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GENETICS AND STRUCTURE OF AUTOANTIGENS INVOLVED IN TYPE 1 DIABETES: A DETAILED REVIEW

KAMBLE PA, SHIMPI JS AND DESAI S*

Department of Pharmacology, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, Maharashtra, India

***Corresponding Author: Dr. Shivani Desai: E Mail: desai.shivani28@gmail.com**

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ABSTRACT

Type 1 diabetes mellitus is a chronic disorder also known as autoimmune diabetes and is characterized by the identification of one or more β cell proteins as an autoantigen (also known as self-antigen) by the immune system of the patient with the same disorder, i.e., CD4+ and CD8+ T cells and/or autoantibodies (self-reactive B cell products). Depending on β-cell-specificity, these autoantigens (AAs) are categorized into two groups. The list of target autoantigen in this autoimmune disorder is continuously rising and remains to be developed. Many autoantigens which are related to this disorder are well studied and play important role in the prognosis, diagnosis, and immunotherapy of Type 1 diabetes mellitus. Its identification helps in understanding pathogenesis and the clinical aspects of the same disorder. Since numerous splendid reviews have completed previously confirmed autoantigens which are associated with Type 1 diabetes mellitus, here we will discuss some newly identified Type 1 diabetes mellitus associated autoantigens that are islet amyloid polypeptide (IAPP), chromogranin A (CgA), pancreatic duodenal homeobox factor 1 (PDX1), and zinc transporter 8 (ZnT8). This review will discuss their identity, structure, genetics, biology, and its relation to diabetes.

Keywords: Type 1 diabetes, Autoantigens, Structure, B cells, Immunogenicity

INTRODUCTION

Diabetes mellitus is a group of metabolic autoimmune disorders **[1]** in which hyperglycemic condition occurs i.e., blood sugar level increases due to defects in insulin secretion and insulin action or both **[2]**. It is a non-communicable heterogeneous disorder and is diagnosed with a large population worldwide. Its prevalence has increased 4 times since 1980. According to WHO, in 2014 the number was 422 million and estimated to be up to 590 million by 2035 **[3]**. There are many types of diabetes mellitus and they have similar signs and symptoms (polyuria, polyphagia, polydipsia, ketoacidosis, and hyperosmolar coma) as well as complications (cardiovascular diseases, neuropathy, retinopathy, nephropathy, foot damage, and skin conditions) but different etiology **[4]**.

Type 1 diabetes mellitus (T1DM)

Type 1 diabetes mellitus or juvenile-onset diabetes is the gene responsible (HLA-DQA1*0301, HLA-DQA1*0302, HLA-DQB-1*0602, and HLA-DQW1.2) progressive autoimmune disorder which mostly affects the children that are below 20 years but now diagnosed at any stage of age with a high mortality rate **[4,5]** and it is caused by the destruction of β cells of islets of Langerhans of pancreas and loss of insulin secretion that mediated by T cells **[2]**. The destruction of β cells is due to the markers that are islet cell autoantibodies, antibodies to insulin, antibodies to glutamic acid decarboxylase-65 (GAD65), antibodies to tyrosine phosphatase Insulinoma antigen-2A (IA-2A), and Insulinoma antigen-2B (IA-2B). These markers are used to diagnose the disorder **[2]**. The children diagnosed with this disorder may have the risk of development of other autoimmune diseases such as hypothyroidism, hypoadrenalism, pernicious anemia, alopecia areata, vitiligo, and other thyro-gastric complex diseases **[4]**. The existence of autoantibodies and autoreactive T cells point out that certain islet antigens are erroneously identified as foreign which initiates immune response leading to conditions involving type 1 diabetes mellitus **[6]**. Autoantigen (AA) is a normal protein or protein complex (sometimes it is DNA or RNA) that is identified by the patient's immune system who is suffering from a specific autoimmune disease including type 1 diabetes mellitus **[7]**. These autoantigens (AAs) are produced due to mutations, the formation of neoantigen (formed by gene-producing self-protein that can mutate and form new immunogenic protein i.e., neoantigen), or exposure of previously hidden self-antigens **[8]**. The major AAs that are diagnosed in patients

with type 1 diabetes mellitus are non-specific islet cell AAs (ICA), insulin, glutamic acid decarboxylase 65 (GAD65), insulinoma antigen-2 (IA-2), zinc transporter (ZnT8). Additional AAs are islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP), chromogranin A (CgA), islet amyloid polypeptide (IAPP), Imogen-38, heat shock protein (HSP), and others that are recently identified are peripherin, tetraspanin-7, prolyl-4-hydroxylase β (P4Hb), glucose-regulated protein 78 (GRP78), urocortin-3, insulin gene enhancer protein islet-1 and pancreatic duodenal homeobox factor I (PDX1) **[6]**. In this review we will target only four AAs, viz., IAPP, PDX1, CgA, and ZnT8 and will describe their basic biology and other features along with relation to type 1 diabetes mellitus.

Role of autoantigens in type 1 diabetes mellitus:

Type 1 diabetes mellitus occurs due to the destruction of insulin-producing β cells. However, its initiation and progression mechanism is not fully understood but it is believed that the AAs against β cells, macrophages, dendritic cells, B lymphocytes, and T lymphocytes are involved in this process **[9]**. The events that are involved in destruction are shown in **Figure 1**.

DETAIL STUDY OF SOME IMPORTANT AUTOANTIGENS IN TYPE 1 DIABETES MELLITUS:

Islet amyloid polypeptide (IAPP)

Islet amyloid polypeptide (IAPP) or amylin is a putative hormone found in β cells of the pancreatic islet in 90% of patients with diabetes mellitus **[10]**. The name was given because it has the unique property to aggregate into insoluble amyloid fibrils **[11]** that show cytotoxic effects on β cells and possess pro-inflammatory properties **[12]**. This aggregated IAPP is found in the pancreatic islet of patients with type 2 diabetes and the pancreatic islet transplanted patients with type 1 diabetes mellitus **[6,13]**. The difference between rodent IAPP and human IAPP (hIAPP) is that rodent IAPP does not aggregate like hIAPP because they possess three proline amyloidogenic regions of the peptide **[12]**. It is the main constituent of islet amyloid and was firstly identified in 1987 in the pancreatic extract of the patient with diabetes and insulinoma **[10]**. The IAPP is co-secreted in parallel to insulin in response to glucose stimulation **[13]** and thus insulin-dependent patients with diabetes and patients with insulin deficiency encounter IAPP deficiency also **[14]**. People with glucose tolerance and hyperinsulinemic condition experience high levels of IAPP

which indicates that amylin level varies with insulin **[14]**.

Structure and physicochemical properties of islet amyloid polypeptide:

It is a peptide hormone and was initially identified as insulinoma amyloid peptide, later named as diabetes-associated peptide (DAP), and now known as islet amyloid peptide or amylin **[11]**. It is a larger, originally purified, and most abundant constituent of the congophilic amyloid deposits which contains 37 amino acid proteins produced by β cells and shows a close similarity with calcitonin-gene-related peptide i.e., CGRP **[10,15]**. The gene of IAPP is located on the short arm of chromosome 12 **[15]** and the polypeptide is amidated at C- terminus **[16]**. The amino acid sequence of IAPP is 43% homologous with αCGRP and 46% homology with the second β from CGRP. Near to the N-terminus, it contains an intermolecular disulfide bridge between Cysteine-2 and Cysteine-7 **[16]**.

It is a hydrophobic polypeptide hormone that contains positively charged residues such as Lysin-1, Arginine-11, and Histidine-18 (based on pH). The acidic residue is absent in IAPP. The isoelectric point (pI) of Tyrosine and Lysine residue is above the pKa. Depending on pKa's of N-terminus, Histidine-18, and the pH, the polypeptide possesses a positive charge at and below the physiological pH with a net charge of 2-4. For the interaction with sulfated proteoglycans of extracellular matrix and with negatively charged non-physiological model membranes, the net positive charge on the molecule is important. The hIAPP sequence contains many residues of Asparagine and Selenocysteine/Threonine for its size, 6 and 10 respectively. Three aromatic residues are also present that are conserved C-terminal Tyrosine and conserved Phenylalanine at 15^{th} position and 23^{rd} position **[16]**.

Synthesis and storage of islet amyloid polypeptide:

The process for the synthesis of IAPP is like that of insulin, both genes contain similar promoter elements and transcription factor PDX1 that regulates both genes in glucose response **[11]**. Its synthesis is carried out by inactive pro-hormones and involves the number of protease cleavages and post-translational modifications **[17]**. It is translated as a preprohormone precursor, i.e., preproIAPP which is in an immature form. The Preprohormone gene contains three exons, of them last two encode the full prepromolecule **[11]**. The preproIAPP (containing 89 amino acid residues out of which 22 are amino acid signal peptide and 2 are short flanking peptides, i.e., C-and N-terminal which later breaks at double basic amino acid residues) **[11]** is converted to

produce proform, i.e., proIAPP (containing 67 residues) by deleting the signal peptide ($hproIAPP_{1-67}$ in human and $mproIAPP_{1-70}$ in mice) **[12]**. In the β cells of mice, $proIAPP_{1-67}$ is cleaved by prohormone convertase enzyme (PC) 1/3 at its C-terminus to produce an N-terminally extended intermediate form, i.e., $proIAPP_{1-48}$. Produced $proIAPP_{1-48}$ is then cleaved by PC2 in secretory granules to generate a mature form of IAPP **[17]** which contains 37 amino acids ($hIAPP_{1-37}$ or $mIAPP_{1-37}$) **[7]**. The cleavage of human IAPP is done by using PC2 and not by PC3 or by furin (Clark *et al*., 1996). The breakdown of proIAPP by PC2 is done at Lysine-10 Arginine-11 position and by PC 1/3 at Lysine-50 Arginine-51 position **[11]**. This whole process of production of IAPP from proIAPP is shown in **Figure 2 [12,16]**.

Role of islet amyloid polypeptide in putative functions [10,14,16]:

1. Metabolism and glucose homeostasis regulation
2. Regulation of insulin action and its secretion
3. Gastric emptying inhibition
4. Vasodilatation and modulation of calcium (Ca^{2+}) metabolism
5. Inhibition of glycogen synthesis and increasing its breakdown

Relation of islet amyloid polypeptide and type 1 diabetes mellitus:

As IAPP is co-located into granules of β cells and co-secreted in the blood in parallel to insulin in response to glucose stimulation, the hyposecretion of IAPP has been observed in type 1 diabetes mellitus. It was found that with the injection of synthetic IAPP and insulin, the glycemic control was improved by the suppression of glucagon secretion **[6]**.

Chromogranin A

Chromogranin A (CgA) is a member of the granin family which is a highly acidic protein and contains 439 amino acid residues processed by 18 signal peptide residues. It has a molecular weight of 48kDa, and it was the first discovered granin glycoprotein **[18,19]**.

Structure and biochemical properties of chromogranin A:

A pre-chromogranin A molecule contains 457 amino acids while, as mentioned above, chromogranin A contains 439 amino acids which are set into one-chain peptide and processed by NH-2 terminal 18 amino acid signal peptide **[19]**. The gene of CgA, i.e., CHGA is a single copy gene present on the 14^{th} chromosome with base pairs 12,194 at q32.12 locus and 8 axons **[18]**. Its primary structure derived from the sequence of cDNA is shown in **Figure 3 [20]**. A high amount of charge, mostly acidic amino acids, are present in their structure, while multiple sites involve two

or more adjacent basic amino acids. In all mammalian species of CgA, there are 7 of these dibasic residues conserved **[20]**. It forms bioactive post-translational fragments shown in **Figure 4 [21]** by the action of prohormone convertase enzyme PC1/3 and PC2 **(Table-I) [18]**.

Due to the presence of excessive negative charges and property of aggregation in presence of cation, CgA binds to calcium with a moderate affinity at multiple sites. As all the granin family members contain multiple dibasic residues and binding sites for calcium in their structure they show similarities in their functions. CgA is more like CgB. In both molecules, at their amino termini, a disulfide-bonded loop structure is present with a highly correspondent amino acid sequence demonstrated in **Figure 3 [20]**.

The role of chromogranin A in type 1 diabetes mellitus:

The comparative results of wild-type non-obese diabetic (NOD) mice and CgA-deficient knockout NOD mice (NOD.CgA$^{-/-}$) show that there is no development of type 1 diabetes in NOD.CgA$^{-/-}$ or it occurs in a very small portion of its population. This shows that CgA causes type 1 diabetes mellitus. The observative results for 12 months show that type 1 diabetes mellitus is developed in > 90% female wild type NOD mice and only 3% symptoms are observed in NOD.CgA$^{-/-}$ female mice. The signs of type 1 diabetes mellitus were not observed in male NOD.CgA$^{-/-}$ mice. After 12 months observational period, pathological examinations were carried out for the insulitis and inflammation of the islet of the pancreas. The obtained results indicated the insulitis in all the wild-type NOD mice in males as well as females but only 20% was developed in NOD.CgA$^{-\backslash-\ mice}$ and the amount of $CD4^+$ and $CD8^+$ T-cells were found in its pancreatic tissues **[18]**.

Uses of chromogranin A [21]:

- It is a neuroendocrine biomarker and used as a diagnostic marker of the tumor and used to monitor the tumor progression as well as regulation during its treatment.
- Used even as a biomarker in neurodegenerative and neuropsychiatric diseases.
- Used as a biomarker in hypertension, cardiovascular diseases with addition of heart, renal, and liver failure conditions.
- It could be a potential diabetic biomarker.

Pancreatic duodenal homeobox-1(PDX1)

Pancreatic duodenal homeobox-1 protein (PDX1) is a β cell-specific novel autoantigen which is also called insulin promoter factor 1 or IDX-1/STF-1/IPF1. It is a homeodomain transcription factor which is a β cell identity and function

regulator. It is a protein containing its antennapedia-like homeodomain in its structure. It contributes to pancreatic development and in the transcription process of the insulin gene **[22]**.

Structure of pancreatic duodenal homeobox-1:

The human PDX1 gene is detected on chromosome 13q12.1 and contains 2 axons, 1 intron, and 6 kb spans. The human PDX1 contains 283 amino acids accompanied by 31K molecular weight. The PDX1 gene of the mouse is detected at the distal end of chromosome 5 while the PDX1 gene of the rat is located on chromosome 12 **[23,24]**. The human PDX1 contains highly conserved protein, and the sequence of protein has higher homology with other species such as 90% with the hamster, 88% with rat, 87% with the mouse, and 68% with Xenopus **[6,25]**.

In the structure of PDX1, the antennapedia-like homeodomain (which has the same amino acid sequence except for one amino acid towards the NH2-terminal) is present in the middle region. This antennapedia like homeodomain is flanked by a proline-rich region on both sides **[22,25]**. Homeodomain is involved in the DNA binding process and protein-protein interaction by the transcriptional activation mechanism. For DNA binding, highly conserved histidine residue at 189^{th} position and KIWFQN motif which is present in helix 3 of homeodomain is required. The NH2-terminal region contains a transactivation domain. This transactivation domain is divided into 3 evolutionarily conserved subdomains namely, subdomain A (13-22amino acids), subdomain B (32-38 amino acids), and subdomain C (60-73 amino acids) **[25-27]**. The COOH- terminal has the inhibition action **[6]**. The structure is illustrated in **Figure 5**.

Regulation of pancreatic duodenal homeobox-1 gene expression:

It is a complex process contain up to 6 kb sequence from the start site of transcription. A proximal E box binds the ubiquitous bHLH protein USF-1 at around 104. Several sites are also present that bind HNF3β which is a forkhead winged-helix transcription factor. For the expression of the PDX1 gene, the HNF3β transcription factor could prime the PDX1 gene by other factors of transcription. PDX1 binds to the TAAT core sequence that contains the site of HNF3β. This shows that the autoregulatory feedback mechanism, but the expression of PDX1 is inhibited by glucocorticoids by interfering with HNF3β. Other factors such as HNF1α and SP1/3 are also involved in the PDX1 gene regulation process **[26]**.

Role of pancreatic duodenal homeobox-1:

It plays a role in early embryonic pancreatic development, differentiation of endocrine lineages, and β cell maturation. In the early development of the pancreas, its expression is detected at the somite stage in mice and the 4-week gestational stage in humans. According to a human case report study, 5 years old female Caucasian had pancreatic agenesis because of the presence of homozygous, a single nucleotide in the PDX1 gene. PDX1 is also important for pancreatic lineage differentiation. In pregnant mice, when PDX1 expression is stopped or blocked via parturition then pancreas development is arrested. Another role of PDX1 is in maintaining the function of β cells as it induces glucose intolerance when PDX1 reduction takes place in mature β cells **[28]**.

Relation of pancreatic duodenal homeobox-1 and diabetes:

Several molecular events are involved in the development of diabetes which affects the ability of β cells to secrete insulin. These events also affect the ability of other cells such as muscle, fat, and liver cells to respond to the actions of insulin **[25]**. Monogenic form of diabetes, i.e., **M**aturity **O**nset **D**iabetes of the **Y**oung (MODY) is marked by autosomal dominant inheritance, pancreatic β cell dysfunction as well as flaws in insulin secretion leading to the development of early-onset diabetes. The six genes responsible for diabetes are MODY1, MODY2, MODY3, MODY4, MODY5, and MODY6. One of them encodes the glycolytic enzyme GK, i.e., glucokinase (MODY2), and the remaining five encodes the transcription factor. The transcription factor encoding gene contains three genes from **H**epatocyte **N**uclear **F**amily (HNF). These are HNF-4α in MODY1, HNF-1α in MODY3, and HNF-1β in MODY5. MODY4 and MODY6 are the results of mutation in the PDX1 gene and NeuroD/B2 gene, respectively. Mutation in any one of these six genes results in MODY. The frameshift mutation and missense mutation in PDX1 lead to decreased binding activity of PDX1 to the insulin promoter and this is accompanied by reduced insulin transcription in response to hyperglycemia (in-vitro) **[25,29-31]**.

Zinc transporter protein 8 (ZnT8)

Zinc transporter protein 8 (ZnT8) is a novel islet autoantigen in both type 1 and type 2 diabetes mellitus. It is a product of the SLC30A8 gene and present in pancreatic β cells where zinc is present in high amounts **[32]**. Zinc is required to crystalize the insulin, storage as well as exocytosis of insulin secretory vesicles **[33]**. ZnT8 transports the zinc from the cytoplasm to intracellular vesicles and extracellular space **[34]** and it is an important constituent required for the insulin maturation process

[35]. It regulates the zinc concentration in β cells and acts as a zinc sensor **[34]**.

Structure of zinc transporter protein 8:

Human ZnT8 shown in **Figure 6** has 2 homodimer isoform which is differentiated by the length difference of 49 amino acid at N-terminal end **[36,37]**. One form (A) contains 369 amino acids while another form (B) contains 320 amino acids according to N-terminal extension **[34,38]**. Its exact structure is not yet clear, but it is believing that according to bacterial Yiip X-ray structure it has a "Y" shaped structure like immunoglobulin architecture **[34,39]**. This dimer is encoded by SLC30A8 located at chromosome 8q14.11 and each monomer contain six transmembrane domains (TMDs), cytoplasmic amino- and carboxy-terminal tails with histidine-rich loop [34,40]. This transmembrane protein act as a Zn^{2+}/H^{+} exchanger **[40]** and transport the zinc ions from cytosol to intracellular vesicles as well as to intracellular space as mentioned above **[38]**. The conformational changes in protein structure result in the induction of antiport mechanism **[34]**.

ZnT8 has 4 binding sites on each promoter. The primary active site A located in the center of TMDs while cytoplasmic binding sites B are located on the surface of the membrane **[34]** and C on the dimer interface of the C-terminal domain (CTD). The C-terminal domain plays a role in protein-protein interaction and as a zinc sensor **[34]**. The amino acid of ZnT8 at 320 position could be arginine (R), tryptophan (W), or glutamine (Q) due to single-nucleotide polymorphism within SLC30A8, that are rs13266634 and rs16889462. According to previous research, rs13266634 has a relation with type 2 diabetes mellitus but not with type 1 diabetes mellitus **[41]** which indicates that it can stratify the risk of type 1 diabetes mellitus in children with positive autoantibodies to ZnT8 (ZnT8A). The 12 rare mutations in SLC30A8 could reduce 65% risk of T2DM and these mutations are 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. This suggests that the SLC30A8 genotype may be a common genetic cause of type 1 diabetes mellitus as well as type 2 diabetes mellitus **[41]**.

Functions/ role of zinc transporter protein 8:

- The basic role of ZnT8 is to maintain the intracellular zinc homeostasis process with other zinc transporters as it is required for the structural stability, storage, and secretion of insulin but it does not affect the content of insulin, size of

the islet, and composition of the cell **[32,41]**.

- According to previous reports, some researchers noticed that ZnT8 could impair the Glucose Stimulated Insulin Secretion (GSIS) while others reported increased or unchanged GSIS. This indicates that ZnT8 has a conflicting effect on GSIS, insulin sensitivity, and glucose tolerance tests **[32,41]**.
- It plays an indeterminate function in the survival of β cells **[41]**.

These contradictory results might be due to interaction between ZnT8 and other influencing factors such as an environmental factor, genetic history, age, sex, and subcellular localization of ZnT8 **[32,41]**.

Zinc transporter protein 8 as an autoantigen:

In serum of a patient with type 1 diabetes mellitus, autoantibodies are detected by utilizing the different fragments of human ZnT8, there are only C-terminal fragments that produce higher sensitivity and specificity (50.4 and 98%). 18.6% autoantibodies are detected against the ZnT8-COOH (ZnT8A-COOH) in patients with type 1 diabetes mellitus but ZnT8-NH_2 was rare. Some studies suggest that the ZnT8 dominant epitope/s may be present at 268-369 amino acid of ZnT8, and which is conformational instead of the linear epitope. Researchers reported that, by using site-directed mutagenesis in the C-terminal of ZnT8, its epitopes are critically dependent on the polymorphism at 325 amino acids by using site-directed mutagenesis in C-terminal ZnT8. It has indicated that there is approximately 50% prevalence of ZnT8A-325R among the patient with new-onset type 1 diabetes which is a bit more than ZnT8A-325W while nearly 30% positive rate of ZnT8A-325Q in type 1 diabetes mellitus but ZnT8A-325Q is not applicable epitope for the same disorder diagnosis because receiver operating characteristic curve of this epitope shows that it cannot significantly differentiate the patients with type 1 diabetes from controls. Hence, the polymorphism of ZnT8 C-terminal at amino acid 325, mainly ZnT8325-R and/or ZnT8-325W, consult an antigenicity and epitope specificity of ZnT8. Moreover, linear epitope R_{325}, R_{332}, E_{333}, K_{336}, and K_{340} participate in another region of antigenicity **[41]**.

The key epitope of the ZnT8-reactive T cell was studied previously. ZnT8 is a protein that is bound to the HLA-DQ8 molecule, mainly 166-179 amino acid of ZnT8 is firstly identified by Chang and Unanue. In the NOD mouse, after the protein immunization by islet antigen-containing

cells, $ZnT8_{330-344}$ and $ZnT8_{345-359}$ were present but the latter were diabetogenic epitopes. Dang *et al*. noticed that some epitopes are bound to HLC-DR4. These are $ZnT8_{8–22}$, $ZnT8_{15–29}$, $ZnT8_{120–134}$, $ZnT8_{134–148}$, $ZnT8_{260–274}$, $ZnT8_{267–281}$, and $ZnT8_{295–309}$, while $ZnT8_{155–169}$ and $ZnT8_{323–337}$ are HLA-DR3 restricted epitopes in human. Albeit in type 1 diabetes mellitus, ZnT8186-194 is the predominant epitope for CD8+ T cells. In type 1 diabetes mellitus, HLA-A*0201-restricted $CD8^+$ T cells epitopes are also identified, and these are $ZnT8_{153-161}$, $ZnT8_{107-115}$, $ZnT8_{115-123}$, and $ZnT8_{145-153}$. In type 2 diabetes mellitus, $ZnT8_{253-261}$ is the most frequently detected epitope than type 1 diabetes mellitus. Most T cell epitopes were mapped against the ZnT8 in the transmembrane region or the C-terminal region, there is no overlapping with the polymorphic region of ZnT8 at amino acid 325 **[41]**.

Table:

Name of peptide	Some features
Pancreastatin ($CgA_{250-301}$)	• **It is a dysglycemic hormone and plays the role in Glucose-stimulated and unstimulated insulin secretion inhibition.** • **It inhibits the glucose uptake and suppresses insulin signaling.** • **Lipid synthesis and leptin secretion in adipocytes are decreased by pancreastatin.**
Vasostatin-1 (CgA_{1-76}) and Vasostatin-2 (CgA_{1-115})	**It shows anti-inflammatory, anti-microbial, cardioprotective, and anti-adrenergic effects.**
Parastatin ($CgA_{357-428}$)	**It plays the role in the inhibition of low Ca^{2+}-stimulated parathyroid secretion in vivo.**
Chromacin ($CgA_{173-194}$)	**Gram-positive as well as Gram-negative bacteria inhibited by the Chromacin.**
Catestatin ($CgA_{352-372}$)	**It is a catecholamine release inhibitory and anti-hypertensive peptide and shows the opposite effects to the parastatin on glucose metabolism and lipogenesis process.** **It also plays a significant role in cardiac function and blood pressure regulation.**
Serpinin ($CgA_{417-442}$)	**The newly discovered fragment has an anti-apoptotic effect, and it also promotes cell survival and myocardial contractility and relaxation**

Table 1: Post-translational fragments and their roles [18]

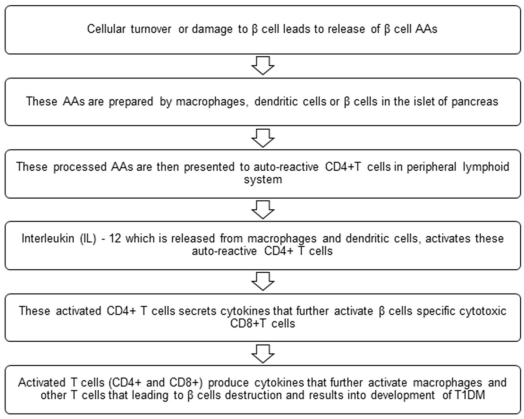

Figure 1: The events involved in the destruction of β cells [9]

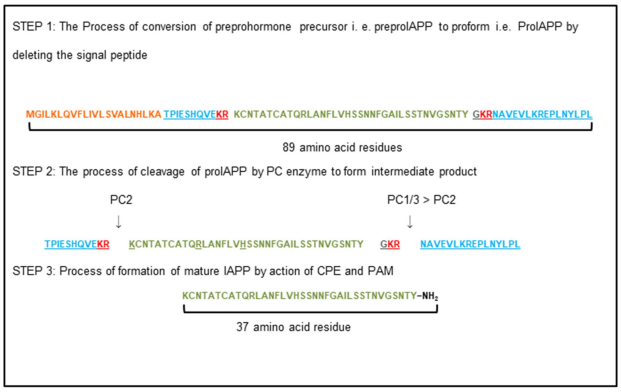

Figure 2: Formation of mature IAPP from preproIAPP. The primary sequence in step 1 with orange color is the 22-residue signal sequence. The underlined regions are 2 short flanking peptides i. e. C- and N – terminals. The preform of IAPP i. e., proIAPP is cleaved by the action of PC1/3 and PC2 at two conserved dibasic sites that are indicated by arrows in step 2. In the last step, mature IAPP is formed by the action of CPE (deleted basic residues that are indicated by red color) and PAM (deleted glycine residue that indicated by dark grey color) [12, 16]

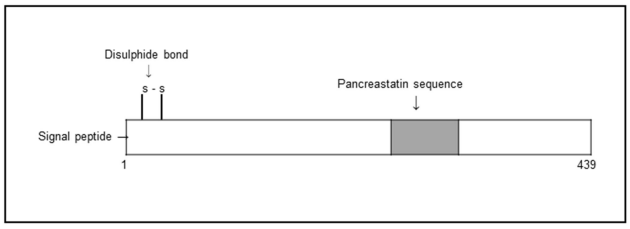

Figure 3: Primary structure of human chromogranin A (hCgA) with dibasic amino acid sites where proteolytic processing may occur, signal peptide. S-S indicates disulfide bond while grey color indicates pancreastatin sequence [20]

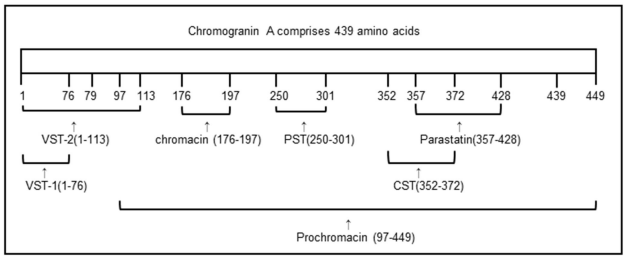

Figure 4: Diagrammatic presentation of chromogranin A containing 439 amino acids and its derived peptides from the stress-activated, diffuse neuroendocrine system [18, 19, 21]

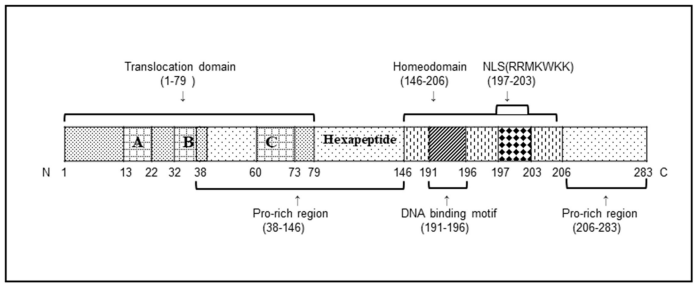

Figure 5: Protein structure of PDX1 gene. The transactivation domain which consists of 1-79 amino acids is indicated by, subdomains A, B, and C are indicated by, the pro-rich region indicated by. The antenna type hexapeptide which contains PFPWMK amino acid is present in the pro-rich region which is located before the homeobox. The homeodomain region which contains 146-206 amino acid indicated by Amino acid 191-196 is DNA binding motif shown The nuclear localization signal i.e. NLS containing 7 amino acids such as RRMKRKK indicated by [25, 26]

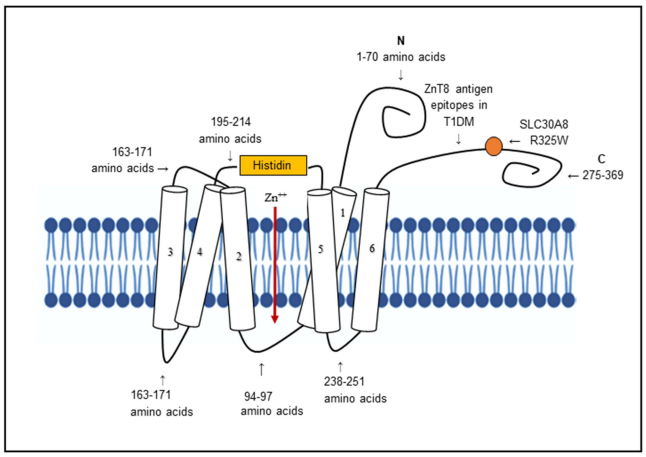

Figure 6: Schematic diagram of zinc transporter protein 8 which is present in insulin secretory granule. The C- and N-terminals are cytosolic and 1-6 numbered transmembrane domains are extracellular [36, 37]

CONCLUSION

Since autoantigens have important theoretical and practical applications in type 1 diabetes mellitus, there has been appreciable work is done to identify and characterize autoantigens that are involved in type 1 diabetes mellitus. In this review, we generally discuss the recently discovered 4 autoantigens namely IAPP, CgA, PDX1, and ZnT8. Numerous autoantigens which are related to type 1 diabetes mellitus are previously recognized, promoting the purpose that immune response to multiple autoantigens participates in the pathogenesis of the same disorder. Identification of $CD4^+$ and $CD8^+$ T cell autoantigens may be the beginning step in the autoimmune cascade. Finding the epitopes of β cell-specific $CD4^+$ and the $CD8^+$ T cell is a key principle of type 1 diabetes mellitus exploration. This assists in cognizance of the pathogenesis of type 1 diabetes mellitus and the new strategy for the development and progression of disorder monitoring. It also helps in the development of therapeutic interventions. It is also believed that additional autoantigens and T cell epitopes associated with type 1 diabetes mellitus will be advanced hereafter.

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