Novel Antigens in Type 1 Diabetes: The Importance of ZnT8

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The presence of circulating islet cell autoantibodies distinguishes type 1A diabetes (T1D) from other diabetic syndromes and determination of autoantigen genes and proteins is instrumental in understanding T1D as a clinical entity and in investigating the pathogenesis of the disease. ZnT8 was recently defined as a candidate autoantigen based on a bioinformatics analysis focused on discovery of β-cell–specific proteins associated with the regulatory pathway of secretion. The native molecule does not lend itself easily to solution-phase autoantibody assays, but ligands based on the predicted domain structure and molecular modeling have led to robust diagnostic procedures showing high specificities and sensitivities that complement current T1D autoantibody assays and add to the predictive value of their measurement. The incorporation of genetic and structural epitope analysis into ZnT8A determinations adds a further dimension to its diagnostic value and understanding of its role in the autoimmune disease process.

Introduction

The hallmarks of type 1 diabetes (T1D) are decreased peripheral glucose uptake and increased hepatic glucose production resulting from marked reduction of insulin secretory capacity and insulin deficiency consequent to autoimmune attack directed at the insulin-secreting pancreatic β cell. Although more than 80% of cases are classifiable as sporadic in nature, there is an underlying genetic susceptibility associated with 20 or more genes of which common polymorphic variants in the major histocompatibility loci account for more than 40% of the inheritability [1,2]. The precise etiology remains unclear but could be linked to common environmental factors, such as childhood enteroviral infections or dietary behaviors. These exert themselves in the context of individual determinants associated with inherent defects in critical immunomodulatory mechanisms [3] and increase the risk of a pathogenic rather than protective immune response to self [4]. Irrespective of whether the autoimmune response is initiated by an unfortunate mismatch of a nondeleted T-cell receptor and a cognate self-peptide, or by a foreign antigen, it is clear that islet cell proteins are critical to progression from autoimmunity to clinical disease, a process that can take more than a decade in most human subjects [5].

Although T1D is primarily considered to result from T-cell–mediated destruction of islet cells, the molecular targets of diabetic autoimmunity were largely identified by serologic studies of circulating autoantibodies. Insulin, glutamic acid decarboxylase 65 (GAD65), and the protein tyrosine phosphatase proteins ICA512 (IA-2) and phogrin (IA-2β) are well-established targets of circulating islet cell autoantibodies, and determination of their prevalence and titers provide sensitive (> 85%) and specific (> 99%) diagnostic tests for prediabetes in humans. The combined measurement of autoantibodies to insulin (IAA), GAD65 (GADA), and IA2 (IA2A), although highly predictive of T1D susceptibility, fall short of being able to determine individual risk in the general population, and the search for new molecular targets is an important goal. The induction of antigen-specific immune tolerance provides an attractive approach to prevention of the disease and one that can be used in combination with approaches based on monoclonal antibodies that modulate or temporarily deplete broad components of the immune system, such as T and B cells. The advent of methodology to interrogate differences in levels of gene expression across multiple tissues and to distinguish single nucleotide polymorphisms (SNPs) across the entire genome introduces new approaches for disease and tissue biomarker discovery, including the identification of tissue and disease-specific autoantigens. In

this article, we review the application of such approaches to the discovery of an independent autoantibody marker of T1D, namely the insulin secretory granule zinc transporter, ZnT-8 [6,7••], the product of the *SLC30A8* gene in humans.

Discovery of ZnT8

Insulin, the first T1D autoantigen identified at the molecular level, was discovered by a candidate gene approach [8]; *GAD65* (*GAD2*) by a combination of biochemical analysis and fortuitous clinical association of T1D with a rare disease affecting GABAergic neurons [9]; *IA2* (*PTPrN*) [10,11] from screening of islet gene expression libraries with T1D patient sera; and *IGRP* (*G6PC2*) from cloning of β-cell–specific genes using a subtractive hybridization approach [12,13]. A common thread linking these discoveries is the relatively specific expression of these proteins to pancreatic β cells at moderate or high levels and intracellular localization of the proteins to the regulated pathway of secretion, often in association with the insulin secretory granule. This prompted us to perform a multidimensional gene expression analysis initially using microarray data from public domain multi-tissue custom arrays (Novartis Gene Atlas V2) [14] to define pancreas and islet specificity. This was followed by analysis of individual gene expression in a murine βTC3 insulinoma line in comparison with the α TC1-6 glucagonoma and mPAC ductal cell lines. Additional data pertaining to islet specificity were derived from analysis of the embryonic *Neurogenin 3* knockout mouse pancreas [15•], which develops normally but is devoid of islets. A list of 68 genes derived in this way included insulin, *GAD65*, *IA2*, *IGRP*, and other documented targets of diabetic autoimmunity [7••]. Of the genes that were not previously implicated in diabetes, high on the list was ZnT8 [7••], a member of the large and evolutionarily conserved family of cation diffusion efflux proteins. ZnT8 appears largely confined to the islet, predominantly in the β cell [6], with some lesser expression in the α cell [15•,16].

Zinc Biology and Disease Associations

ZnT8 is the product of one of nine known *ZnT* genes (*SLC30A1-8* and *SLC30A10*) that are expressed in humans, of which six (ZnT 1, 3, 6, 7, 8, and 10) are detectable at the mRNA level in islets. There are an additional 14 Zn importer genes of the *SLC39A* family in mammalian cells that act to counter the export of Zn or its sequestration in intracellular organelles mediated by the *SLC30A* family members [17]. The genes are often tissue-specific, have specific intracellular localization, and are tightly regulated [18]. Inactivation of *Slc30A* and *Slc39A* family members in mice is associated with various pathologies ranging from embryonic lethality ($ZnT1^{-/-}$) [19] and ($Zip4^{-/-}$) [20] to tissue-specific phenotypes such as production of Zn-deficient milk (*ZnT4[lm]*) [21], acrodermatitis enteropathica ($Zip4^{+/-}$) [22], and impaired growth that can be compensated with dietary Zn supplementation ($Znt7^{-/-}$) [23]. Others such as $ZnT3^{-/-}$ [24] show remarkably weak phenotypes in spite of specific tissue and subcellular distribution. Mice with a triple knockout of *Zip1*, *-2*, and *-3* have no phenotype [25] except under conditions of dietary Zn deficiency [26].

Zinc is an important structural component of many proteins; 200 different Zn binding protein motifs that mediate in protein/protein interactions, protein/DNA interactions, and metal ion–mediated catalysis were described. Free Zn^{2+} reportedly blocks the K_{ATP} channel in the pancreatic α and β cells and were implicated in paracrine signaling from the β cell to the α cells and the priming of glucagon release stimulated by a decrease in extracellular glucose [27,28]. ZnT8 is postulated as important for providing Zn to allow for the proper maturation, storage, and secretion of insulin [29]. Diet-induced Zn deficiency in rats reduces the ability to secrete insulin after a glucose tolerance test [30] and Zn-deficient hamsters exhibit reduced glucose tolerance, albeit without a change in insulin production [31]. The extent to which any of these processes reflect physiologic events remains to be elucidated [32]. In the immunology area, Zn deficiency was implicated in cytokine signaling [33] and potentially as an intracellular messenger in its own right [34]; free Zn^{2+} can mimic several events mediated by epidermal growth factor, brain-derived neurotrophic growth factor, and other growth factor receptors. It is conceivable that at the high concentrations associated with insulin, Zn could affect antigen presentation through an aggregation mechanism, and one is reminded of the adjuvant effects of Al^{3+} [35] and the ability of Be^{2+} to form specific peptide adducts that, in the context of specific HLA alleles, can be presented to self-reactive T cells and cause pulmonary berylliosis [36,37].

Autoantibodies to ZnT8

Assays for ZnT8 antibodies (ZnT8A) are performed using ^{35}S Met-labeled ZnT8 produced by in vitro transcription and translation in solution phase followed by isolation of the immune complexes with protein A agarose beads [7••]. The procedures are similar to those that were successfully applied to insulin, GAD65, and IA2 and use similar amounts of sample (2.5–5 μL of serum) [38]. Bead-bound radioactivity is determined by liquid scintillation counting after washing by centrifugation filtration procedures. Up to 1200 samples can be assayed by a single technician over a 2-day period. Nonradioactive procedures are under development and considerable interest exists in reducing these technologies to a point-of-service assay. ZnT8 presents some additional challenges for autoantibody assays,

50% of the sequence of 369 amino acids that characterize the native molecule is embedded in the phospholipid bilayer that it spans six times [6]. The first 70 and the last 100 amino acids are predicted to form globular domains with a cytosolic orientation and both possess antigenic epitopes. The N-terminus is typically recognized by up to 20% of new-onset diabetic sera with 98% specificity, and the C-terminus around 70% sensitivity with a significantly higher specificity (99.5%) [7••]. Further optimization of the current assays and the introduction of multivalent antigen preparations promise to increase the sensitivity of the assay to the range of 80% without sacrifice of specificity. The sensitivity is comparable to IAA, GADA, and IA2A. Also important is the observation that ZnT8A provide an independent measure of autoreactivity with 25% to 30% of subjects negative for IAA, GADA, and IA2 being ZnT8A-positive. It is a more robust assay than IAA but does not supplant it because IAA reactivity is greater in younger subjects than ZnT8A. Conversely, ZnT8A are more prevalent in subjects older than 8 years of age [39]. ZnT8A show less of an association with common diabetes-susceptibility haplotypes than does GADA (notably DR3-DQ2) and IA2A (notably DR4-DQ8) [40,41], suggesting that it reports on a different subpopulation of individuals. ZnT8A correlates weakly with IA2A and not at all with GADA and IAA. Its major strength clinically is that when it is measured alongside the classical T1D autoantibodies, it increases the overall sensitivity of detection of autoimmunity to greater than 90% and increases predictive value, particularly for individuals with a single classical T1D autoantibody who otherwise have a risk of disease development only marginally greater than controls [7••].

ZnT8A detected at the time of disease onset show little or no crossreactivity with their nearest orthologues ZnT3 or ZnT4, even though the two are expressed in islets [15•]. More distantly related ZnT members are likewise not reactive. Autoantibodies reactive with ZnT8 probes were undetectable in type 2 diabetes, rheumatoid arthritis, lupus erythematosus, and multiple sclerosis but present in low frequency in patients with Addison's disease and celiac disease, two conditions associated with increased risk of T1D development [7••]. Similarly, patients with Stiff Man Syndrome who have an elevated risk of developing T1D and show a high prevalence and titers of GADA [42] had low titers of ZnT8A and IA2A in 2 of 12 cases (Solimena and Hutton; Unpublished data). Because ZnT8 is not expressed in the tissues primarily affected in these diseases (adrenal cortex, small intestine, and GABAergic neurons of the musculature), we would surmise that these individuals had an underlying pancreatic insulitis. This is consistent with the observation that ZnT8A are detected in a high proportion of patients followed prospectively to T1D and may precede clinical disease from 6 months to more than 15 years. The common feature in this instance is likely to share common genetic risk factors that predispose to autoimmunity.

ZnT8A Epitope Mapping and *SLC30A8* Genetics

Identification of the amino acid sequences and conformations recognized by autoantibodies is important in terms of assay optimization and providing critical information relevant to understanding the presentation of the antigen to B cells and potentially in the design of antigen-specific therapeutic agents. These studies are facilitated in the case of ZnT8 by the availability of the crystal structure of the bacterial iron transporter Ziip [43••] and the fact that the C-terminal region of the mouse ZnT8 molecule, which is more than 80% identical in sequence, does not react significantly with human autoantibodies. Incidentally, the nonobese diabetic mouse, a useful T1D experimental model does not exhibit antibodies to human or mouse ZnT8 (Wenzlau, Davidson, and Hutton; Unpublished data). A three-dimensional model of the C-terminus of ZnT8 was generated with the PHYRE server (Structural Bioinformatics Group, Imperial College London, UK; http://www.sbg.bio.ic.ac.uk/phyre) and used to direct site-directed mutagenesis studies to better define the residues responsible for binding of human autoantibodies. A remarkable finding from these investigations was the observation that a single amino acid substitution in mouse ZnT8 (Gln_{324} > Arg) is sufficient to restore reactivity for a subset of T1D ZnT8 autoreactive sera. The equivalent amino acid residue in humans is polymorphic, Arg in 75% of European Caucasians, 98% African Americans, and 50% Asians, or alternatively Trp (SNP rs13266634). An additional nonsynonymous SNP exists in the same aa position because of variation in the second nucleotide of the same codon (C > A encoding Arg_{325} > Gln; SNP rs16889462) and occurs in less than 1% of Europeans, in about 9% of African Americans, and in 1% to 2% of Asian populations.

Recombinant human ZnT8 probes with Arg, Trp, or Gln at aa_{325} each proved recognizable by sera from new-onset T1D and prediabetic individuals, although the prevalence of antibodies measured with these variants show a consistent quantitative and sometimes qualitative variation, typically 50% with Arg_{325}, 45% with Trp_{325}, and 40% with Gln_{325} (Table 1; Fig. 1). About 15% of individuals react only with Arg_{325} probes, 7.5% with Trp_{325}, and none with Gln_{325} alone within the assay sensitivity limits. Quantitative analysis of the response levels fits the hypothesis that a restricted number of ZnT8 antibody epitopes exists, one of which is dependent on the presence of Arg at aa_{325}, another on Trp, and a third class that is unaffected by aa_{325} and can bind probes with Arg, Trp, or Gln in this position equally well. It is conceivable that the naturally occurring Gln_{325} variant may be targeted in

Table 1. ZnT8 autoantibody responses in new-onset patients stratified by aa_{325} reactivity and specificity*

	Rs13266634 genotype	Arg_{325} reactive	Arg_{325} restricted	Arg_{325} only	Trp_{325} reactive	Trp_{325} restricted	Trp_{325} only	Gln_{325} reactive	Gln_{325} only	Any probe
Patients, *n* *(% total)*	425	208 (48.9)	125 (29.4)	70 (16.5)	157 (29.9)	69 (16.2)	23 (5.4)	120 (28.2)	2 (0.5)	240 (56.4)
CC	220 (51.8)	121 (58.2)	81 (64.8)	62 (88.6)	53 (33.8)	1 (1.4)	1 (4.3)	57 (47.5)	1 (50)	125 (52.0)
CT	177 (40.0)	79 (38.0)	43 (34.4)	8 (11.4)	80 (51.0)	47 (68.1)	7 (30.4)	53 (44.2)	1 (50)	91 (37.9)
TT	35 (8.2)	8 (3.8)	1 (0.8)	0 (0)	24 (15.3)	21 (30.4)	15 (65.2)	10 (8.3)	0 (0)	24 (10.0)
P chi-squared	—	0.064	0.002	< 0.0001	0.0004	< 0.0001	< 0.0001	0.796	NS	0.596

*Samples obtained within 2 weeks of diagnosis (age, 12.5 ± 8.5 years; range, 0.53–65 years) were assayed with ZnT8 C-terminal probes ($aa_{265–369}$) and stratified as outlined in the Figure 1 legend. The number and percentage of individuals in each category is shown in the first row and subdivided into genotypes in subsequent rows. Statistics are based on a 3 × 2 chi-squared analysis of genotypes (Prism 5 software). NS—not significant.

individuals bearing that allele in non-Caucasian populations, which warrants further investigation because it was also apparent that autoreactivity to the specific aa_{325} variant was dependent on its encoding by the genome. In these terms, immunologic autoreactivity to ZnT8 is truly reactivity to a self-encoded antigen, an observation that is contraindicative of the commonly held view that autoreactivity arises through a process of molecular mimicry initiated by epitopes in foreign proteins that are similar but not identical to self. These conclusions based on the prevalence of autoreactivity to the three aa_{325} probes against the genotype determined by analysis of SNP rs13266634 are further reinforced by quantitative analysis of the levels of antibodies observed in new-onset T1D patients, notably whether heterozygous individuals encoding both antigenic variants of ZnT8 are tolerized by the presence of the alternative allele or equally likely to develop autoreactivity to either allele.

Figure 1 and Table 1 show that reactivity to the rare Gln_{325} variant construct was unaffected by the SNP rs13266634, consistent with the premise that the epitope is not dependent on which residue is at aa_{325}. Responses only to the Arg_{325} construct were principally confined to individuals with the CC genotype and similarly Trp_{325}-only responses with the TT genotype and to a lesser extent, heterozygotes. Calculation of the Arg_{325} and Trp_{325}-restricted responses (defined by the numerical difference between Arg_{325} or Trp_{325} probe reactivity and Gln_{325} probe reactivity) were distributed somewhat differently in that they revealed that heterozygous individuals had significant levels of antibody responses directed at the Arg_{325} or Trp_{325} isoepitopes. The levels of Arg_{325}-restricted antibodies were significantly higher in homozygous CC individuals than in heterozygotes, suggestive of an effect of expression level of the antigen isoepitope on the strength of the response; however, this was not the case for Trp_{325}-restricted antibodies. In essence, about 30% of patients responded to non-aa_{325} epitopes, 30% had antibodies restricted to Arg_{325}, and 15% to 20% were restricted to the Trp_{325} isoepitope, the latter responses being determined by the frequency of the corresponding encoding allele. Collectively, about 60% responded to any epitope in this group.

The heterogeneity of the ZnT8A responses poses important questions regarding the design and performance of assays. The precision of the Arg_{325} or Trp_{325}-restricted autoantibody measurements are affected by the magnitude of the responses themselves, the response relative to other isoepitope reactivity of the sample, and the dynamic range and calibration of the assays. The Arg_{325} or Trp_{325}-restricted signals can be quenched by adding recombinant Arg_{325} or Trp_{325}-C-terminal proteins to the assays, which should provide the basis of future assays that involve measurement of a difference signal with a single probe rather than a comparison of different probes. Linear peptides are ineffective as competing agents, indicating that the epitopes are conformational in nature, and further investigation is required to map the other amino acids in the C-terminus that contribute to the reactivity and the avidity of antibody binding. For many studies, the issue of complexity of the epitopes will be unimportant and a single assay that can integrate the signals coming from the different isoepitopes will be of greater interest. Current approaches include incorporating multiple, individually synthesized probes in a single assay or the generation of hybrid molecules, such as the N-C terminal construct and the generation of multimeric constructs based on tandem repeats of the immunoreactive forms separated by a suitably flexible linker. Preliminary data with the latter indicate that the sensitivity of ZnT8A measurements can be increased from 50% to levels above 70% without sacrificing assay specificity (> 99.5%) by improving the avidity of binding of the autoantibodies. ZnT8 naturally exists as a dimer and further indications show new epitopes that bridge monomers may also be detectable by this strategy [43••].

From a clinical perspective, it will be interesting to look at the emergence of epitope-specific responses in populations of differing ethnic origin because it is clear that the allele frequencies encoding ZnT8 aa_{325} vary significantly within European populations [44] and between

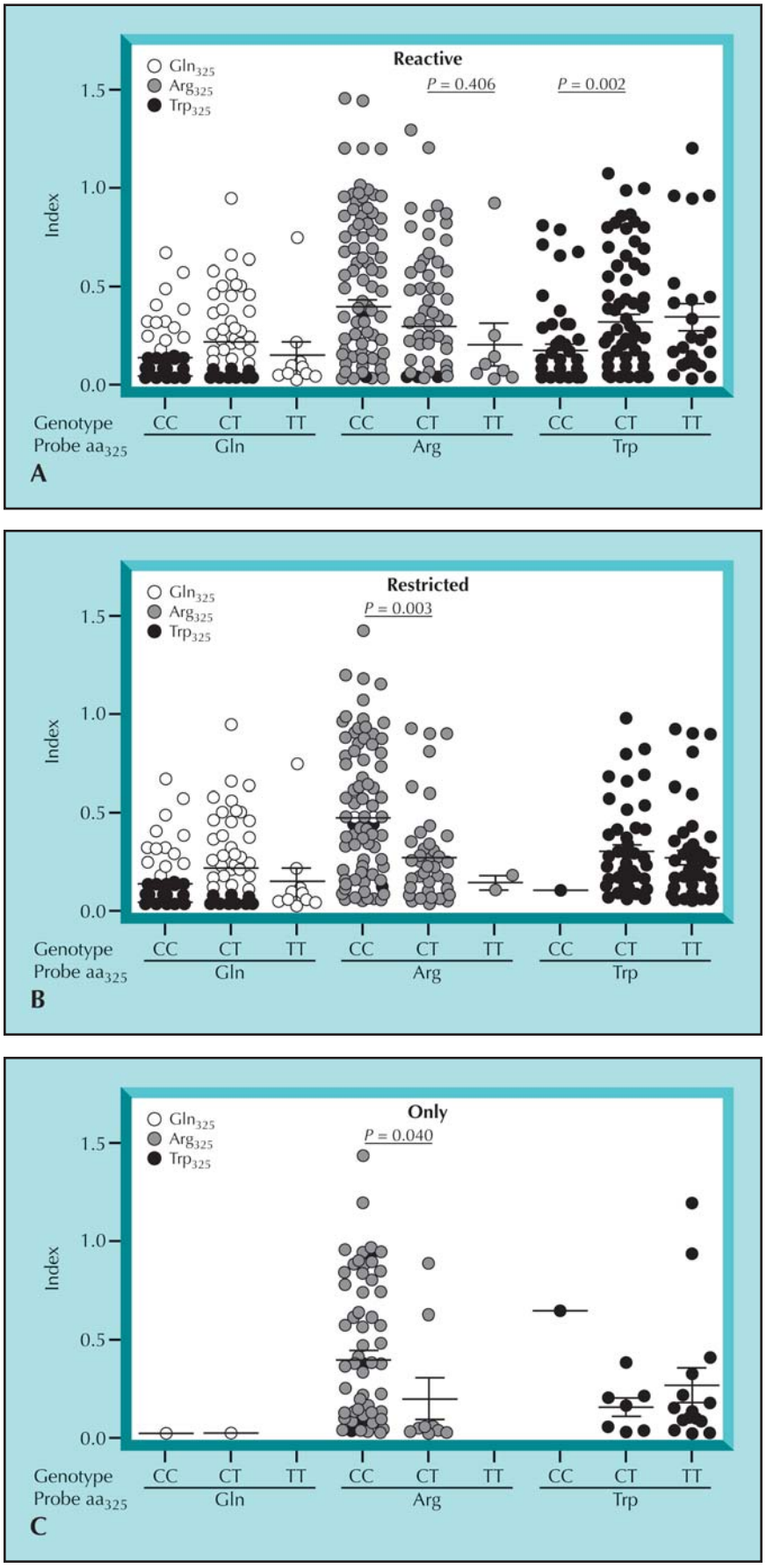

Figure 1. Levels of aa_{325}-restricted and non-restricted ZnT8 autoantibody in new-onset patients. Antibody responses were determined as outlined in Table 1 and expressed as the immunoprecipitation index (diabetic sample counts per minute [cpm] minus control cpm)/(total assay cpm minus control cpm) for each individual assayed with probes encoding Gln_{325}, Arg_{325}, or Trp_{325}. **A,** Data are stratified by genotype and aa_{325} probe reactivity index alone. **B,** Shows signal differences between Arg_{325} and Gln_{325} probe signals and Trp_{325} and Gln_{325} probe signals (Gln_{325} reactivity is displayed for reference). **C,** Data are shown where a response to a single probe was observed (Gln_{325}, Arg_{325}, or Trp_{325}). *P* values are derived from Mann-Whitney nonparametric tests and are shown where significant.

more distantly separated ethnic groups. Data from a small Japanese population clearly demonstrated the existence of Arg_{325} or Trp_{325}-restricted responses within new-onset T1D patients and those with disease of longer duration [45]. By contrast, no ZnT8A were found in individuals with "fulminant diabetes," which may have a different etiology. The major allele frequency for SNP rs13266634 (75% C) in the principally Europid-Caucasian population we studied is similar to that in control European populations, and no dramatic skewing toward one allele or another exists in terms of diabetes incidence or ZnT8A response [46••]. However, an indication exists that the homozygous CC allele may be overrepresented in individuals who develop clinical disease at an early age (< 4 years) than those who become diabetic more typically around the age of puberty [47•]. High ZnT8A levels usually do not appear before 5 years of age, and a trend toward Arg_{325}-restricted versus Trp_{325}-restricted responses exists, which is consistent with the reported skewing toward CC homozygosity of the rs13266634 SNP in this subgroup. It will be interesting to study the emergence of ZnT8 aa_{325}-restricted antibodies in prediabetes prospectively in homozygous and heterozygous populations because it may provide clues to the mechanism of epitope spreading and possible role of B-lymphocyte populations in the process.

ZnT8 and Type 2 Diabetes

A series of recent genome-wide association studies show a significant association of the same polymorphism at aa_{325} with human type 2 diabetes [48••,49] in which the major Arg_{325}-encoding C allele confers a minor risk (odds ratio, 1.08–1.12) of disease. In a large group of subjects with a family history of T2D but no apparent disease, the minor Trp_{325}-encoding T-allele was correlated with increased insulin resistance measured by hyperinsulinemic-euglycemic clamp but was attributable to greater abdominal adiposity. No differences in oral glucose tolerance were observed with any of the *SLC30A8* genotypes, although subjects homozygous for the C allele exhibited a decreased insulin response in intravenous glucose tolerance tests compared with heterozygote and TT homozygotes. An increased plasma proinsulin-to-insulin ratio during oral glucose tolerance tests of individuals at increased risk of type 2 diabetes was also associated with the risk-conferring Arg_{325}, C allele [44]. The latter observation fits with in vitro studies in rat insulinoma cells transfected with human ZnT8 cDNAs, which showed that proinsulin secretion was enhanced relative to processed insulin by the Arg_{325} variant, and that secretory responses to overexpression of Arg_{325} or Trp_{325} variants were increased [50]. One could conceive that alteration of the level of expression or intrinsic differences in the ZnT8 ion transporter activity could have profound effects on the biogenesis of secretory granules and composition of the secretory products or susceptibility of the β cell to an unfolded protein response and thus endoplasmic reticulum stress; all factors that may affect the antigenicity of the β-cell constituent proteins and thus susceptibility to autoimmune attack [51]. However, T1D susceptibility loci were not documented on chromosome 8 in the region where *SLC30A8* is located, neither in humans nor in the syntenic region in mice. Without being associated with incidence or initiation of autoimmunity, the polymorphism might still affect the disease severity by affecting the susceptibility of the islet to metabolic stress that appears with the loss of first-phase insulin secretion and emerging glucose intolerance that accompanies the T1D onset. In such a scenario, increased susceptibility to β-cell failure and β-cell death would aggravate the metabolic disturbance and further contribute to insulitis by additional recruitment of inflammatory mediators. This could be manifest in a more rapid progression of β-cell loss and residual β-cell secretory capacity after clinical onset.

Conclusions

The discovery of ZnT8 as a fourth major diabetes autoantigen in humans provided an additional metric for disease incidence and progression, a potential new target for evaluating cell-based autoimmunity, and a potential biomarker of islet β-cell mass and function. The relationship between kinetics of ZnT8A emergence and progression of autoimmunity differ from those of insulin, GAD65, and IA2A, and ZnT8A is not associated the same as with diabetes susceptibility genes within HLA loci; thus, measurement of ZnT8A provides an independent disease marker and adds predictive value to autoantibody measurements in prediabetes. The existence of a nonsynonymous SNP that acts as a determinant of the specificity of antibodies provides an intriguing link to the genetics of type 2 diabetes as well as highlighting the oligoclonality of the B-cell response, a phenomenon that deserves further scrutiny. ZnT8A measurements are rapidly being incorporated into selection criteria for current clinical studies and trials. The sensitivity of ZnT8A assays is likely further enhanced by epitope mapping and molecular engineering, and new assay formats need to be developed for clinical applications outside of a laboratory setting. Currently, little evidence exists of spontaneous autoimmunity directed against ZnT8 in animal models, such as the nonobese diabetic mouse, but potential exists for using "humanized" mice in further investigation of the cell-mediated responses alongside direct studies in human subjects.

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Disclosure

No potential conflicts of interest relevant to this article were reported.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Concannon P, Erlich HA, Julier C, et al.: **Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families.** *Diabetes* 2005, **54:**2995–3001.
2. Erlich H, Valdes AM, Noble J, et al.: **HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families.** *Diabetes* 2008, **57:**1084–1092.
3. Kukreja A, Cost G, Marker J, et al.: **Multiple immunoregulatory defects in type-1 diabetes.** *J Clin Invest* 2002, **109:**131–140.
4. Arif S, Tree TI, Astill TP, et al.: **Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health.** *J Clin Invest* 2004, **113:**451–463.
5. Barker JM, Barriga KJ, Yu L, et al.: **Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY).** *J Clin Endocrinol Metab* 2004, **89:**3896–3902.
6. Chimienti F, Devergnas S, Favier A, Seve M: **Identification and cloning of a beta-cell-specific zinc transporter, ZnT8, localized into insulin secretory granules.** *Diabetes* 2004, **53:**2330–2337.
7. •• Wenzlau JM, Juhl K, Yu L, 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:**17040–17045.

This paper identifies ZnT8 as an autoimmune target in T1D. It shows prevalence studies at different ages of onset and documents appearance in prediabetic individuals.

8. Palmer JP, Asplin CM, Clemons P, et al.: **Insulin antibodies in insulin-dependent diabetics before insulin treatment.** *Science* 1983, **222:**1337–1339.
9. Baekkeskov S, Aanstoot HJ, Christgau S, et al.: **Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase.** *Nature* 1990, **347:**151–156.
10. Rabin DU, Pleasic SM, Palmer-Crocker R, Shapiro JA: **Cloning and expression of IDDM-specific human autoantigens.** *Diabetes* 1992, **41:**183–186.
11. Bonifacio E, Lampasona V, Genovese S, et al.: **Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies.** *J Immunol* 1995, **155:**5419–5426.
12. Arden SD, Zahn T, Steegers S, et al.: **Molecular cloning of a pancreatic islet-specific glucose-6-phosphatase catalytic subunit-related protein.** *Diabetes* 1999, **48:**531–542.
13. Lieberman SM, Evans AM, Han B, et al.: **Identification of the beta cell antigen targeted by a prevalent population of pathogenic CD8+ T cells in autoimmune diabetes.** *Proc Natl Acad Sci U S A* 2003, **100:**8384–8388.
14. Su AI, Wiltshire T, Batalov S, et al.: **A gene atlas of the mouse and human protein-encoding transcriptomes.** *Proc Natl Acad Sci U S A* 2004, **101:**6062–6067.
15. • Juhl K, Sarkar SA, Wong R, et al.: **Mouse pancreatic endocrine cell transcriptome defined in the embryonic Ngn3-null mouse.** *Diabetes* 2008, **57:**2755–2761.

This paper shows downloadable microarray data used in defining pancreatic β-cell–specific proteins.

16. Gyulkhandanyan AV, Lu H, Lee SC, et al.: **Investigation of transport mechanisms and regulation of intracellular Zn2+ in pancreatic alpha-cells.** *J Biol Chem* 2008, **283:**10184–10197.
17. Cousins RJ, McMahon RJ: **Integrative aspects of zinc transporters.** *J Nutr* 2000, **130(5S Suppl):**1384S–1387S.
18. Aydemir TB, Blanchard RK, Cousins RJ: **Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations.** *Proc Natl Acad Sci U S A* 2006, **103:**1699–1704.
19. Andrews GK, Wang H, Dey SK, Palmiter RD: **Mouse zinc transporter 1 gene provides an essential function during early embryonic development.** *Genesis* 2004, **40:**74–81.
20. Dufner-Beattie J, Weaver BP, Geiser J, et al.: **The mouse acrodermatitis enteropathica gene Slc39a4 (Zip4) is essential for early development and heterozygosity causes hypersensitivity to zinc deficiency.** *Hum Mol Genet* 2007, **16:**1391–1399.
21. Huang L, Gitschier J: **A novel gene involved in zinc transport is deficient in the lethal milk mouse.** *Nat Genet* 1997, **17:**292–297.
22. Andrews GK: **Regulation and function of Zip4, the acrodermatitis enteropathica gene.** *Biochem Soc Trans* 2008, **36(Pt 6):**1242–1246.
23. Huang L, Yu YY, Kirschke CP, et al.: **Znt7 (Slc30a7)-deficient mice display reduced body zinc status and body fat accumulation.** *J Biol Chem* 2007, **282:**37053–37063.
24. Cole TB, Robbins CA, Wenzel HJ, et al.: **Seizures and neuronal damage in mice lacking vesicular zinc.** *Epilepsy Res* 2000, **39:**153–169.
25. Kambe T, Geiser J, Lahner B, et al.: **Slc39a1 to 3 (subfamily II) Zip genes in mice have unique cell-specific functions during adaptation to zinc deficiency.** *Am J Physiol Regul Integr Comp Physiol* 2008, **294:**R1474–R1481.
26. Dufner-Beattie J, Huang ZL, Geiser J, et al.: **Mouse ZIP1 and ZIP3 genes together are essential for adaptation to dietary zinc deficiency during pregnancy.** *Genesis* 2006, **44:**239–251.
27. Franklin I, Gromada J, Gjinovci A, et al.: **Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release.** *Diabetes* 2005, **54:**1808–1815.
28. Zhou H, Zhang T, Harmon JS, et al.: **Zinc, not insulin, regulates the rat alpha-cell response to hypoglycemia in vivo.** *Diabetes* 2007, **56:**1107–1112.
29. Chimienti F, Favier A, Seve M: **ZnT8, a pancreatic beta-cell-specific zinc transporter.** *Biometals* 2005, **18:**313–317.
30. Quarterman J, Florence E: **Observations on glucose tolerance and plasma levels of free fatty acids and insulin in the zinc-deficient rat.** *Br J Nutr* 1972, **28:**75–79.
31. Boquist L, Lernmark A: **Effects on the endocrine pancreas in Chinese hamsters fed zinc deficient diets.** *Acta Pathol Microbiol Scand* 1969, **76:**215–228.
32. Ravier MA, Rutter GA: **Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic alpha-cells.** *Diabetes* 2005, **54:**1789–1797.
33. Haase H, Ober-Blobaum JL, Engelhardt G, et al.: **Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes.** *J Immunol* 2008, **181:**6491–6502.
34. Yamasaki S, Sakata-Sogawa K, Hasegawa A, et al.: **Zinc is a novel intracellular second messenger.** *J Cell Biol* 2007, **177:**637–645.

35. Eisenbarth SC, Colegio OR, O'Connor W, et al.: **Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants.** *Nature* 2008, **453:**1122–1126.
36. Fontenot AP, Falta MT, Freed BM, et al.: **Identification of pathogenic T cells in patients with beryllium-induced lung disease.** *J Immunol* 1999, **163:**1019–1026.
37. Amicosante M, Fontenot AP: **T cell recognition in chronic beryllium disease.** *Clin Immunol* 2006, **121:**134–143.
38. Yu L, Cuthbertson DD, Maclaren N, et al.: **Expression of GAD65 and islet cell antibody (ICA512) autoantibodies among cytoplasmic ICA+ relatives is associated with eligibility for the Diabetes Prevention Trial-Type 1.** *Diabetes* 2001, **50:**1735–1740.
39. Bingley PJ, Bonifacio E, Mueller PW: **Diabetes Antibody Standardization Program: first assay proficiency evaluation.** *Diabetes* 2003, **52:**1128–1136.
40. Mayr A, Schlosser M, Grober N, et al.: **GAD autoantibody affinity and epitope specificity identify distinct immunization profiles in children at risk for type 1 diabetes.** *Diabetes* 2007, **56:**1527–1533.
41. Williams AJ, Aitken RJ, Chandler MA, et al.: **Autoantibodies to islet antigen-2 are associated with HLA-DRB1*07 and DRB1*09 haplotypes as well as DRB1*04 at onset of type 1 diabetes: the possible role of HLA-DQA in autoimmunity to IA-2.** *Diabetologia* 2008, **51:**1444–1448.
42. Solimena M, Folli F, Denis-Donini S, et al.: **Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus.** *N Engl J Med* 1988, **318:**1012–1020.
43.•• Lu M, Fu D: **Structure of the zinc transporter YiiP.** *Science* 2007, **317:**1746–1748.
This paper shows successful resolution of the structure of a cation efflux family member. The multispanning membrane protein from *Escherichia coli*, although not strikingly homologous, shares a common fold with the ZnT family members.
44. Boesgaard TW, Zilinskaite J, Vanttinen M, et al.: **The common SLC30A8 Arg325Trp variant is associated with reduced first-phase insulin release in 846 non-diabetic offspring of type 2 diabetes patients-the EUGENE2 study.** *Diabetologia* 2008, **51:**816–820.
45. Kawasaki E, Uga M, Nakamura K, et al.: **Association between anti-ZnT8 autoantibody specificities and SLC30A8 Arg325Trp variant in Japanese patients with type 1 diabetes.** *Diabetologia* 2008, **51:**2299–2302.
46.•• Wenzlau JM, Liu Y, Yu L, et al.: **A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes.** *Diabetes* 2008, **57:**2693–2697.
This paper demonstrates that T1D autoantibodies to ZnT8 vary according to ZnT8 genotype related to the common polymorphism encoding aa_{325}. This is the same SNP implicated in type 2 diabetes susceptibility.
47.• Gohlke H, Ferrari U, Koczwara K, et al.: **SLC30A8 (ZnT8) polymorphism is associated with young age at type 1 diabetes onset.** *Rev Diabet Stud* 2008, **5:**25–27.
This paper documents the differences in SLC30A8 genotype associated with early onset of T1D.
48.•• Sladek R, Rocheleau G, Rung J, et al.: **A genome-wide association study identifies novel risk loci for type 2 diabetes.** *Nature* 2007, **445:**881–885.
This paper discusses the first gene association study reporting association of *SLC30A8* with type 2 diabetes.
49. Zeggini E, Weedon MN, Lindgren CM, et al.: **Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes.** *Science* 2007, **316:**1336–1341.
50. Chimienti F, Wheeler MB, Nicholson TJ, et al.: **Single nucleotide polymorphism rs13266634 modifies the Zn transporter activity of SLC30A8/ZnT8 in clonal pancreatic beta cells.** *Diabetologia* 2008, **Suppl 1:**S47.
51. Scheuner D, Kaufman RJ: **The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes.** *Endocr Rev* 2008, **29:**317–333.