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Assessment of Insulin Gene VNTR INS -23/Hph1 (rs689) Polymorphism and its Relation with Type 1 Diabetes Mellitus in Egyptian Children: Review Article

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ABSTRACT

Chronic autoimmune illness, including type 1 diabetes mellitus (T1DM), are common in young people especially children and teenagers. The classic "trio" of symptoms: polydipsia, polyphagia, and polyuria, are observed when pancreatic β cells are lost, leading to insulin insufficiency and hyperglycemia. The likelihood of developing T1DM and type 2 diabetes mellitus (T2DM) is six and three times higher in individuals with a family history of these conditions, respectively, than in those who are unrelated. The multifactorial etiology of T1DM likely involves a combination of environmental and genetic factors that either initiate or permit the autoimmune response against the β-cells. HLA genes exert the greatest impact on the likelihood of developing TIDM. Polymorphisms in the insulin (INS) gene's noncoding region (IDDM2) influence the likelihood of developing T1DM, second only to HLA in importance. An insulin gene mini-satellite called a variable nucleotide tandem repeat (VNTR) is present at the 5′ end of this locus. The size of the VNTR determines its classification into three primary groups: class I (26–63 repeats), class II (about 80 repeats), and class III (140–200 repeats). Insulin gene polymorphisms can be used as markers because they are in a linkage disequilibrium with the VNTR region. This review article aims to assess the Insulin Gene VNTR INS -23/Hph1 (rs689) Polymorphism in Type 1 Diabetes Mellitus patients.

Keywords: Insulin Gene VNTR INS -23/Hph1 (rs689) Polymorphism; T1DM; Paediatrics; Autoimmunity; Genetic Risk Score (GRS)

Type 1 diabetes mellitus

T1DM is the most common chronic autoimmune disease in young people, particularly in children and teenagers. The classic "trio" of symptoms, which includes increased thirst, increased hunger, and increased urine frequency a long with hyperglycemia, occurs when pancreatic β cells are lost, resulting in insulin deficiency and their bodies are unable to produce enough insulin, and people with T1DM need insulin injections on a daily basis. The reason behind this is that replacing insulin from external sources is an immediate necessity following the autoimmune destruction of pancreatic β cells [1]. Although the precise causes of these autoimmune responses are unknown, the public tends to blame "environmental factors" for the disease's development. Based on numerous studies and extensive scientific investigation, the present scientific consensus states that a range of environmental factors initiates autoimmune response to β-cells in genetically predisposed individuals. When activated, cytotoxic CD8+ and autoreactive CD4+ T-cells infiltrate the islets, it leads to islet β-cell apoptosis [2].

Compared to the general population, families with a history of the disease have at least three times the rate of T2DM and six times the rate of T1DM. Environmental variables and genetic susceptibility interact in intricate ways to form the pathophysiology of T1DM. The unique environment dictates which genetic variations or alleles are linked to a person's risk of getting T1DM or their ability to avoid the condition. The specific context determines the alleles or genetic variants that are associated with a person's risk of developing T1DM or their capacity to avoid developing the disease. When it comes to determining the chance of developing TIDM, HLA genes are the most influential. However, research has shown that more than fifty loci outside the HLA region affect T1D. Genetics, and more especially the relative abundance of risk and protective alleles, dictate when T1DM manifests in a person. There are two major genetic factors that affect the risk of developing T1DM: HLA and INS,IDDM2.The insulin gene at this specific locus contains a mini-satellite known as a VNTR at its 5′ end [3].

Class I VNTRs have 26-63 repeats, class II VNTRs have 80-100 repeats, and class III VNTRs have 140-200 repeats; these three groups are classified according to size. Variation in the insulin gene can be identified using markers that are in linkage disequilibrium with the VNTR region. As an example, consider the 223HphI A>T single-nucleotide polymorphism (SNP) in the INS gene. It was discovered to be in a state of considerable linkage disequilibrium with respect to the VNTR alleles. Specifically, the A allele was linked to the short (class I) allele and the T allele to the long (class III) allele [4]. The literature is contradictory when it comes to the link between the A and T alleles and the risk of TIDM. Some studies have found that the A allele increases the risk of diabetes, while others have found that the T allele protects against it. Still others have shown that the T allele is the one linked to risk, and still others deny that either allele plays a role in TIDM risk. Ultimately, risk susceptibility is a result of a combination of hereditary and environmental factors. Depending on the ethnic group, the relative frequency of each allele can vary, and exposure to different local environments can affect the amount a

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gene polymorphism contributes to the overall risk of a disease. Therefore, it is not possible to generalise the results from one population to another [5].

- **Global epidemiology of type 1 diabetes**

T1DM occurs when the beta cells of the pancreas, which produce insulin, are destroyed by the body's own immune system. New studies have helped us better understand T1DM's global epidemiology by revealing trends in incidence, prevalence, mortality, and complications [6].

Children younger than 15 years old exhibited a wide range of geographical heterogeneity in T1DM incidence, from 0.02 to more than 50 cases per 100,000. [7].

Researchers need to keep an eye on the data constantly because incidence trends might shift over time and differ between demographics and geographical locations. According to the T1D Index, almost 35,000 deaths in people younger than 25 were caused by not having a diagnosis of T1DM. An estimated 8.4 million people were living with prevalent T1DM in 2021, with prevalence rates varying between 1.5 and 534 cases per 100,000 citizens. The T1D Index, which used a Markov model for the first time, aims to quantify the worldwide burden of T1D and provides the data used in this analysis. Based on variations in diabetes care accessibility, the T1D Index model indicates that health-adjusted life years vary significantly among nations. There is some variation in life expectancy and mortality rates linked with T1DM, according to previous population-based research. This variation is influenced by factors such as period, geography, and the quality of management. Expert organisations have recognised that the results of T1DM epidemiological surveillance should drive public health initiatives and resource allocation in order to enhance patient outcomes and diagnosis rates on a worldwide level [8].

- **Pathogenesis of type 1 diabetes**

Hereditary and environmental factors contribute to the complex pathophysiology of T1DM by causing the loss of immunological tolerance, which in turn targets the pancreatic beta cells responsible for generating insulin. Hypoinsulinemia and hyperglycemia result from the coordinated destruction of beta cells by autoreactive T cells and autoantibodies [8].

- **Destruction of beta cells**

T1DM develops when the pancreatic beta cells gradually lose both size and function. This process might start months, or even years, before a patient is formally diagnosed, since many autoantibodies can be detected before symptoms manifest. Investigating the pancreas of people with T1DM histologically reveals insulitis, which is defined by the invasion of the islets by immune cells such as T lymphocytes, B lymphocytes, macrophages, and dendritic cells. Beta cells are killed off by cytokines released by these immune cells. When hyperglycemia is noticed, the beta cell death rate will have ranged from 81% to 95%. T cells (CD4+ and CD8+) engage in direct targeting of beta cells that secrete processed autoantigen peptides via the HLA class I and II pathways. Insulin, zinc transporter 8, insulinoma-associated antigen 2, and glutamic acid decarboxylase 65 (GAD65) are among the beta cell antigens that are targeted.

It is believed that beta cells are damaged by environmental triggers, such as viral infections, which release autoantigens that activate autoreactive T cells [8].

An autoimmune mechanism involving both cells and humoral components destroys pancreatic beta cells in T1DM. The islets are invaded by autoreactive CD4+ and CD8+ T lymphocytes, which then produce inflammatory cytokines and directly target the beta cells in this multi-pronged onslaught. In addition to beta cells producing autoantibodies that activate complement, immune cells that expand and attract additional immune cells keep insulitis at bay. As apoptosis keeps happening at a faster rate than beta cell regeneration, the number of functioning beta cells eventually drops to a negligible level [9].

- **Loss of insulin secretion**

Beta cell autoimmunity destroys insulin-producing and secreting cells, which disrupts glucose homeostasis. In T1DM, pancreatic beta cells are damaged by pro-inflammatory cytokines released by immune cells that invade the insulitis islets. This ability to tightly regulate insulin release in response to changes in blood glucose levels is impaired early in the pathogenesis. Normally, beta cells are very good at this, but they interfere with the signal transduction pathways that regulate insulin release in response to glucose. At first, beta cells make up for it by increasing their number and insulin production per cell. However, inadequate basal and postprandial insulin secretion occurs when secretory function is unable to counteract the degree of beta cell death [10].

- **Role of T Cells**

Beta cell peptides are recognized by CD4+ T helper cells using HLA class II molecules. Within the islet infiltration, these cells transform into inflammatory Th1 and Th17 subsets and release cytokines like IFN-γ, TNF-α, and IL-17. These cytokines enhance local inflammation and draw in extra immune cells, playing a significant role in the autoimmune reaction that targets beta cells. The help that Th1 and Th17 cells provide to B cells promotes the generation of autoantibodies. When CD8+ cytotoxic T lymphocytes contact with the autoantigen peptides on HLA class I, they kill beta cells.The secretion of granzymes, perforin, and pro-apoptotic cytokines is the mechanism by which they destroy cells. Normally, regulatory T cells would be able to suppress autoimmune responses. However, in T1DM , these cells are unable to control the expansion of pathogenic T cells [11].

Investigated, as a potential immunotherapy approach is the manipulation of the ratio of autoreactive to regulatory T cells. Other subsets of T cells may exacerbate beta cell damage, in addition to the more common CD4+ and CD8+ T cells. In response to lipid antigens, NKT cells within islets can release

inflammatory cytokines. Gamma-delta (γδ) T cells may invade islets at an early stage in the development of T1DM due to their expansion in the peripheral blood of these patients. More explanation of their responsibilities is necessary. There are various levels of autoreactive T cells that contribute to the autoimmune attack that causes beta cell dysfunction and destruction [12].

- **Role of autoantibodies:**

Autoantibodies that specifically target islet autoantigens are defining features of T1DM. Their presence point to persistent autoimmunity even in the absence of a clinical diagnosis. The usual targets of the islet autoantibodies include insulin, GAD65, IA-2, and ZnT8. These are common sights. B cells in the pancreatic lymph nodes and islet infiltration, with the assistance of T cells, produce these autoantibodies. Their specificity and sensitivity make them valuable markers, regardless of whether they directly cause beta cell death or not. In pathogenesis, autoantibodies do not work in reverse but rather as immune complexes that set off inflammatory reactions and complement cascades [12]. Additionally, they help get antigens into the hands of autoreactive T cells. Those at risk for developing T1DM may be able to gauge the severity of their injury from T cells by looking at their autoantibodies. Their detection has gained significant importance in the field of pre-symptomatic disease staging, risk stratification in T1DM patient relatives, and participant recruitment in prevention trials. However, autoantibodies cannot trigger T1DM unless autoreactive T cells are involved as well. The ability to differentiate between self- and non-self-antigens (negative selection) is conferred upon T lymphocytes by central tolerance mechanisms that allow them to mature in the thymus after originating from progenitor cells in the bone marrow. Different affinities allow regulatory T cells (Tregs) and pathogenic T cells to identify self- or beta cell antigens, which may explain why they serve different purposes [12]. When they are in circulation, mature T lymphocytes may be exposed to their unique peptide-MHC/HLA complex. T1D develops when T cells target insulin, GAD55, and other beta cell proteins. Antigen presenting cells express beta cell peptides on MHC/HLA, which trigger lymph node activation, migration to islets, and the antigen-specific destruction of beta cells by T lymphocytes. Tregs suppress these events, which is a component of peripheral tolerance. Insulin insufficiency, high blood sugar, and T1DM occur when the immune system is unable to stop the autoimmune assault on beta cells. Because most signalling happens locally in organs like the pancreas and lymph nodes, biomarkers cannot pick it up in its current form **in Figure 1** [12,13].

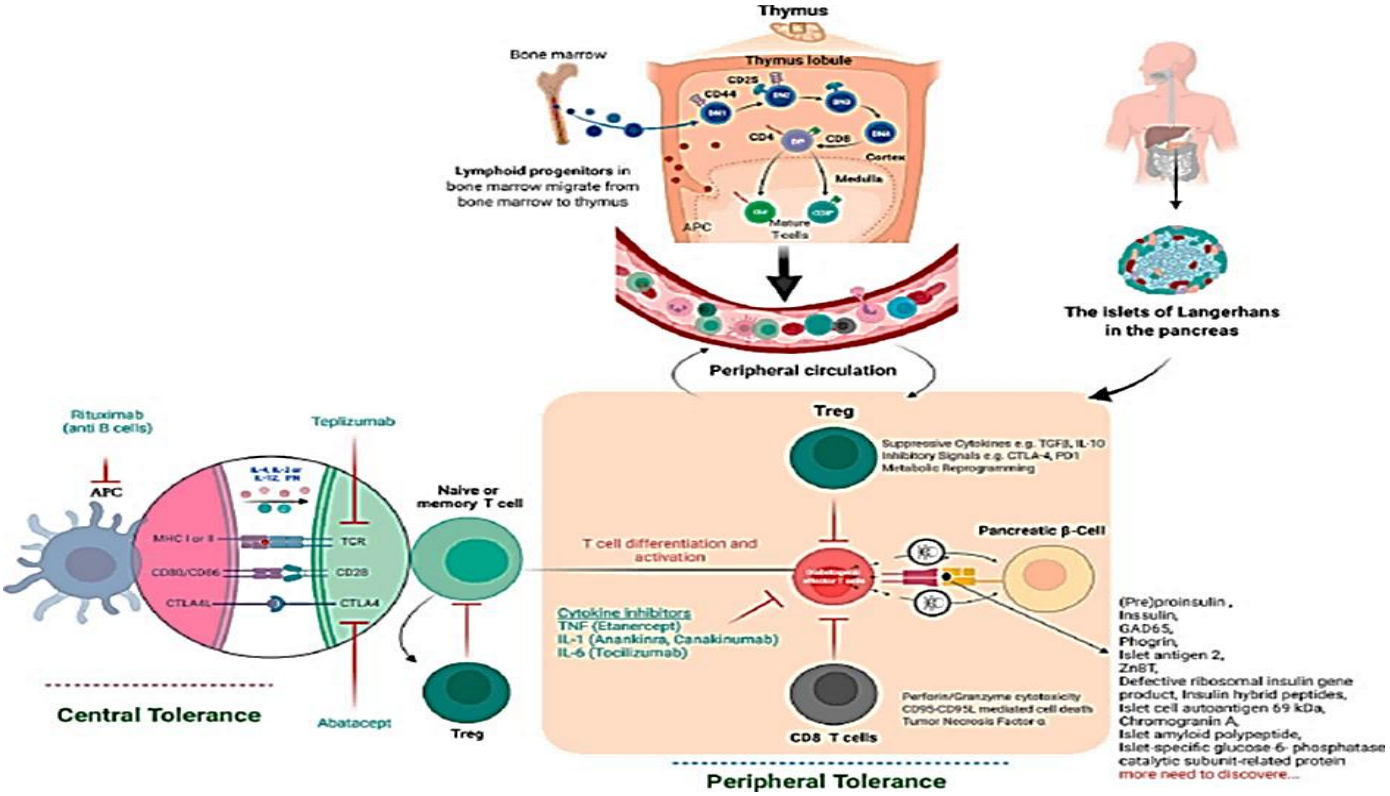

Figure 1: Pathogenesis of T1DM autoimmunity

- ***Etiology***

Table 1: Contributing factors in type 1 diabetes mellitus pathogenesis [14]

A. Genetic factors
1. HLA
2. Insulin-VNTR
3. CTLA-4
4. Other genetic associations (PTPN22, AIRE, FoxP3, STAT3)
B. Epigenetic factors
1.DNA methylation
C. Environmental factors
1. Viruses (rubella, enteroviruses)
2. Diet (cow's milk, cereals, omega-3 fatty acids, vitamin D)
3. Gut microbiota
D. Immunologic factors
1. Immune tolerance (central, peripheral, Tregs)
2. Cellular immunity
3. Humoral immunity (GAD65, IA-2, IAA, ZnT8)

➢ **Genetic factors**

T1DM is a complicated autoimmune disease with a high hereditary component. On chromosome 6, you may find the major histocompatibility complex (MHC) area, which is also called the human leucocyte antigen (HLA) region. This region houses the main genes that cause T1DM.

Immune system function relies on this area. Inheritable risk factors for T1DM include the HLA complex, which accounts for 40-50% of all such variables. Insulin (Ins-VNTR, IDDM 2) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) are two additional genes on chromosome 2 and 11 that enhance the hereditary susceptibility to T2DM, respectively. The HLA system consists of three components. Classes A, B, and C are encoded by genes in the class I area.

Additionally, HLA-DR, -DQ, and -DP are encoded in the class II region. The class III region concludes with genes encoding proteins related to the complement system and tumor necrosis factor (TNF) family. HLA-encoded class I and class II molecules bind and display polypeptide antigens so that HLA-specific T cells can recognize them. CD8+ T lymphocytes can locate and destroy the antigenic target because the majority of cells express peptide antigens linked to HLA class I molecules.

Only immune cells express the HLA class II molecules that CD4+ T cells recognize. These cells initiate the immune response and promote cellular cooperation **(Figure 2)** [13].

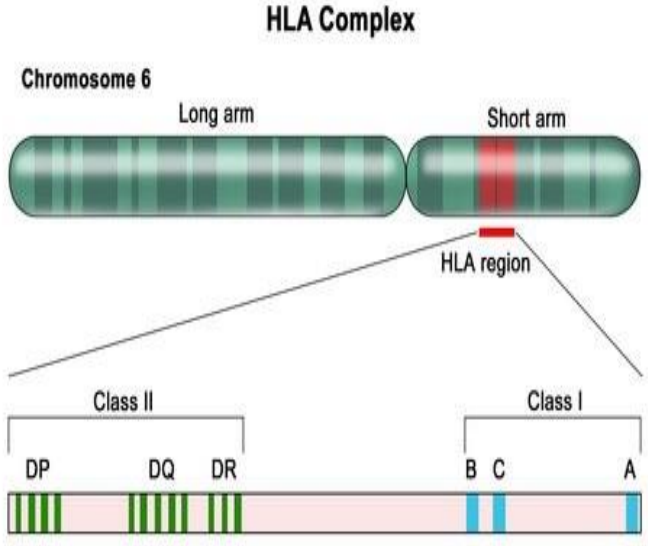

Figure 2: Human chromosome 6 with amplification of the HLA region [14]

○ **Genetic factors associated with the HLA Region**

While several studies have linked more than 60 distinct genetic loci to T1DM, the polymorphisms in the HLA region continue to have a disproportionately large role in determining susceptibility to the disease. An autoimmune reaction and the onset of T1DM are linked to two groups of genes in the HLA region. One set of genes encodes A, B, and C class I α chains, while the other set encodes DR, DQ, and DP class II α and β chains, respectively. There are two sets of genes that code for α chain. Class II HLA loci are located at the centromeric end of the short arm of chromosome 6, while Class I loci are located at the telomeric end of chromosome 6 [15].

Even though there is a lot of variation at the HLA class II locus on chromosome 6, different alleles still produce unique haplotypes. Combinations of HLA class II antigens determine whether a haplotype is protective or high-risk. Class II HLA DR-DQ haplotypes are the primary genetic risk factors for T1DM. The risk of T1DM is significantly elevated by the DR3-DQ2 and DR4-DQ8 haplotypes, which are associated with mutations in the HLA-DRB1, DQA1, and DQB1 genes. Islet cell autoimmunity is most common in those with the heterozygous DR3-DQ2/ DR4-DQ8 genotype, which increases the risk of type 1 diabetes. Some haplotypes may be more common in some populations than others because of the strong linkage disequilibrium. The high frequency of the risk-associated DR4-DQ8 and DR3 DQ2 haplotypes in the Finnish population may be one factor contributing to the high prevalence of T1DM.

Research has demonstrated that class I -A and -B alleles significantly influence both the age at which T1D begins and the rate at which the disease progresses, even when class II HLA is not considered. Since having an HLA-B allele raises the risk of T1DM, it is reasonable to assume that the HLA class I genes are crucial in controlling the demise of B cells. Children with IAA- or GADA-initiated autoimmunity may be at increased risk for developing systemic autoimmunity due to their HLA class II genotypes. Both the protective and susceptibility DR-DQ haplotype frequencies have been shifting recently. Environmental factors are becoming more important, particularly in industrialised nations, and as a result, children with T1D are more likely to have protective and mild-risk HLA haplotypes [16].

- **Genetic factors outside the HLA region**

Additional risk factors for T1D outside of the HLA area on chromosome 6 have been identified at over 40 loci. New, non-HLA genetic risk variables have been discovered thanks to recent large-scale GWAS (Genome- Wide Association Studies) [17]. The intricate interplay between HLA variation and alterations in immune cell receptor structure and function has been the subject of much research. Some variation occurs in non-HLA related parts of the genome. One of the major gene loci linked to T1D is the insulin gene (IDDM2 locus). A haplotype at the IDDM2 locus, which includes a variable number tandem repeat (VNTR) and genetic variation at the (rs689) single nucleotide polymorphism (SNP) upstream from the INS gene transcription start site (TSS), poses a risk [17].

A number of nucleotide variations in the insulin gene's promoter region have been linked to an increased risk of developing T1DM. The short repeating VNTR region inside the INS gene is significantly correlated with illness development. The INS gene also had two other loci linked to T1DM: rs698 and rs3842753. Insulin tolerance could be influenced by changes in insulin mRNA and protein expression, which could be brought about by variations in the INS gene that lead to increased insulin expression in the pancreas [18].

In autoimmune illnesses, it has been used to successfully apply a genetic risk score (GRS) to predict disease development, despite the fact that most individual polymorphisms do not significantly predict this condition. Research has demonstrated that GRS, which is the weighted average of all hazards associated with specific SNPs, can predict the rate of T1D progression and the rate of progression from islet autoimmunity to T1DM in children. There are non-HLA genetic variables that account for more than 80% of the heritability of T1DM [16].

- **Environmental factors**

T1D has been more common over the last 30 years, even though fewer people are carrying high-risk HLA haplotypes. This suggests that modern environmental factors contribute to the development of the illness through intricate gene-environment interactions [19].

- **Gut microbiota**

Several factors can influence the gut microbiome. These include the method of delivery, the timing of breastfeeding, the introduction of solid foods to the infant's diet, the usage of drugs and antibiotics, and even emotional stress. By the age of three, the makeup of a child's gut microbiome has stabilized, and from then on, it acts just like an adult's. When the immune system matures and the first T1DM autoantibodies manifest, the gut microbiome is also maturing [20].

- **Viral infections**

Most people with T1DM have enteroviruses and Coxsackie B virus in their pancreatic islets, which can trigger an immune response and speed up the disease's progression by molecularly mimicking human islet cell autoantigens, according to some researchers who believe that infectious agents contribute to the development of autoimmunity and type 1 diabetes. There have been claims that certain immunizations, such the measles shot, can reduce the likelihood of developing T1DM. The risk of T1DM is dramatically decreased in children who have received the measles vaccine. Similarly, a lower incidence of T1DM was associated with receiving a flu vaccination [21].

- **Immunologic factors**

Islet cell autoantibodies typically manifest months or even years before symptoms of T1D become apparent. Autoimmunity and B-cell dysfunction can be detected early on by these autoantibodies. Autoantibodies that target insulin (IAA), 65 kDa glutamic acid decarboxylase (GAD65), insulinoma-associated antigen 2 (IA 2A), or zinc transporter 8 (ZNT8) are characteristic of T1D. Depending on the hereditary risk linked with HLA, the quantity and quality of islet autoantibodies can differ. When it comes to autoimmunity in children, the role of the HLA class II genotype is similar in cases when IAA or GADA begins it. Seroconversion-measured IAA and IA-2A

autoantibodies are significantly linked with HLA class II DR4-DQ8 risk haplotype, insulin-coding gene polymorphisms, and PTPN22 gene polymorphisms. Upon seroconversion, GADA autoantibodies are much higher in children with the HLA-DR3-DQ2 haplotype. There is no evidence that HLA class I or II genotypes are significantly related to persistent ZnT8A formation [22].

Children with diabetes or numerous autoantibodies had higher autoantibody levels for the first year after seroconversion. Autoantibody levels are either remaining stable or decreasing after the first year of seroconversion, especially with IAA. Previous study has demonstrated that those with high GADA levels have a significant increased chance of getting various autoantibodies. Islet autoantibody levels can be used for accurate T1D prediction, according to other studies as well [17].

- **Interaction between islet cell autoantibodies and autoreactive regulatory t cells**

Autoantibodies do not influence B-cell damage on their own, even though islet cell autoantibodies are the main indicators of disease progression. T1DM occurs when the pancreas's B-cells are activated and killed by autoreactive T cells, leading to hyperglycaemia and insulin insufficiency. As with other autoimmune diseases, T1D can develop when there is a disruption in the frequencies or functions of Treg cells. More advanced stages of T1D progression may be associated with changes in subsets of Tregs. Immune-dysregulation poly-endocrinopathy enteropathy, including autoimmune enteropathy and type 1 diabetes, may result from FOXP3 dysfunction, which is essential for Treg proper function. Possible biomarkers of disease progression include changes in FOXP3 Treg profiles [23].

Insulin gene

T1DM has a complex etiology that includes both hereditary and environmental factors. While certain genes in the MHC class II family have been associated with type 1 diabetes, it is also true that genes outside of the MHC family, such the insulin gene, can increase the likelihood of getting the disease. Without a predisposition to the condition in one's family, the risk of developing T1DM increases. A very tiny percentage of patients (about 10-15%) have a close relative with the disease. Despite only affecting 0.4% of the population overall, T1DM significantly increases the lifetime risk of getting the condition in 6% of children, 5% of siblings, and 50% of monozygotic twins. Always thought of as a prime candidate susceptibility gene, the insulin gene plays a pivotal role in the aetiology of both types of diabetes[24]. Consequently, we will summarise what is currently known regarding insulin gene expression and regulation and how these factors may impact T1DM risk .

- **The insulin gene, its promoter**

A compact 1,425 bp gene situated on chromosome 11, the insulin gene (INS) in humans consists of three exons divided by two introns. The 5' untranslated sequence makes up the entire first exon of this gene, while exons 2 and 3 contain the entire coding region that encodes the 110 amino acid long proinsulin precursor protein. Specifically, signal peptidase removes the N-terminal part of the proinsulin precursor protein sequence, which allows pancreatic beta cells to secrete proinsulin. Once prohormone convertase enzymes have released the internal C-peptide, the two-chain insulin hormone is synthesized. Disulphide bridges link this hormone's A- and B-chains. For peptides with a structure comparable to insulin, the human genome has ten genes. This set of genes include three relaxins (RLN1, RLN2, and RLN3), two insulin-like growth factors (IGF1 and IGF2), and four insulin-like factors (INSL3, INSL4, INSL5, and INSL6). Genomic data provided light on the sequencing and timing of the gene duplication events that gave birth to this gene family. However, phylogenetic research is still lacking in explaining the relationships between these genes because their proteins are so small [25].

- **Insulin gene transcription factors**

New data suggests that INS transcription can happen in more than just pancreatic β-cells, challenging the traditional belief that it could only happen in these cells. Various additional tissues and cells can initiate INS transcription and produce small amounts of proinsulin or insulin. This has been demonstrated in specific cells throughout embryonic development in several organs and systems, including the thymus, peripheral lymphoid organs, central and peripheral nervous system, adrenal glands, retina, yolk sac, intestines, and cow's milk gland. Although some insulin is produced in organs other than the pancreas, the basic ability to store and release insulin in response to glucose stimulation is only present in β-cells, and most cells in the body do not contain the genes necessary to produce insulin secretases. A complex system of regulatory processes, which includes the gene's promoter and various transcription factors, enables this level of modest expression. The formation of a functional transcriptional complex occurs exclusively in pancreatic β-cells when certain transcription factors bind to specific promoter regions. Inviting more

transcription co-activators is another step in this process. Displayed here are these transcription factors together with the promoter areas where they interact.

Figure 3 include the INS promoter and transcription factors that bind to certain promoter sites, the HUMTHO1 gene, the IGF-II gene, and the IGF2 gene at the bottom. (Top) The organization of the insulin gene containing the most important polymorphism sites [26].

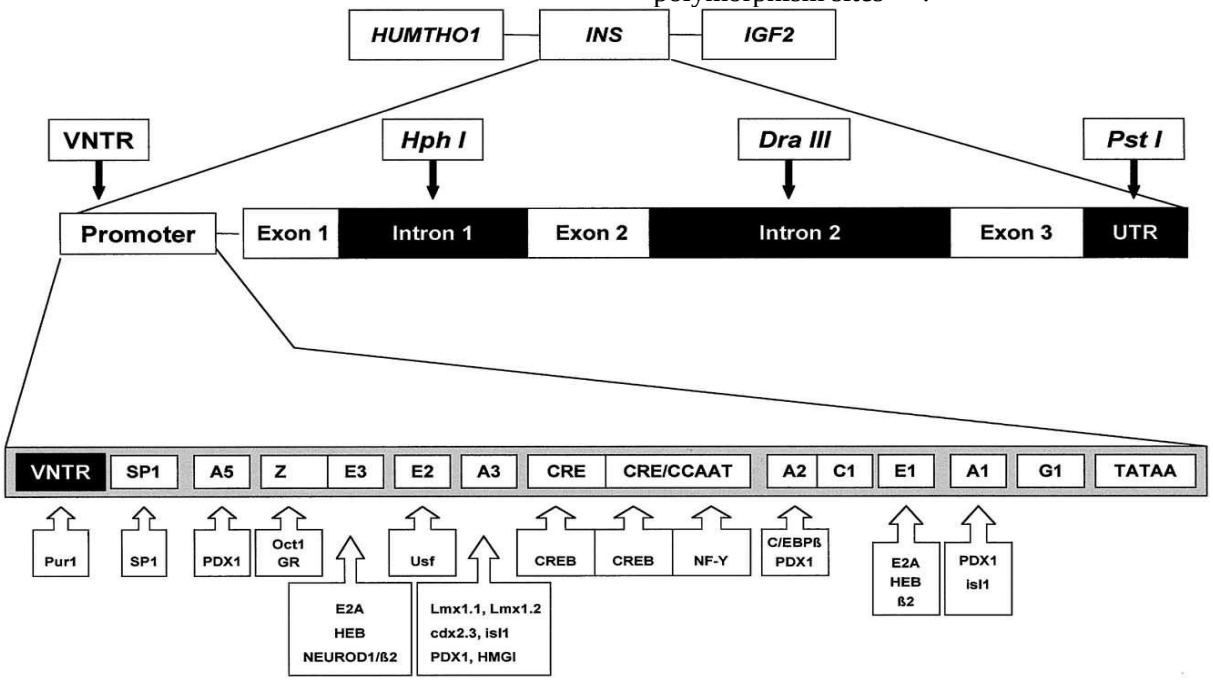

Figure 3: Schematic representation of the chromosome 11p15 region [26]

One of the significant effects of glucose is the phosphorylation, nuclear translocation, and binding of the homeodomain transcription factor PDX-1 to the A5, A3, and A1 motifs in the promoter. Enzymes E47 and NEUROD1/BETA2 form a dimer, and in the presence of glucose, their binding strength to the E elements increases. When attached to An elements, this dimer and PDX-1 promote transcription [26].

- **Insulin gene polymorphism**

Different people or groups of people can have different variations of the same DNA sequence, a phenomenon known as polymorphism. As a population, it experiences a change in its DNA sequence at a frequency of 1% or greater. Mutations that affect only one nucleotide are the most prevalent kind of polymorphism, which is also known as an SNP.

As genetic signatures in populations, SNPs allow researchers to study the susceptibility to diseases and other traits [27].

- **Insulin gene mutations**

A mutation occurs when the normally occurring DNA sequence at a specific gene locus is altered. Both hereditary mutations (germline mutations) and somatic mutations (mutations acquired during an individual's lifetime) are types of mutations. Different types of DNA mutations fall into different categories. When one nucleotide is changed, deleted, or added, it is called a point mutation. Chromosome structural changes occur alongside point mutations. Similar to mutations, polymorphisms can involve changes in one or more nucleotides. The SNP is a prime example of a common polymorphism [28].

➢ **Types of insulin gene mutations**

○ **Insulin gene mutations affecting insulin gene transcription and translation**

Mechanisms involving transcription and translation closely regulate insulin synthesis in pancreatic beta cells. In response to glucose stimulation, preproinsulin production can increase by a factor of 30 in only one hour, indicating the existence of distinct regulating mechanisms. Genomic data shed light on the sequencing and timing of the gene duplication events that gave birth to this gene family, which contains ten genes for peptides with a structure comparable to insulin, including three relaxins (RLN1, RLN2, and RLN3), two insulin-like growth factors (IGF1 and IGF2), and four insulin-like factors (INSL3, INSL4, INSL5, and INSL6) [29].

○ **Insulin gene mutations affecting proinsulin folding in the ER (Endoplasmic reticulum)**

It is anticipated that over 70% of all autosomal dominant insulin gene mutations impact the normal ER proinsulin folding pathway. Experiments have shown that approximately half of these mutants cause proinsulin misfolding in the ER. Preproinsulin-C96Y

mutation is the most studied type of this insulin gene mutation. The C96Y mutation causes beta cell death by causing proinsulin misfolding in the ER, which in turn induces ER stress, according to in vivo and in vitro studies [30].

- **Mutations affecting insulin binding to the insulin receptor**

An increased ratio of circulating insulin to C-peptide occurs when the mutant insulins secreted into the bloodstream have a longer half-life due to a defect in their binding to insulin receptors. This, in turn, significantly impairs insulin clearance. Insulin clearance in vivo is facilitated, in large part, by receptor-mediated uptake, which results in physiological degradation of insulin, as demonstrated here. Due to gene deletion, a changed start codon, a changed polyadenylation signal that would impact mRNA stability, and mutations in the promoter region, insulin biosynthesis is reduced in individuals with recessive mutations in the insulin gene [30].

The association between insulin gene variable number of tandem repeats (VNTR) and T1D

With a prevalence of around 10%, the INS gene is the second gene associated with type 1 diabetes. It helps the body store energy by encoding the precursor to insulin. Rather than metabolizing glucose, insulin aids storage of the fuel in the form of glycogen or fat. Insulin consists of two polypeptide chains associated by disulphide bonds; these chains are A and B. One gene, the INS gene, is responsible for producing insulin, as opposed to several genes that contribute to the production of other proteins. Humans only have one insulin gene, unlike certain species like rats and mice which have two. The INS gene secretes preproinsulin, a dormant insulin precursor, to initiate the insulin production process. By removing a signaling peptide, it is transformed into proinsulin, another inert substance. The last step in making insulin from proinsulin is to extract the C-peptide, which is responsible for binding chains A and B together [31].

Nevertheless, insulin biosynthetic network disruptions can result in certain diseases when the INS gene is mutated. Endoplasmic reticulum (ER) stress causes mutant proinsulin to be produced by the INS gene, particularly the C96Y point mutation, which in turn causes the death of β-cells and type 1 diabetes. Numerous other point mutations have also been discovered in animals and humans that cause type 1 diabetes. Varying numbers of tandem repeats (VNTRs) in the promoter region of the INS gene belong to one of three categories: I, II, or III [3].

The first VNTR contains 26–63 repeats, the second 80, and the third 140–210. The prevalence of VNTR I is high among Caucasians, but VNTR III is moderate and VNTR II is incredibly rare. T1D is more common in VNTR I homozygotes compared to VNTR III homozygotes, whereas VNTR II protects against the disease. The autoimmune regulator's effects on insulin thymic expression are determined by a change in the INS gene's promoter region. A higher chance of getting T1DM is associated with carrying the VNTR I allele. Among other things, this genotype lowers insulin and insulin precursor tolerance and blocks insulin transcription. In the thymus, the production of insulin messenger RNA is enhanced by the VNTR II allele [31].

The surrogate marker HphI T/A SNP at locus -23 (rs689) was used to identify the INS-VNTR. The A and T alleles of INS-VNTR class I and III, respectively, were identified by -23 HphI. Results from restriction fragment length polymorphism (RFLP) testing genotyped the -23 HphI T/A. People of European descent who have the A/A genotype at genomic location -23 in the INS gene are less likely to secrete insulin in response to weight increase. Because the -23T> is so common in Japan, it may explain why their insulin secretion rates are lower than those in Europe [32].

Furthermore, rs689 was linked to the age at which T1DM was first detected; being homozygous for the T1DM protective T allele delays the start of T1D by about two years. Research into the INS gene has shown that it influences the average age of diagnosis. There was a correlation between the rs689 polymorphism and autoantibodies that target insulin. Older onset T1DM cases rarely contain these autoantibodies. Homozygosity for the protective T allele at rs689 delayed diagnosis of T1DM in 100 affected sib-pairs (ASP) families studied by the T1D Genetics Consortium (T1DGC). Having the T allele at rs689 postpones the start of T1D is supported by the results [32].

The variable number of tandem repeats (VNTR) in the insulin gene is located around half a kilo base upstream of the codon. That particular polymorphic repeat is characterized by a consensus sequence of fourteen to fifteen base pairs (ACAGGGGTCTGGGG). There are three different classes that it can belong to: class I, which is small and found in approximately 70% of Caucasians but in more than 90% of Japanese; class II, which is intermediate and unusual; and class III, which is huge and found in approximately 30% of Caucasians. A gene called the insulin VNTR, which is also called the IDDM2 susceptibility locus, is associated with type 1 diabetes. Almost all insulin-producing genes are silenced in pancreatic b-cells [33].

An exception to this rule is the thymus. New research suggests that the thymus plays a key role in the development of self-tolerance or negative selection through the expression of self-antigens. This mechanism may also be responsible for the expression of genes for self-molecules like insulin. It was found that the VNTR allele correlates with insulin mRNA levels in the thymus. The thymus transcribes class III VNTR alleles at far higher quantities than class I alleles. Whether autoreactive T-lymphocytes are negatively selected for or self-tolerance develops depends on the

insulin gene VNTR allele, which may affect thymic production of self-antigens. Insulin tolerance could thus be regulated by this gene. The surrogate marker Hph1 T/A SNP at locus -23 (rs689) was used to identify the INS-VNTR. Restrictive fragment length polymorphism (RFLP) analysis was used to genotype the -23 HphI T/A(Figure 4) [34].

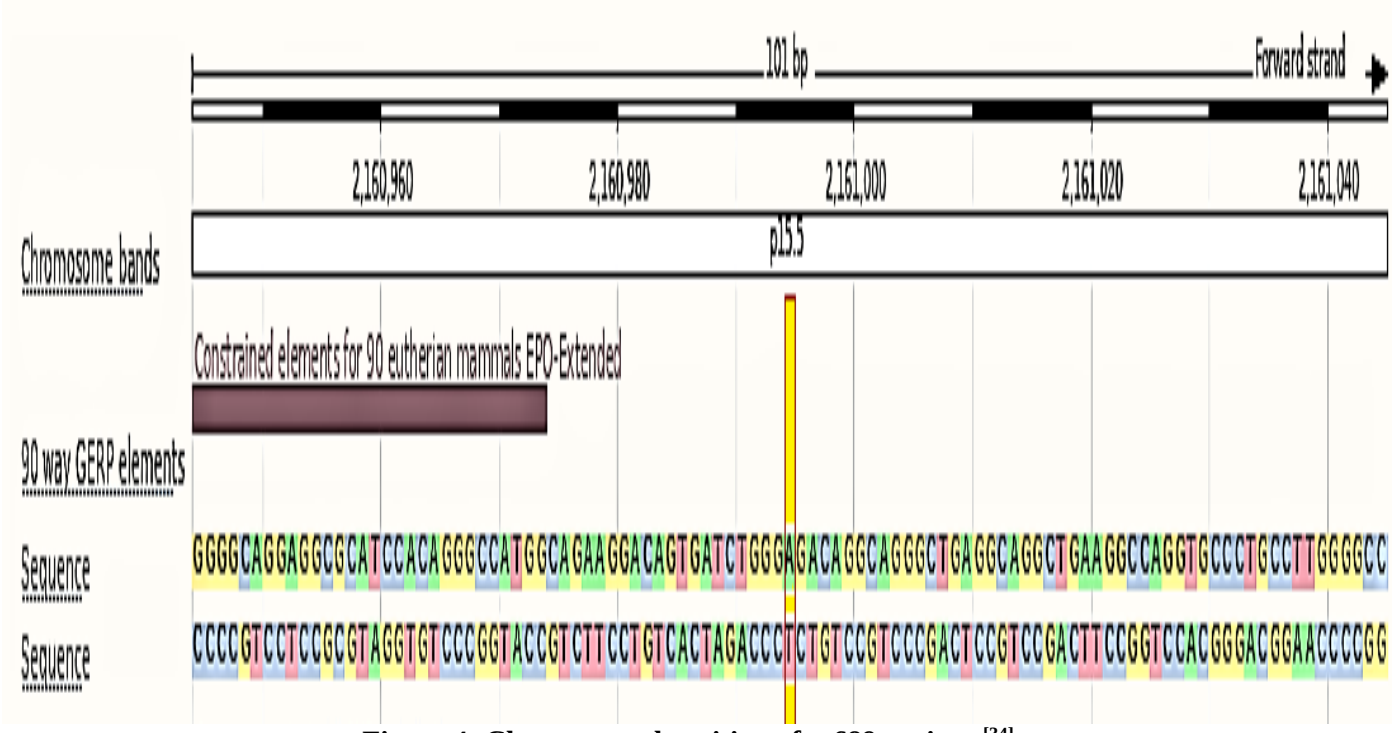

Figure 4: Chromosomal position of rs689 variant [34]

There are two major types of VNTR alleles, and the rs689 SNP is a good surrogate for both. The presence or absence of the INS-VNTR Class I and III alleles were identified using the -23 Hph1 A and T alleles, respectively. Not only does rs689 link T1DM to metabolic syndrome, but it has also been linked to type 2 diabetes and a less common form of diabetes called latent autoimmune diabetes of adulthood (LADA) [35].

CONCLUSION

There is evidence that INS VNTR increases the likelihood of acquiring T1DM. The age of onset of T1DM is correlated with INS VNTR specific susceptibility, according to another discovery. The autoimmune destruction of pancreatic β cells is the main cause of T1DM, and it is thought that autoimmune disorders, which result from not being able to control one's immune system, are connected to the role of T1DM susceptibility genes. Despite not encoding any protein, the INS VNTR is believed to regulate insulin gene transcription due to its proximity to the insulin gene promoter. People whose INS VNTR is more common in younger generations may be more likely to develop T1D than those whose condition shows up later in life.

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