

**EMERGING ROLE OF ZINC TRANSPORTER-8
AUTOANTIBODIES (ZnT8A) IN TYPE 1 DIABETES
MELLITUS- A REVIEW**

ABSTRACT:

Zinc, an important micronutrient for the storage, structural stabilization, secretion and action of insulin, is present in highest concentration in pancreas. The transport of zinc occurs through the zinc transporter-8 (ZnT8) to the insulin secretory vesicles. Zinc Transporter-8 Autoantibodies (ZnT8A) has been found to be associated with Type 2 Diabetes Mellitus. Recently it is recognized as a new autoantigen in Type 1 Diabetes Mellitus (T1DM) and its autoantibodies have been found in 50-60% of individuals with T1DM. Moreover, ZnT8A exhibit humoral auto reactivity which is not displayed by any of the other islet autoantigen like glutamine decarboxylase(GAD), insulin or tyrosine phosphate-related molecules(IA-2). Immunity against ZnT8 is dependent on clinical characteristics, which may provide evidence for early recognition highlighting the importance of this transporter in the pathogenesis of T1DM.

Information regarding this article was retrieved through PubMed, Google Scholar and other search engines available in the University by using the keywords zinc, ZnT, ZnT8, SLC30A8 (Solute carrier 30 member 8) and Type 1 Diabetes Mellitus. Information was gathered through original researches, reviews and epidemiological studies published up to August 2019. The aim of this review is to summarize the emerging role of ZnT8A in diagnosis and understanding the genetic basis of Type 1 Diabetes Mellitus.

KEYWORDS : *Diabetes Mellitus, Type 1; insulin; Zinc .*

INTRODUCTION:

The progress of Type 1 Diabetes Mellitus (T1DM) and the role of zinc transporter-8 autoantibodies (ZnT8A) has been under consideration for the homeostasis of zinc for decades. Researchers observed that zinc supplementation has shown favorable effects in the prevention of T1DM [1]. It was actually not deliberated as the classical organ-specific disease as it is now recognized to be [2]. Type 1 Diabetes Mellitus (T1DM) is a multifactorial autoimmune disease that targets destruction of insulin secreting pancreatic β cells [3] [4]. It is a chronic disease mostly diagnosed in children and adolescents but can appear in adults at any age [5] [6].

Genetic makeup and environmental causes are known to contribute in the different clinical characteristics and incidence rates of T1DM among populations supported by the fact that T1DM clusters in families. A

40 number of environmental agents including dietary factors (consumption of cow's milk and formula milk,
41 exposure of gluten, vitamin D deficiency) and viral agents (causing lymphopenia) may play a potential
42 role in the autoimmunity of β -cell [2] [7]. Coxsackievirus B, rubella, enterovirus, cytomegalovirus,
43 rhinovirus, mumps and adenovirus have been implicated in inducing certain cases of the disease [8] [9].
44 The specific etiology of T1DM remains unclear but is considered as cell-mediated disease that occurs
45 from immune dysfunction with consequent loss of tolerance to β cell antigens and destructive lymphocytic
46 infiltration of the islets. As a result of which insulin deficiency occurs leading to the impaired glucose
47 homeostasis and ultimately hyperglycemia with symptoms [3].

48 The population of diabetics is anticipated to increase from 425 million to 629 million from 2017 to 2045
49 [10]. A Finnish study showed that 22% of the children diagnosed with T1DM have a positive family history
50 [11]. A monozygotic twin of an individual with T1D is more prone to develop diabetes than a dizygotic
51 twin; additionally, there is no difference in the appearance of autoimmunity against β cell between siblings
52 and dizygotic twins [12]. The risk of T1DM development increases with multiple first-degree relatives [13].

53 The hallmark of T1DM is the antigen-specific T cells but identification of the occurrence of pathogenic T
54 cells in vivo is challenging. Multiple autoantigens for islet are commonly employed as the standard
55 diagnostic and predictive marker in T1DM development [1]. To clarify the etiology in idiopathic T1DM, a
56 number of researches are being done on new pancreatic antigens, such as zinc transporter 8 (ZnT8), islet
57 amyloid polypeptide, chromogranin A and pancreatic duodenal homeobox factor-1. These antigens may
58 help in the development of new treatment options [14] [15]. The earlier established autoantibodies against
59 insulin, glutamic acid decarboxylase (GAD) and tyrosine phosphatase-like islet antigen 2 (IA2) are
60 standard for the diagnosis of T1DM, zinc transporter 8 autoantibody (ZnT8A) is lately identified as another
61 major biomarker for T1D diagnosis through bioinformatics, expanding the panel of T1D diagnostic
62 autoantibodies [16]. In this review paper, we aim to summarize the emerging role of ZnT8A in T1DM.

63

64 **Review Method:**

65 This review work was done by extensive study of the literature on the emerging role of ZnT8A in T1DM.
66 Information regarding this article was retrieved through PubMed, Google Scholar and other search
67 engines available in the University by using the keywords zinc, ZnT, ZnT8, SLC30A8 and Type 1
68 Diabetes Mellitus. Information was gathered through original researches, reviews and epidemiological
69 studies published up to August 2019.

70

71 **RESULT AND DISCUSSION**

72 Zinc is an essential micronutrient for all the biological processes. Its homeostasis is regulated by the
73 transporters and zinc transporter-8 (ZnT8) plays a vital role in the secretion of insulin. ZnT8 is currently
74 known as an autoantigen in T1DM development and ZnT8A has already been associated with Type 2
75 Diabetes Mellitus. Therefore, we recapitulated the role of zinc, its transporter and ZnT8A in the
76 development of Type 1 Diabetes Mellitus.

77

78 **Zinc transporters:**

79 Zinc transporters in mammals come from two major families, ZIP (SLC39) family and ZnT (SLC30) family.
80 The family members of ZIP promote the influx of zinc from the intracellular compartments and from the
81 outside of the cells into the cytosol while the members of the family of ZnT allow the transport of zinc from
82 the cytosol to the outside the cell or into the lumen of intracellular organelles [17]. To regulate the cellular
83 zinc homeostasis, both ZnT and ZIP groups of transporters work in coordinated manner but in an
84 opposite direction [18].

85 **Zip:**

86 There are 14 **Zrt-/Irt-like protein (ZIP)** transporters encoded by the human genome and have now been
87 identified at all phylogenetic levels. The transporters are designated as ZIP1 to ZIP14 and are encoded by
88 the genes SLC39A1 to SLC39A14 respectively [19]. ZIP transporters are expected to have 8
89 transmembrane domains (TMD) and comparable expected topologies. This topology has been
90 established for yeast.

91 The presence of histidine-rich region in most of the members of this family is between the TMD III and IV
92 as a long loop region that is proposed to be zinc-binding domain. Most members of the ZIP family share
93 an analogous proposed topology with both C and N terminals extra-cytoplasmic [17]. The principal
94 function of Zip transporter is that it helps in the influx of zinc from the lumen of intracellular compartments
95 and from the extracellular space into the cytosol [20] **that is represented in Fig 1.**

96 **ZnT:**

97 In mammals, the **Solute carrier family 30 (SLC30)** family encodes for 10 members as Zinc transporters
98 (ZnTs). The transporters are designated as ZnT1 to ZnT10 and are encoded by the genes SLC30A1 to
99 SLC30A10 respectively. This family belongs to the zinc transporters that facilitate the transport of zinc
100 from the cytosol to the outside of the cell or into the lumen of intracellular organelles. Most transporters of
101 this family are expected to have six transmembrane domains (TMD), except for ZnT-5 which contains an
102 additional TMD and are suggested to have cytoplasmic N and C termini. Like the ZIP family, it also
103 contains a long histidine-rich loop between TMD IV AND V that acts as a zinc-binding domain [17] [21]
104 [22].

105 There is a large C- terminal Domain (CTD) on ZnTs with a copper chaperone-like architecture, in spite of
106 nonexistent sequence homology. The significance of this protein has been related to diabetes as the
107 researchers have found an association of ZnT8 variants with the risk of diabetes. Particularly, the W325R
108 variants affects the thermostability increasing diabetes risk. Likewise, ZnT8 is recently recognized as a
109 major autoantigen in T1DM and has been suggested that the conformational change of the CTD alters
110 the recognition of antigen linked [23].

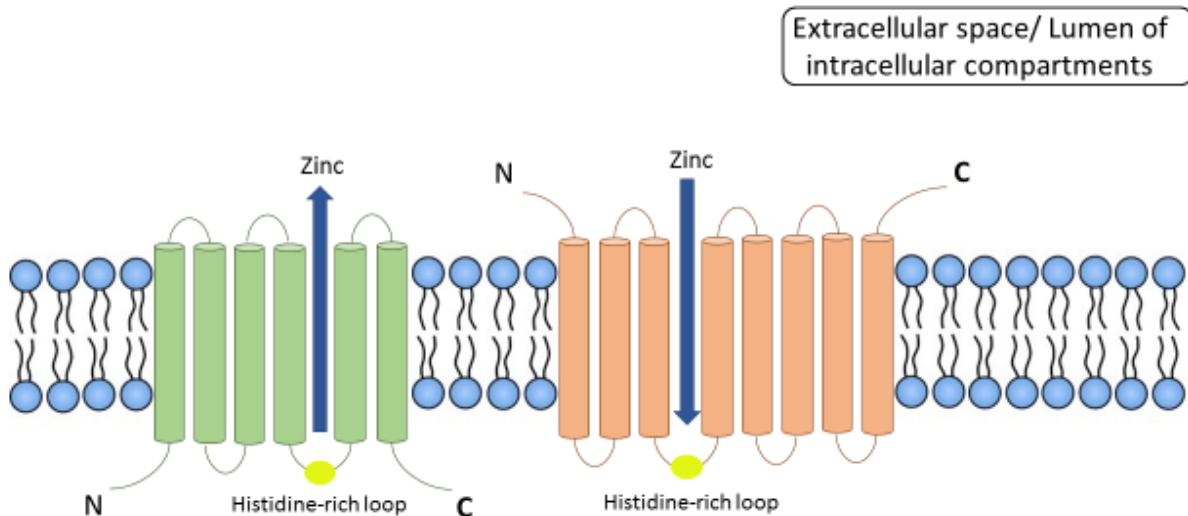


Figure 1: Homeostasis of zinc is maintained by two groups of transporters ZnT (SLC30A gene family) with six transmembrane domains and ZIP (SLC39A gene family) zinc transporters with eight transmembrane domains.

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114 **Insulin Processing and Secretion by ZnT8:**

115 Pancreas has the highest concentration of zinc ions and the concentration of free zinc ions is highest in
116 the secretory vesicles. They are essential for the structural stabilization, secretion, storage and insulin
117 action [1] [24]. Insulin hormone plays a vital role in the homeostasis of glucose as it is the only hormone
118 that lowers the concentration of blood glucose. Because of this reason insulin deficiency causes severe
119 metabolic disorders like T1DM and uncompensated Type 2 Diabetes Mellitus (T2DM). Electrical activity
120 plays a critical role in the secretion of insulin. The β cells that secrete insulin are present in the pancreatic
121 islet of Langerhans. Insulin secretion occurs as a result of elevated intracellular Ca^{2+} by the extracellular
122 influx of Ca^{2+} via Ca-channels (voltage dependent) [25].

123 Insulin is a polypeptide that is secreted from the pancreatic beta cells [3]. The synthesis of preproinsulin is
124 initiated in the cytosol. The newly synthesized preproinsulin is then guided to the ER for translocation by
125 its signal peptide [26]. Pre proinsulin is converted to proinsulin by the cleavage of signal sequence.

126 The assembly of two zinc ions and calcium ions occurs in the proinsulin forming a hexameric proinsulin in
127 the Golgi apparatus [1]. In B-chain (His B10) at 10th amino acid, histidine coordinates with the two central
128 zinc ions [3]. Prohormone convertase (PC1/3, PC2) and exoprotease carboxypeptidase E are the
129 proteolytic enzymes that cause the excision of the C-peptide forming insulin hexamer [1].

130 Zinc is also required for its transport into the insulin secretory granules and cytoplasm by zinc
131 transporters. In the pancreatic β -cells of rat model of insulinoma, zinc was recognized as a K_{ATP} channel
132 activator. It activates hyperpolarization of membrane potential and decreases the voltage-dependent Ca^{2+}
133 current. Once there is elevation of glucose in the cells, elevation of intracellular ATP occurs. ATP
134 elevation inhibits the activity of K_{ATP} channel, activating the electrical activity by increase in intracellular
135 Ca^{2+} leading to the release of insulin [25]. In the β -cells, there is co-secretion of zinc with it into the
136 extracellular space of islet during the exocytosis of insulin. At high pH of blood, zinc is then released from
137 the insulin and these ions provide negative feedback to the α -cells for glucagon release during glucose
138 deprivation by closing the α -cell K_{ATP} channel [1]. During the exocytosis of insulin, frequent exposure of
139 ZnT8 antigen occurs on glucose stimulated insulin secretion. Hence, it is supposed that ZnT8 exposure
140 can exacerbate or trigger the production of autoantibodies against ZnT8 antigens in genetically
141 susceptible individuals [27].

142

143 **SLC30A8 Gene Polymorphism:**

144 After thousands of genetic studies for more than forty years, HLA region on the human genome remains
145 the risk determinant for Type 1 Diabetes [28]. It shows association of disease susceptibility with multiple
146 genetic loci encoding Major histocompatibility complex class II glycoproteins [18]. According to a recent
147 genetic study, more than 60 risk loci showed association with the development of T1DM [29]. Some other
148 genetic studies documented that 2 types of genes are involved in Type 1 DM: HLA genes and non-HLA
149 genes. The HLA gene includes HLADR3, DQB1(also denoted as DR3-DQ2), DR4, DQB1(also denoted
150 as DR4-DQ8) and the non-HLA genes include Insulin gene(INS), Protein tyrosine phosphatase, non-
151 receptor type 22 (PTPN22), Cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and Solute carrier
152 family 30 A8 (SLC30A8) [2].

153 Two unique SLC30 genes were identified, SLC30A8 and SLC30A10 that extend the SLC30 family to 10
154 members from genomic databank [30]. The SLC30A8 gene is located on 8th Chromosome on q arm at
155 position 24.11 and it encodes for ZnT8 auto-antigen that is made of 369 amino acids [31]. ZnT8 is a six
156 transmembrane protein that facilitates the efflux of Zn^{+2} from the cell and stores it intracellularly [18].

157 Autoantibodies against ZnT8 are produced and detected before the disease onset. Single Nucleotide
158 Polymorphism (SNP) rs13266634 C/T is known to be responsible for the altered immune response to
159 ZnT8. In the presence of β cell dysfunction and impaired autoimmunity, this SNP displays a susceptible
160 role in the development of T1DM [27]. SLC30A8 genotypes were strongly associated with ZnT8

161 autoantibodies in T1DM. In a cohort on the offspring of Diabetic parent, children who were ZnT8 positive
162 in their follow-up had at least 1C allele out of which 82% were homozygous (CC genotype) [32].

163 An Asian study showed that stratification of T1DM risk can be done by SLC30A8 in ZnT8A-positive
164 children [33]. Another one reported a significant association of R325W C allele with an increased risk in
165 the development of GDM and the postpartum T1DM [34]. Therefore, it is proposed that individuals
166 carrying C- allele are at increased risk for developing Type 1 Diabetes Mellitus.

167

168 **Autoimmunity Against ZnT8:**

169 The role of essential trace element zinc in autoimmunity for regulation of biological processes has been
170 known for many years along with enzyme regulation at cellular level. Zinc homeostasis is critical for the
171 influence of innate and adaptive immunity by modifying the host defense and immune response [35] [36].
172 Researchers observed that zinc has a protective function for β -cells of pancreas from cytokine-induced
173 destruction in Type 1 Diabetes Mellitus and the deficiency induces lymphopenia, thymic atrophy, natural
174 killer cell activity, suppression of cytolytic T-cells and delayed hypersensitivity reactions [1].

175 Literature suggested that the initiation of the autoreactive cascade is by the immunologic response to
176 antigen. There is a binding of antigen to the antigen presenting cells (APCs) by a groove in major
177 histocompatibility complex(MHC) class II molecules. In T1DM, APCs are then presented to antigen
178 receptors on helper T cells or autoreactive CD4 inducers which in turn stimulates immune facilitated injury
179 to the β cell of pancreas. Furthermore, B7 protein on APC binds to CD28 and Lymphocyte functional
180 antigen-3 (LFA-3) enhancing the T-cell activation by costimulatory pathway. Some other molecules also
181 contribute in the immune response such as interleukin-2 binding to its receptor (IL-2R). The balance of
182 both the regulatory (Treg) and effector (Teff) T lymphocytes are crucial for conserving body cells [37] [2].

183 There is an association of ZnT8 with β -cell survival [38]. Zinc transporter 8 down regulation usually
184 promotes cell survival of beta-cells [39]. Zinc content is increased in β -cells by the overexpression of
185 ZnT8 that protects them from zinc depletion induced cell death [38]. However, some researchers
186 suggested that the overexpression of ZnT8 makes the cells susceptible to IL-1 β induced apoptosis and
187 ZnT8 expression is regulated by IL-1 β [40]. It is proposed that ZnT8 might play an active role in
188 regulating the cell survivals by expressing at a subcellular level that is supported by the fact that at
189 position 325(aa325) the amino acid substitution encodes for a polymorphism [1].

190

191 **ZnT8 in Three Sub-Types of Type 1 Diabetes:**

192 It is eminent that there are at least three subtypes of type 1 diabetes in Japan, acute-onset, slow onset,
193 and fulminant type 1 diabetes [1]. Type 1 Diabetes Mellitus (T1DM) is usually developed in childhood but
194 a greater proportion is diagnosed in their adulthood exhibiting different manifestations [41]. About 90% of
195 the patients demonstrated acute-onset form in both childhood and adolescent-onset type 1 diabetes while
196 the rest of the patients showed slow-onset form. In childhood, fulminant T1DM is scarce [1]. On the other
197 hand, 2/3rd of the patients showed slow-onset form and nearly 20% presented with ketoacidosis or ketosis
198 were classified as fulminant type 1 diabetes. It was suggested in a study that ZnT8 is identified as a major
199 autoantigen in T1DM [42].

200 In Caucasian patients of T1 Diabetes Mellitus, it was previously reported that 60-80% of them were
201 positive for the ZnT8 autoantibodies (ZnT8A) [43]. In Japanese population, it was also observed that 58%
202 and 20% patients were positive for ZnT8A with acute-onset and slow-onset T1DM respectively [1].

203 ZnT8A was positive in 58% T1DM patients with acute-onset and in 20% with slow- onset T1DM [41]. In
204 comparison, the sera obtained from fulminant T1DM were non-reactive to ZnT8 construct demonstrating

205 that ZnT8 cannot be used as a diagnostic marker in fulminant type 1 diabetes [1]. ZnT8A can be used in
 206 the diagnosis of acute and slow onset diabetes but not in fulminant T1DM.

207

208 **ZnT8 as a Diagnostic Tool:**

209 Clinical disease (T1DM) is typically preceded by humoral auto-reactivity and takes months or years, and
 210 the progression to clinical manifestations is marked by intramolecular and intermolecular epitope
 211 spreading [7]. It has been suggested to utilize measurements of combination markers for the diagnosis of
 212 Type 1 (autoimmune) diabetes. Monitoring of GAD65, IA-A2, insulin autoantibodies in combination with
 213 ZnT8 autoantibodies in diabetic subjects allowed confirmation of autoimmunity in more than 96% of the
 214 cases [3]. ZnT8 share some common features with insulin, GAD, IA-2 which are as follows:

- 215 1. Constituents of the secretory pathway
- 216 2. Membrane proteins
- 217 3. Tissue specific gene expression
- 218 4. Evidence of alternative splicing

219 Almost 10% of the new T1DM patients are negative for any GADA, ICA, IA-A2 or IAA and addition of
 220 ZnT8 autoantibody variants can improve the diagnostic sensitivity in adults [4]. In another study, several
 221 patients with presumed diagnosis of T1D lack GAD65A and IA2A autoantibodies, establishing the
 222 autoimmune basis sometimes becomes difficult [7]. In a Turkish study, all the well-defined pancreatic
 223 antigens (ICA, GAD, Insulin and IA-2) are positive for 80% of new onset T1D patients and almost 13% of
 224 cases were ZnT8 positive all of which were negative for GADA, IA-2A and IA [37]. The three variants of
 225 the autoantibodies are ZnT8RA, ZnT8WA or ZnT8QA and a study result showed that the three ZnT8WQA
 226 improved the 2% of diagnostic sensitivity in patients of T1DM. by improving it from 93-95% [4]. Some
 227 studies stated that ZnT8 plays a decisive role in the diagnosis of T1DM and it is the only autoantibody
 228 present in some patients.

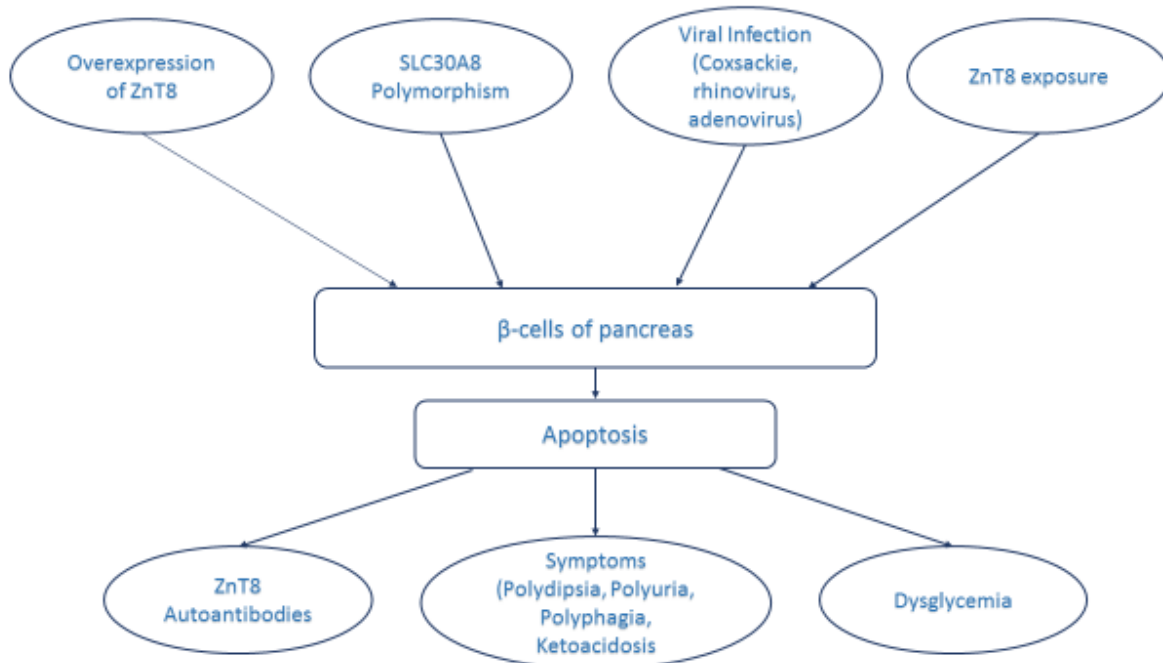
229 ZnT8 is prevalent in Type 1 Diabetics in different populations and is more frequently seen in children than
 230 in adults (Table 1). Therefore, there is a need to improve the diagnostic criteria and add ZnT8 as a
 231 standard autoantibody for the diagnosis of T1DM.

232 **Table 1: Studies showing prevalence of 3 Autoantibodies (GADA, IA-2A and ZnT8A) and ZnT8A**
 233 **alone in the patients of Type 1 Diabetes Mellitus (T1DM).**

Studies	Country	Patients showing positivity of 3 Autoantibodies			Only ZnT8A
		GADA	IA-2A	ZnT8A	
Yang et al. [44]	China 539	53.4%	25.8%	24.1%	5.4%
Gomes et al. [7]	Latin America 629	68.3%	64.8%	68.7%	24.1%
Niechcial et al. [45]	Poland Children=218 Adults=149	69.7% 75.4%	80.7% 44.0%	81.1% 34.8%	2.9% 5.0%
Boudiaf et al. [46]	Algeria 160	53.125%	32.5%	46.25%	8.75%
Rogowicz et al. [5]	Poland 35 years< =66 35 years> =53	35 years< 81.8% 35years> 77.3%	35 years< 51.5% 35 years> 34%	35 years< 45.4% 35 years> 34%	—
Garnier et al. [47]	France Children=119 Adults=109	68% 60%	41% 25%	61% 34%	7% 9%

234

235 Antibodies formed against ZnT8 (ZnT8A) are regarded as an independent autoimmunity demonstrator in
236 T1DM diagnosis [37]. “Prediabetes” individuals who are ZnT8 autoantibody positive and positive for any
237 one autoantibody of T1D are at increased risk for development of the T1D than those who are positive for
238 any two autoantibodies excluding ZnT8. Therefore, monitoring all these systemic autoantibodies is the
239 valid biomarker for identification of prodromal phase of T1DM as their appearance precedes years or
240 even decades before onset [3]. We have proposed the mechanism of development Type 1 Diabetes
241 Mellitus shown in Fig 2.



242

Figure 2: Proposed mechanism of development of Type 1 Diabetes Mellitus

243

244 **PATHWAYS IDENTIFYING OTHER GENES:**

245 The variations in innate immunity pathways are detectable in genetically susceptible individuals [48].
246 Previously, PPI (Protein Protein Interaction) was used for the network analysis for finding candidate
247 genes in diseases [49]. Weighted Gene Co-Expression Analysis(WGCNA) is used in a study to find the
248 association with the disease and was found that IL-1 was significantly increased in newly diagnosed
249 T1DM patients. Some other genes that showed some relevance by this approach are IL-1 β , FAS, CXCL8
250 etc [50]. There was a hypothesis that Toll-like receptors are involved in the initial phase of diabetes and
251 found that TLR-induced IL-1 β and IL-6 were frequently involved in children.

252 Enriched pathways among different diseases were observed and links between T1D and IL-2- mediated
253 signaling genes has been found. Prioritization of IL-2-mediated signaling genes showed 7 non-MHC
254 candidate disease loci with strong evidences. Four of them are validated by other studies and 3 (*RAF1*,
255 *MAPK14* and *FYN* are assumed to be novel loci in T1D for future studies [51]. Understanding of Type 1
256 diabetes was done by another study based on conventional GWAS, gene expression, PPI and STRING
257 data. They concluded that 8 (*IFNGR1*, *CD83*, *IL17RD*, *IL27RA*, *TRAF3IP2*, *PLCG2*, *CXCR7* and *MYO1B*)

258 regulated genes in the network sheltered SNPs associated with T1DM [52]. New insight to the pathways
259 and genes behind T1DM pathogenesis may offer the plan to develop innovative treatment strategies [53].

260 **CONCLUSION:**

261 ZnT8 is recently identified as a major target of autoantibodies in Type 1 Diabetes Mellitus patients. ZnT8A
262 is the only antibody in some of the individuals with negative autoantibody profile for T1DM and recurrent
263 exposure of ZnT8 can trigger the formation of ZnT8A in genetically susceptible individuals supports that
264 ZnT8 measurement should be more widespread for a better and earlier diagnosis. The individuals
265 carrying C- allele are at increased risk for developing Type 1 Diabetes Mellitus. Further studies are
266 required on antigenic determinants of ZnT8 to make the autoantibody measurement an expedient tool for
267 early diagnosis of T1DM. Better understanding of the new pathways and genes involved in the
268 pathogenesis of T1DM may help to advance inventive strategies.

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