine monitoring of T1DM patients receiving treatment. Additionally, the presence of AAbs, such as GADA, IA-2A, and ZnT8A, is the frequently-used inclusion criteria for intervention trials in T1DM.[73] However, GADA and IA-2A fail to reflect the progression of T1DM.[9] Although ZnT8A is positively associated with the loss of β cell function in T1DM,[69,71] the anti-CD20 monoclonal antibody rituximab had only slight effects on ZnT8A, as well as GADA and IA-2A.[74] Taken together, these results suggest ZnT8A may be suitable for screening T1DM patients, rather than therapeutic monitoring.

Zinc Transporter-8: A Potential Therapeutic Target?

As a novel islet autoantigen, an intriguing question is whether ZnT8 can be used as a therapeutic target for T1DM. It has been shown that zinc, which is regulated by ZnT8, could modulate gene expression within the immune cells, such as T cells and natural killer cells,[75] and prevents cytokine-induced β cells destruction.[27] Additionally, $CD4^+$ T cells could recognize ZnT8 peptide presented by islet antigen presenting cells in NOD mice,[37] indicated that ZnT8 directly participates in the development of islet autoimmunity. Moreover, antibodies recognizing ZnT8 could cross-react with *Mycobacterium avium subspecies paratuberculosis* (MAP) epitopes, such as $ZnT8_{186–194}$ (VAANIVLTV) and $MAP3865c_{133–141}$ (LAANFVVAL), $ZnT8_{178–186}$ (MIIVSSCAV) and $MAP3865c_{125–133}$ (MIAVALAGL),[76] which means that ZnT8 may be a target protein after viral infections. Thus, ZnT8 treatment might inhibit the islet autoimmunity, and ZnT8 may be a potential therapeutic target for T1DM.[32]

The next question is how ZnT8 can be "druggable" targeted? First, a number of clinical trials of immunomodulatory therapies based on islet autoantigens, including oral and nasal insulin or alum-formulated recombinant human GAD65, are undergoing in T1DM patients.[73] Although there are no relevant literature about the ZnT8-specific immunotherapy in T1DM, ZnT8 might induce immune tolerance as well as GAD65, which is supported by the diabetogenic role of ZnT8 in NOD mice.[37] Second, ZnT8 transporter activity increment and ZnT8 pharmacological inhibitors would be a useful therapeutic option.[77] However, ZnT8 inhibitors not only inhibit ZnT8 activity, but also down-regulate insulin biosynthesis, storage, and secretion.[28] The direct function of ZnT8 in metabolism and β cell survival is still uncertain.[28] Thus, regulating ZnT8 function to prevent or treat T1DM needs more evidence. The third possible strategy is zinc supplementation. Recently, a meta-analysis found that zinc supplementation (3–240 mg/d, median 30 mg/d) significantly reduced fasting glucose concentrations and HbA1c, but not insulin concentrations, in diabetic patients in the overall, ungrouped analysis.[78] However, zinc supplementation seems to have no effect on glucose metabolism in T1DM patients.[78] There are many problems to be solved, such as the dose and the timing of zinc supplementation before the large scale clinical application. Finally, no matter what ZnT8-specific therapy plan is chosen, one of the first issues to consider is patient selection. The positive rate of ZnT8A in Asians is lower, which means ZnT8 might not be the major diabetogenic autoantigen in Asians. Therefore, the disparity caused by ethnicity must also be taken into consideration before choosing ZnT8-specific therapy for T1DM.

Conclusions

As a novel islet autoantigen, ZnT8 has widely clinical applications in T1DM. (1) For the general population, the genotypes of *SLC30A8* help to evaluate the risk of the different types of diabetes (T1DM and T2DM) progression. (2) ZnT8-specific T cells response, especially Th1 response, contribute to distinguish T1DM from T2DM. Combined ZnT8A and ZnT8-sepcific T-cell response yield higher diagnostic sensitivity of T1DM. (3) Among FDR, early detection of ZnT8A and other islet autoantibodies, especially IA-2A, is recommended. If positive, the 5-year diabetes risk is nearly 50%. (4) For LADA patients, ZnT8A is a valuable biomarker for the better β cells function, especially the high level of ZnT8A-325R. Additionally, decreases in ZnT8A-325W and the TT genotype of *SLC30A8* rs13266634 SNP are also the risk factors for insulin therapy. (5) ZnT8A may be suitable for screening and

diagnosing T1DM, rather than therapeutic monitoring. (6) Due to the function of ZnT8 in maintaining intracellular zinc homeostasis and the diabetogenic role of ZnT8 in T1DM, ZnT8 may be a potential therapeutic target for T1DM.

Nevertheless, many challenges still lie ahead. First, a thorough evaluation of clinical and immune features, such as genetic background and the antibody affinity, should be done in ZnT8A positivity subjects. Second, what is the optimal screening and diagnostic strategy for T1DM based on AAbs and other biomarkers, such as biochemical and metabolic biomarkers, islet autoantigens-specific T cells response, and genetic background? However, optimization and establishment of a more sensitive and accurate detection method for ZnT8A and ZnT8-specific T cells is still required, in particular, to achieve synchronous detection of multiple AAbs in a single assay. The purpose of this is to standardize the detection protocol among labs. The strategy and clinical efficacy of ZnT8-specific therapy for T1DM are other concerns that need to be addressed. Further challenges include the understanding of the in-depth mechanism of ZnT8 and ZnT8-specific T cells in the pathogenesis of diabetes, which could open up new avenues for the prevention and/or cure of T1DM.

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Conflicts of interest

There are no conflicts of interest.

References

1. International Diabetes Federation. IDF Diabetes Atlas. 6th ed. Brussels, Belgium: International Diabetes Federation; 2013.
2. Xu Y, Wang L, He J, Bi Y, Li M, Wang T, *et al.* Prevalence and control of diabetes in Chinese adults. JAMA 2013;310:948-59.
3. Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, *et al.* Prevalence of diabetes among men and women in China. N Engl J Med 2010;362:1090-101.
4. Zhou Z, Xiang Y, Ji L, Jia W, Ning G, Huang G, *et al.* Frequency, immunogenetics, and clinical characteristics of latent autoimmune diabetes in China (LADA China study): A nationwide, multicenter, clinic-based cross-sectional study. Diabetes 2013;62:543-50.
5. In't Veld P. Insulitis in human type 1 diabetes: A comparison between patients and animal models. Semin Immunopathol 2014;36:569-79.
6. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, *et al.* Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med 2012;209:51-60.
7. Arvan P, Pietropaolo M, Ostrov D, Rhodes CJ. Islet autoantigens: Structure, function, localization, and regulation. Cold Spring Harb Perspect Med 2012;2:a007658.
8. Di Gialleonardo V, de Vries EF, Di Girolamo M, Quintero AM, Dierckx RA, Signore A. Imaging of ß-cell mass and insulitis in insulin-dependent (Type 1) diabetes mellitus. Endocr Rev 2012;33:892-919.
9. Mallone R, Roep BO. Biomarkers for immune intervention trials in type 1 diabetes. Clin Immunol 2013;149:286-96.
10. Winter WE, Schatz DA. Autoimmune markers in diabetes. Clin Chem 2011;57:168-75.
11. Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, *et al.* The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A 2007;104:17040-5.
12. Ziegler AG, Nepom GT. Prediction and pathogenesis in type 1 diabetes. Immunity 2010;32:468-78.
13. Chujo D, Foucat E, Nguyen TS, Chaussabel D, Banchereau J, Ueno H. ZnT8-Specific CD4+ T cells display distinct cytokine expression profiles between type 1 diabetes patients and healthy adults. PLoS One 2013;8:e55595.
14. Dang M, Rockell J, Wagner R, Wenzlau JM, Yu L, Hutton JC, *et al.* Human type 1 diabetes is associated with T cell autoimmunity to zinc transporter 8. J Immunol 2011;186:6056-63.
15. Énée É, Kratzer R, Arnoux JB, Barilleau E, Hamel Y, Marchi C, *et al.* ZnT8 is a major CD8+T cell-recognized autoantigen in pediatric type 1 diabetes. Diabetes 2012;61:1779-84.
16. Mallone R, Brezar V, Boitard C. T cell recognition of autoantigens in human type 1 diabetes: Clinical perspectives. Clin Dev Immunol 2011;2011:513210.
17. Nakayama M, Abiru N, Moriyama H, Babaya N, Liu E, Miao D, *et al.* Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. Nature 2005;435:220-3.
18. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, *et al.* A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445:881-5.
19. Gohlke H, Ferrari U, Koczwara K, Bonifacio E, Illig T, Ziegler AG. SLC30A8 (ZnT8) polymorphism is associated with young age at type 1 diabetes onset. Rev Diabet Stud 2008;5:25-7.
20. Xu K, Zha M, Wu X, Yu Z, Yu R, Xu X, *et al.* Association between rs13266634 C/T polymorphisms of solute carrier family 30 member 8 (SLC30A8) and type 2 diabetes, impaired glucose tolerance, type 1 diabetes – a meta-analysis. Diabetes Res Clin Pract 2011;91:195-202.
21. Achenbach P, Lampasona V, Landherr U, Koczwara K, Krause S, Grallert H, *et al.* Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. Diabetologia 2009;52:1881-8.
22. Flannick J, Thorleifsson G, Beer NL, Jacobs SB, Grarup N, Burtt NP, *et al.* Loss-of-function mutations in SLC30A8 protect against type 2 diabetes. Nat Genet 2014;46:357-63.
23. Pound LD, Hang Y, Sarkar SA, Wang Y, Milam LA, Oeser JK, *et al.* The pancreatic islet ß-cell-enriched transcription factor Pdx-1 regulates Slc30a8 gene transcription through an intronic enhancer. Biochem J 2011;433:95-105.
24. Egefjord L, Jensen JL, Bang-Berthelsen CH, Petersen AB, Smidt K, Schmitz O, *et al.* Zinc transporter gene expression is regulated by pro-inflammatory cytokines: A potential role for zinc transporters in beta-cell apoptosis? BMC Endocr Disord 2009;9:7.
25. El Muayed M, Billings LK, Raja MR, Zhang X, Park PJ, Newman MV, *et al.* Acute cytokine-mediated downregulation of the zinc transporter ZnT8 alters pancreatic beta-cell function. J Endocrinol 2010;206:159-69.
26. Smidt K, Jessen N, Petersen AB, Larsen A, Magnusson N, Jeppesen JB, *et al.* SLC30A3 responds to glucose- and zinc variations in beta-cells and is critical for insulin production and *in vivo* glucose-metabolism during beta-cell stress. PLoS One 2009;4:e5684.
27. Kawasaki E. ZnT8 and type 1 diabetes. Endocr J 2012;59:531-7.
28. Davidson HW, Wenzlau JM, O'Brien RM. Zinc transporter 8 (ZnT8) and ß cell function. Trends Endocrinol Metab 2014;25:415-24.
29. Lampasona V, Petrone A, Tiberti C, Capizzi M, Spoletini M, di Pietro S, *et al.* Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes: Non Insulin Requiring Autoimmune Diabetes (NIRAD) 4. Diabetes Care 2010;33:104-8.
30. Skärstrand H, Lernmark A, Vaziri-Sani F. Antigenicity and epitope specificity of ZnT8 autoantibodies in type 1 diabetes. Scand J Immunol 2013;77:21-9.
31. Wenzlau JM, Frisch LM, Hutton JC, Davidson HW. Mapping of conformational autoantibody epitopes in ZNT8. Diabetes Metab Res Rev 2011;27:883-6.
32. Wenzlau JM, Liu Y, Yu L, Moua O, Fowler KT, Rangasamy S, *et al.* A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes 2008;57:2693-7.
33. Wenzlau JM, Moua O, Liu Y, Eisenbarth GS, Hutton JC, Davidson HW. Identification of a major humoral epitope in Slc30A8 (ZnT8). Ann N

Y Acad Sci 2008;1150:252-5.
34. Kawasaki E, Uga M, Nakamura K, Kuriya G, Satoh T, Fujishima K, *et al.* Association between anti-ZnT8 autoantibody specificities and SLC30A8 Arg325Trp variant in Japanese patients with type 1 diabetes. Diabetologia 2008;51:2299-302.
35. Vaziri-Sani F, Delli AJ, Elding-Larsson H, Lindblad B, Carlsson A, Forsander G, *et al.* A novel triple mix radiobinding assay for the three ZnT8 (ZnT8-RWQ) autoantibody variants in children with newly diagnosed diabetes. J Immunol Methods 2011;371:25-37.
36. Chang KY, Unanue ER. Prediction of HLA-DQ8beta cell peptidome using a computational program and its relationship to autoreactive T cells. Int Immunol 2009;21:705-13.
37. Nayak DK, Calderon B, Vomund AN, Unanue ER. ZnT8-reactive T cells are weakly pathogenic in NOD mice but can participate in diabetes under inflammatory conditions. Diabetes 2014;63:3438-48.
38. Scotto M, Afonso G, Larger E, Raverdy C, Lemonnier FA, Carel JC, *et al.* Zinc transporter (ZnT) 8 (186-194) is an immunodominant CD8+T cell epitope in HLA-A2+type 1 diabetic patients. Diabetologia 2012;55:2026-31.
39. Wu X, Xu X, Gu R, Wang Z, Chen H, Xu K, *et al.* Prediction of HLA class I-restricted T-cell epitopes of islet autoantigen combined with binding and dissociation assays. Autoimmunity 2012;45:176-85.
40. Li S, Li H, Chen B, Lu D, Deng W, Jiang Y, *et al.* Identification of novel HLA-A 0201-restricted cytotoxic T lymphocyte epitopes from Zinc Transporter 8. Vaccine 2013;31:1610-5.
41. Rolandsson O, Palmer JP. Latent autoimmune diabetes in adults (LADA) is dead: Long live autoimmune diabetes! Diabetologia 2010;53:1250-3.
42. Sanjeevi CB, Schatz DA, Atkinson MA. The IDS conducts standardization of assays for the measurement of autoantibodies and of T cells in autoimmune diabetes. Preface. Ann N Y Acad Sci 2008;1150:xiii.
43. Trabucchi A, Faccinetti NI, Guerra LL, Puchulu FM, Frechtel GD, Poskus E, *et al.* Detection and characterization of ZnT8 autoantibodies could help to screen latent autoimmune diabetes in adult-onset patients with type 2 phenotype. Autoimmunity 2012;45:137-42.
44. Ziegler AG, Bonifacio E, BABYDIAB-BABYDIET Study Group. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. Diabetologia 2012;55:1937-43.
45. Parikka V, Näntö-Salonen K, Saarinen M, Simell T, Ilonen J, Hyöty H, *et al.* Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. Diabetologia 2012;55:1926-36.
46. Vaziri-Sani F, Oak S, Radtke J, Lernmark K, Lynch K, Agardh CD, *et al.* ZnT8 autoantibody titers in type 1 diabetes patients decline rapidly after clinical onset. Autoimmunity 2010;43:598-606.
47. Wenzlau JM, Walter M, Gardner TJ, Frisch LM, Yu L, Eisenbarth GS, *et al.* Kinetics of the post-onset decline in zinc transporter 8 autoantibodies in type 1 diabetic human subjects. J Clin Endocrinol Metab 2010;95:4712-9.
48. Petruzelkova L, Ananieva-Jordanova R, Vcelakova J, Vesely Z, Stechova K, Lebl J, *et al.* The dynamic changes of zinc transporter 8 autoantibodies in Czech children from the onset of Type 1 diabetes mellitus. Diabet Med 2014;31:165-71.
49. Richardson CC, Dromey JA, McLaughlin KA, Morgan D, Bodansky HJ, Feltbower RG, *et al*. High frequency of autoantibodies in patients with long duration type 1 diabetes. Diabetologia 2013;56:2538-40.
50. Andersson C, Larsson K, Vaziri-Sani F, Lynch K, Carlsson A, Cedervall E, *et al.* The three ZNT8 autoantibody variants together improve the diagnostic sensitivity of childhood and adolescent type 1 diabetes. Autoimmunity 2011;44:394-405.
51. Yang L, Luo S, Huang G, Peng J, Li X, Yan X, *et al.* The diagnostic value of zinc transporter 8 autoantibody (ZnT8A) for type 1 diabetes in Chinese. Diabetes Metab Res Rev 2010;26:579-84.
52. Kawasaki E, Nakamura K, Kuriya G, Satoh T, Kobayashi M, Kuwahara H, *et al.* Zinc transporter 8 autoantibodies in fulminant, acute-onset, and slow-onset patients with type 1 diabetes. Diabetes Metab Res Rev 2011;27:895-8.
53. Kawasaki E, Nakamura K, Kuriya G, Satoh T, Kobayashi M, Kuwahara H, *et al.* Differences in the humoral autoreactivity to zinc transporter 8 between childhood- and adult-onset type 1 diabetes in Japanese patients. Clin Immunol 2011;138:146-53.
54. Huang G, Xiang Y, Pan L, Li X, Luo S, Zhou Z. Zinc transporter 8 autoantibody (ZnT8A) could help differentiate latent autoimmune diabetes in adults (LADA) from phenotypic type 2 diabetes mellitus. Diabetes Metab Res Rev 2013;29:363-8.
55. Luo S, Zhang Z, Li X, Yang L, Lin J, Yan X, *et al.* Fulminant type 1 diabetes: A collaborative clinical cases investigation in China. Acta Diabetol 2013;50:53-9.
56. Andersen MK, Härkönen T, Forsblom C, Groop PH, Knip M, Tuomi T. Zinc transporter type 8 autoantibodies (ZnT8A): Prevalence and phenotypic associations in latent autoimmune diabetes patients and patients with adult onset type 1 diabetes. Autoimmunity 2013;46:251-8.
57. Sørgjerd EP, Skorpen F, Kvaløy K, Midthjell K, Grill V. Prevalence of ZnT8 antibody in relation to phenotype and SLC30A8 polymorphism in adult autoimmune diabetes: Results from the HUNT study, Norway. Autoimmunity 2013;46:74-9.
58. Yu L, Boulware DC, Beam CA, Hutton JC, Wenzlau JM, Greenbaum CJ, *et al.* Zinc transporter-8 autoantibodies improve prediction of type 1 diabetes in relatives positive for the standard biochemical autoantibodies. Diabetes Care 2012;35:1213-8.
59. De Grijse J, Asanghanwa M, Nouthe B, Albrecher N, Goubert P, Vermeulen I, *et al.* Predictive power of screening for antibodies against insulinoma-associated protein 2 beta (IA-2beta) and zinc transporter-8 to select first-degree relatives of type 1 diabetic patients with risk of rapid progression to clinical onset of the disease: Implications for prevention trials. Diabetologia 2010;53:517-24.
60. Vermeulen I, Weets I, Asanghanwa M, Ruige J, Van Gaal L, Mathieu C, *et al.* Contribution of antibodies against IA-2ß and zinc transporter 8 to classification of diabetes diagnosed under 40 years of age. Diabetes Care 2011;34:1760-5.
61. Andersson C, Vaziri-Sani F, Delli A, Lindblad B, Carlsson A, Forsander G, *et al.* Triple specificity of ZnT8 autoantibodies in relation to HLA and other islet autoantibodies in childhood and adolescent type 1 diabetes. Pediatr Diabetes 2013;14:97-105.
62. Ingemansson S, Vaziri-Sani F, Lindblad U, Gudbjornsdottir S, Törn C, Diss-Study Group. Long-term sustained autoimmune response to beta cell specific zinc transporter (ZnT8, W, R, Q) in young adult patients with preserved beta cell function at diagnosis of diabetes. Autoimmunity 2013;46:50-61.
63. Gorus FK, Balti EV, Vermeulen I, Demeester S, Van Dalem A, Costa O, *et al.* Screening for insulinoma antigen 2 and zinc transporter 8 autoantibodies: A cost-effective and age-independent strategy to identify rapid progressors to clinical onset among relatives of type 1 diabetic patients. Clin Exp Immunol 2013;171:82-90.
64. Delli AJ, Vaziri-Sani F, Lindblad B, Elding-Larsson H, Carlsson A, Forsander G, *et al.* Zinc transporter 8 autoantibodies and their association with SLC30A8 and HLA-DQ genes differ between immigrant and Swedish patients with newly diagnosed type 1 diabetes in the Better Diabetes Diagnosis study. Diabetes 2012;61:2556-64.
65. Borg H, Gottsäter A, Landin-Olsson M, Fernlund P, Sundkvist G. High levels of antigen-specific islet antibodies predict future beta-cell failure in patients with onset of diabetes in adult age. J Clin Endocrinol Metab 2001;86:3032-8.
66. Liu L, Li X, Xiang Y, Huang G, Lin J, Yang L, *et al.* Latent autoimmune diabetes in adults with low-titer GAD antibodies: Similar disease progression with type 2 diabetes: A nationwide, multicenter prospective study (LADA China Study 3). Diabetes Care 2015;38:16-21.
67. Hameed S, Ellard S, Woodhead HJ, Neville KA, Walker JL, Craig ME, *et al.* Persistently autoantibody negative (PAN) type 1 diabetes mellitus in children. Pediatr Diabetes 2011;12:142-9.
68. Kawasaki E, Nakamura K, Kuriya G, Satoh T, Kuwahara H, Kobayashi M, *et al.* Autoantibodies to insulin, insulinoma-associated antigen-2, and zinc transporter 8 improve the prediction of early insulin requirement in adult-onset autoimmune diabetes. J Clin Endocrinol Metab 2010;95:707-13.
69. Andersen ML, Vaziri-Sani F, Delli A, Pörksen S, Jacobssen E, Thomsen J, *et al.* Association between autoantibodies to the Arginine variant of the Zinc transporter 8 (ZnT8) and stimulated C-peptide

levels in Danish children and adolescents with newly diagnosed type 1 diabetes. Pediatr Diabetes 2012;13:454-62.
70. Nielsen LB, Vaziri-Sani F, Pörksen S, Andersen ML, Svensson J, Bergholdt R, *et al.* Relationship between ZnT8Ab, the SLC30A8 gene and disease progression in children with newly diagnosed type 1 diabetes. Autoimmunity 2011;44:616-23.
71. Sorensen JS, Vaziri-Sani F, Maziarz M, Kristensen K, Ellerman A, Breslow N, *et al.* Islet autoantibodies and residual beta cell function in type 1 diabetes children followed for 3-6 years. Diabetes Res Clin Pract 2012;96:204-10.
72. Moosavi M, Séguin J, Li Q, Polychronakos C. The effect of type 2 diabetes risk loci on insulin requirements in type 1 diabetes. Horm Res Paediatr 2012;77:305-8.
73. Lernmark A, Larsson HE. Immune therapy in type 1 diabetes mellitus. Nat Rev Endocrinol 2013;9:92-103.
74. Yu L, Herold K, Krause-Steinrauf H, McGee PL, Bundy B, Pugliese A, *et al.* Rituximab selectively suppresses specific islet antibodies. Diabetes 2011;60:2560-5.
75. Bonaventura P, Benedetti G, Albarède F, Miossec P. Zinc and its role in immunity and inflammation. Autoimmun Rev 2015;14:277-85.
76. Masala S, Paccagnini D, Cossu D, Brezar V, Pacifico A, Ahmed N, *et al.* Antibodies recognizing *Mycobacterium avium* paratuberculosis epitopes cross-react with the beta-cell antigen ZnT8 in Sardinian type 1 diabetic patients. PLoS One 2011;6:e26931.
77. Pearson E. Zinc transport and diabetes risk. Nat Genet 2014;46:323-4.
78. Capdor J, Foster M, Petocz P, Samman S. Zinc and glycemic control: A meta-analysis of randomised placebo controlled supplementation trials in humans. J Trace Elem Med Biol 2013;27:137-42.