Association of Effect of Insulin Gene Polymorphism VNTR INS -23/Hph1 (rs689) in Egyptian Children with Type 1 Diabetes Mellitus.

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ABSTRACT
Type 1 diabetes mellitus (T1D) is considered as one of the most frequent chronic illnesses in childhood. It results from insulin deficiency due to autoimmune destruction of pancreatic beta cells. The prevalence of T1D in the overall population is 0.4%. People with a 1st -degree relative diagnosed with T1DM possess an around fifteen-fold elevated relative lifetime probability of developing T1D themselves. Noneth eless, a minimum of eighty-five percent of kids diagnosed with T1D lack a familial history of the condition. Polymorphisms in noncoding region of insulin gene impact susceptibility to or protection from T1D. This locus encompasses a VNTR, additionally referred to as the insulin gene mini-satellite, situated at 5' terminus of insulin gene. The VNTR region consists of variable tandem repeat sequences of 14-15 base pairs, with the consensus sequence 5'-ACAGGGGTGTGGGG-3'. 3 primary classes of VNTRs are categorized by their size: class I (twenty-six to sixty-three repeats), class II (around eighty repeats), & class three (140–200 repeats). The insulin gene -23/Hph1 A more than T (rs689) single-nucleotide polymorphism (SNP), a variant within human insulin gene, demonstrates a strong association disequilibrium with variable nucleotide tandem repeat alleles; A allele is associated with the short (class I) VNTR, while T allele is associated with long (class three) variable nucleotide tandem repeat allele.

Introduction

Diabetes mellitus defined as an important global health problem. Diabetes mellitus frequency increases quickly across all age groups (1).

In 2021, the IDF calculated that approximately 108,300 kids & adolescents under fifteen years were newly diagnosed with T1D, while around 651,700 kids & adolescents were living with the disease globally (2).

The frequency of childhood T1D remains significantly affected by geography, with rates varying between 1.9 and 2.2 per 100,000 individual years within Japan & China, correspondingly, and 52.2 per 100,000 in Finland, wherever the greatest frequency was recorded for many years. The non-European people of Qatar, Kuwait, Algeria, & Saudi Arabia are included in 4 of the top ten nations with the greatest frequency of childhood T1D, as stated in the most recent edition of the International Diabetes Federation (3).

Genetic predisposition & environmental factors are believed to interact to identify the progression of illness, as the pattern of inheritance is complicated. In monozygous twins with ongoing monitoring, the concordance rate for T1D is greater than fifty percent, whereas it is six to ten percent in dizygous twins.

This is comparable to the rate observed in non-twin siblings (4).

The association between T1D & HLA region on chromosome 6p21 has been recognized for more than forty years, & it is responsible for around thirty to fifty percent of the familial aggregation of T1DM (5).

The remaining genetic risk for T1DM may be explained by the other non- human leukocyte antigen genes or loci, which contribute smaller impacts to the illness's probability. Over sixty risk loci have been determined through GWAS. The insulin gene (INS) on chromosome 11p15, non-receptor type twenty-two (PTPN22), Interleukin two Receptor Subunit Alpha genes, protein tyrosine phosphatase, & cytotoxic T-lymphocyte associated protein (CTLA-4) are all included in, or play a role in, immune regulation in pancreatic beta-cell and/or numerous immune cell populations. Insulin gene on chromosome 11p15 is the most significant non-HLA genetic contribution (6).

The literature demonstrates a lack of consistency in the correlation between the A & T alleles and the possibility of developing T1D. The T allele is believed to be protective, while the A allele has been suggested to be correlated with an elevated probability of developing diabetes in certain studies (7)(8)(9)(10)(11).

However, other documents suggest which the T allele is the one correlated to probable susceptibility to T1D (12)(13). Conversely, some deny that either allele has an impact on the probability of developing T1D (14)(15).

The goal of this research was to assess if there is a relationship among insulin gene polymorphism & progress of T1D trying for preventing it.

T1D

Introduction:

T1D is a chronic autoimmune disorder noticeable by increased blood glucose concentrations leading to deficiencies in insulin caused by pancreatic islet beta-cells destruction . (16)

Epidemiology:

T1DM prevalence is around 0.2–0.5% in industrialized countries (17).

Around 96,000 kids under the age of fifteen are known to acquired T1D yearly on globally. In the majority of western nations, T1D is responsible for more than ninety percent of childhood diabetes; however, additional types of diabetes, for example T2DM & monogenic diabetes, additionally present (18).

For example, Northern Europe, Finland, & Canada show the highest frequency probability (19). The illness frequency between Caucasians residing in Europe is approximately twenty times higher, & the incidence of HLA susceptibility genes in the general people is associated with the frequency rates.

The cause of this variation is not yet entirely understood; however, it can be associated with genetic susceptibility, environmental factors, & lifestyle choices, such as hygiene & childhood infections. (20)

Pathogenesis:

T1DM is defined by the chronic immune-mediated destruction of pancreatic beta -cells, which results in a partial or, in the majority of cases, an absolute deficiency of insulin. The most of cases (type 1A) are the outcome of autoimmune-mediated pancreatic beta-cell destruction, that happens at a variable rate & develops into clinically symptomatic if around ninety percent of pancreatic beta-cells are destroyed. (21)

Etiology:

The cause of T1D is multifactorial, likely involving a combination of genetic & environmental variables that initiate or facilitate the autoimmune response against beta-cells. This occurrence frequently occurs years prior to the eventual onset of dysglycaemia (22)

Genetic Factors:

T1D is a multifaceted autoimmune disorder & distinguished by an established genetic component. The primary genes that contribute to T1D are situated in the major histocompatibility complex (MHC) region, that is frequently mentioned to as the HLA (human leucocyte antigen) region & is situated on chromosome 6. This region is essential for the proper functioning of immune system. The genetic risk of T1D progression is accounted for by forty to fifty percent of the HLA complex polymorphic alleles. The CTLA-4 on chromosome two & the insulin gene (Ins-VNTR, IDDM 2) polymorphisms on chromosome eleven contribute to fifteen percent of the genetic predisposition. (23)

Three regions comprise the HLA system. The genes that encode molecules HLA-A, -C, & -B are located at class one region. Furthermore, the class two region encodes HLA-DR, -DP, & -DQ. Lastly, the class three region is characterized by the presence of genes that encode proteins from the complement system & Tumor Necrosis Factor family. Purpose of human leukocyte antigen -encoded class two & class one molecules is to bind peptide antigens & present them for identification by antigen-specific T lymphocytes. Cytotoxic T lymphocytes identify peptide antigens correlated with human leukocyte antigen class one molecules, that subsequently destroy the antigenic target. These antigens are created by the majority of cells. Although HLA class two molecules are exclusively expressed by immune cells, they are detected by CD4+ T cells, that initiate the immune response & stimulate cellular cooperation. (24)

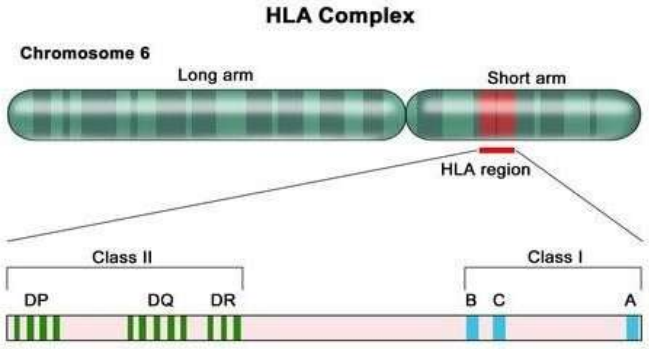

Figure (1): Human chromosome 6 with amplification of the HLA region (25)

Genetic Factors Correlated with the HLA Region:

While numerous investigations have correlated T1D to more than sixty individual genetic loci, polymorphisms of human leukocyte antigen region continue to be the most important contributors to genetic susceptibility to T1DM. Human leukocyte antigen region genes that were discovered to be correlated with development of to T1D & responsible for in autoimmune response may be categorized into two groups: 3 genes which encode class one alfa chain antigens (C, A, & B) & 3 gene pairs of class two beta & alfa chains (DQ, DR, & DP). the telomeric end is the location of highly polymorphic class I loci .Whereas the HLA class two loci are situated at the centromeric end of the short arm of chromosome six. (5)

The genotype DR3-DQ2/DR4-DQ8 is heterozygous & is correlated with the greatest probability of development from islet cell autoimmunity to clinical T1DM. Some haplotypes are inherited more frequently compared to others & may be excessive represented in particular people as a result of the powerful LD. The high incidence rate of T1D in Finland is partially attributed to the prevalence of the risk-correlated haplotypes DR4-DQ8 & DR3 DQ2. (26)

Genetic Factors outside the Human Leukocyte Antigen Region:

Over forty loci located outside human leukocyte antigen region of chromosome six have been determined to be modifiers of clinical T1DM possibility. Detection of new non- human leukocyte antigen genetic risk factors has been made available by the large-scale GWAS that have been conducted lately. (27)

The complex relationship among alterations to immune cell receptor function & structure & HLA variation was the subject of extensive research. Non-HLA regions are the locations of the remaining genetic variation. Insulin gene (IDDM2 locus) is considered the 2nd most significant gene locus in T1DM. The possibility at IDDM2 locus is the result of a haplotype that includes a VNTR & genetic variation at the (rs689) single-nucleotide polymorphism, the two of which are situated upstream from the TSS of the INS gene. (28)

Gut microbiota:

The advanced decrease of function of B-cell in T1D may be attributed to a variety of environmental factors, involving those that are primarily correlated with alterations to the gut microbiome. Gut microbiome is a critical modulator of type 1 diabetes mellitus, although its exact function within development of autoimmunity remains uncertain. Gut microbiome of diabetic cases was the subject of awareness over the past decade due to functional alterations, microbial contact, & the living environment. (29)

The development of islet autoimmunity is preceded by alterations within taxonomic structure of gut microbiome. Reduced diversity of gut microbes in T1D is a consequence of these taxonomic alterations to the gut microbiome composition. In comparison to people in good health, kids who are positive for a minimum one islet cell autoantibody as well as that subsequently advance to T1D throughout the monitoring have a lesser Shannon diversity index & a greater Bacteroidetes/Firmicutes ratio of gut microbiome. (30)

Viral Infections:

Autoimmunity & the pathogenesis of T1D can be influenced by a variety of pathogens, particularly viruses. Coxsackie B virus & enteroviruses, that are existing in pancreatic islets of the majority of T1D cases, have the potential to accelerate the development of the illness by activating the immune system, due to molecular mimicry of human islet cell autoantigens (31).

SARS-CoV-2 infections & prevalence of T1D in kids, in addition to the development to diabetic ketoacidosis, have been the subject of many investigations throughout the COVID-19 pandemic of 2020. The virus was observed to have caused damage to insulin-producing cells. (26)

Immunologic Factors:

T1D is correlated with islet cell autoantibodies development that manifest months or years prior to the onset of symptoms. These autoantibodies function as the initial biomarkers of the progression of autoimmunity & B-cell dysfunction. T1D is correlated with specific autoantibodies that target insulin (IAA), zinc transporter 8 (ZNT8), sixty-five kDa glutamic a` decarboxylase (GAD65), or insulinoma-correlated antigen two (IA 2A) (32)

The HLA DR3 DR4 DQ2 DQ8 haplotypes are typically the 1st to show these autoantibodies in kids. IAA & GAD are the two most prevalent autoantibodies in childhood, while IA-2A & ZnT8 autoantibodies typically are not seen as the initial manifestation. Nevertheless, they are all prevalent at the time of the disease's diagnosis. An elevated frequency of islet autoantibodies' presence throughout the 1st two years of life is suggested by information collected from longitudinal investigations which have monitored people from childbirth to the initial manifestations of clinical . (33)

islet autoantibodies amount & level are both influenced by the HLA-related genetic risk. autoimmunity expansion in kids with insulin autoantibodies & glutamate decarboxylase autoantibodies -initiated autoimmunity is significantly influenced by human leukocyte antigen class two genotype.

HLA class two DR4-DQ8 risk haplotype & insulin-coding polymorphisms & non-receptor type twenty-two genes are significantly correlated with IAA & IA-2A autoantibodies detected at seroconversion. Glutamate decarboxylase autoantibodies autoantibodies are significantly more prevalent during seroconversion in kids with the HLA-DR3-DQ2 haplotype. There were no documented significant correlation among persistent ZnT8A progress & human leukocyte antigen class two & one genotypes. (34)

Interaction between islet cell autoantibodies & autoreactive Treg cells:

While the 1ry indicator for development of an illness is islet cell autoantibodies., damage to B-cells is unaffected by autoantibodies alone. T1D is the outcome of activation & destruction of B-cells in the pancreas by autoreactive T cells, which leads to hyperglycemia & insulin insufficiency. (35)

In cases with type 1 diabetes, deficiencies in immunosuppressive regulatory T cells are the cause of unregulated activation & expansion of autoreactive helper T cells or cytotoxic T cells. According to the most recent findings, regulatory T cells are a very specific subpopulation of T cells that are responsible for keeping homeostasis & self-tolerance by inhibiting immune response & activating autoreactive cells. (35)

The primary mediators that are believed to have an impact on pathogenesis of T1D are autoreactive T cells. In clinical studies, T-cell subsets may serve as biomarkers for therapy effectiveness. The number of T helper cells is elevated both prior to & during the diagnosis of T1D, & they can act as biomarkers for predicting the illness. (34)

The progression of autoimmune illnesses, involved T1D, can be facilitated by dysregulation in the frequencies or functions of Treg cells. Alterations in Treg subsets may be associated with the development of T1D to more advanced stages. FOXP3 is important for the correct functioning of Tregs, & their dysfunction may result in immune-dysregulation poly-endocrinopathy enteropathy, involving autoimmune enteropathy & T1D. Changes in the profiles of FOXP3 Tregs may function as a possible indicator for the advancement of the illness. (36)

Insulin hormone

Insulin defined as a pancreatic hormone that is released by beta-cells. Insulin is composed of twenty-one aa` residues in "A" chain & thirty amino acid residues in "B" chain, that are associated by disulfide bonds. Preproinsulin is synthesized & subsequently converted to proinsulin. Proinsulin is subsequently converted to insulin & C-peptide, which are stored in secretary granules & released upon consumption. (37)

Insulin Hormone Biosynthesis:

The synthesis of mature bioactive insulin in pancreatic beta cells necessitates 3 significant steps that last between thirty & 150 minutes: A process that results in the formation of proinsulin involves the translation of preproinsulin, insulin precursor, in cytoplasm, followed by its translocation across the ER membrane & its subsequent proteolytic processing by signal peptidase on luminal side of

endoplasmic reticulum membrane. Proinsulin experiences oxidative folding in the oxidizing ER environment, resulting in the formation of 3 evolutionarily conserved disulfide bonds (A6-A11, B19-A20, & B7-A7). These bonds enable proinsulin to achieve transport-competence for exit from endoplasmic reticulum. Intracellular trafficking of proinsulin via Golgi apparatus to secretory granules enables the proteolytic processing of proinsulin by prohormone convertases (PC2 & PC1/3) & carboxypeptidase E for producing mature insulin & C-peptide. These products are produced on stimulation & stored in insulin secretory granules. (38). Synthesis of insulin is controlled at translational & transcriptional levels. A series of events in Beta-cells that culminate in the fusion of secretory granules with plasma membrane are responsible for insulin secretion. Glucose is the primary stimulus for insulin secretion, but other nutrients, including free fatty a` & aa`, may improve glucose-induced insulin secretion (39).

Insulin Mechanism of Action:

Insulin's action is started by its binding to receptor of glycoprotein located on cell's surface. Receptor is consisted of an alpha-subunit which binds a beta-subunit & hormone that is an tyrosine-specific protein kinase, insulin-stimulated. It is hypothesized that the activation of this kinase will produce a signal that will ultimately lead to insulin's activity on protein, lipid, & glucose metabolism. It seems that the growth-promoting properties of insulin are achieved by activating receptors for the family of correlated insulin-like growth factors. (40)

Physiological Role of Insulin:

Insulin is the 1ry hormone responsible for the regulation of cellular energy. It is necessary for intracellular transportation of glucose into insulin-dependent tissues, involving adipose tissue & muscle. Adipose tissue fat breakdown is inhibited & its synthesis is stimulated by the signaling abundance of exogenous energy. Glucose entry in muscle cells facilitates the synthesis & storage of glycogen, in addition to the utilization of carbohydrates as the immediate energy source for muscle contraction, instead of fatty a` (or aa`). Consequently, insulin stimulates glycogen synthesis & lipids in muscle cells, while simultaneously inhibiting gluconeogenesis synthesis & lipolysis from muscle amino acids. Insulin is anabolic in muscle if an adequate amount of amino acids is current (41).

Insulin gene

The pathogenesis of T1D is multifactorial, with genetic predisposition & environmental factors contributing to its development. The MHC class two genes are recognized for their correlated with T1D; however, there are additionally non-MHC genes, including the insulin gene, that increase susceptibility to T1DM. (42)

The insulin gene has consistently been regarded as a suitable susceptibility gene due to its central role in the pathogenesis of both forms of diabetes. Consequently, we will examine the present level of knowledge regarding regulation & expression of the insulin gene & the potential impact on susceptibility to T1D.

The insulin gene, its promoter:

The human insulin gene is a small gene, spanning 1,425 base pairs, that is situated on chromosome eleven. It is comprised of three exons that are separated by 2 introns. Coding region of this gene, that encodes 110 aa` long proinsulin precursor protein, is distributed across exons two & three, with 1st exon consisting entirely of 5' untranslated sequence. A signal peptide is found in N-terminal part of the sequence of proinsulin precursor protein. This peptide is responsible for enabling secretion from pancreatic beta-cells. This peptide is removed by signal peptidase, which then converts it into proinsulin. (42)

Information derived from the locations of these genes in the genome has significantly resolved the sequence & timing of the gene duplication events that resulted in the formation of this gene family. This is despite the fact that phylogenetic analysis is incapable to provide a comprehensive explanation for the relationships that exist among these genes due to the short protein length characteristics. (43)

Insulin gene transcription factors:

It was previously believed that INS transcription was only limited to pancreatic beta-cells. However, mounting proof is discovered which a variety of additional tissues & cells are capable of actively transcribed human insulin gene & the production of few concentrations of proinsulin/insulin. This was observed in peripheral & CNS of fetus, as well as in retina, adrenal gland, gut, mammary gland, yolk sac, & particular cells that were discovered in thymus & peripheral lymphoid organs (44).

Figure (4) illustrates the interaction between these transcription factors & specific regions of promoter. The top panel shows the genes coding for thyrosine hydroxylase (HUMTHO1), INS, & insulin-like growth factor 2, while middle panel illustrates insulin gene structure with the most important polymorphic loci (variable nucleotide tandem repeat). Bottom panel shows the human insulin gene promoter & transcription factors that associate with particular promoter elements.

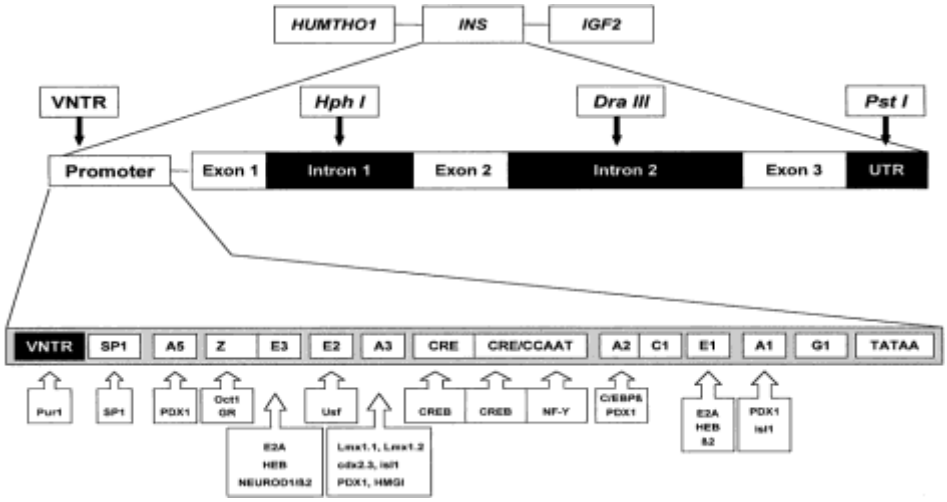

Figure (2): Schematic representation of the chromosome 11p15 region (45)

Insulin gene polymorphism:

Polymorphism is the existence of two or more variant forms of a specific deoxyribonucleic a` sequence that may be found in different individuals or people. It is a deoxyribonucleic a` sequence variation that is present in people at an incidence of one percent or greater. Variation at a single nucleotide (similarly defined as single-nucleotide polymorphism, or a SNP) is the most prevalent form of polymorphism. single-nucleotide polymorphism are utilized as genetic signatures in populations to investigate the predisposition to specific traits, such as illnesses. (46)

The INS variable number of tandem repeats & T1D:

The variable nucleotide tandem repeat of INS is situated around 0.5 kb upstream of insulin gene. This polymorphic repeat is categorized as class I (small, frequency around seventy percent in Caucasians, however above ninety percent within Japanese), class two (rare, intermediate), or class three (large, frequency around thirty percent in Caucasians). It is composed of a fifteen to fifty bp unit of consensus sequence (ACAGGGGTCTGGGG). The insulin VNTR, which is also known as the IDDM2 susceptibility locus, was discovered to be correlated with T1D. There are only a small number of exceptions to the severely limited insulin gene expression in pancreatic b-cells. (10)

Class 3 variable nucleotide tandem repeat alleles exhibit markedly higher transcription concentrations in thymus relative to class one alleles. The thymic expression of self-antigens & their concentrations affect the development of self-tolerance or negative selection of autoreactive T-lymphocytes; consequently, the INS variable nucleotide tandem repeat allele may modulate tolerance to insulin by influencing its expression in thymus.. (47)

The INS-VNTR has been recognized by its surrogate indicator Hph1 T/A SNP at locus -23 (rs689). The -23 HphI T/A has been genotyped by restriction fragment length polymorphism (RFLP) analysis (48)

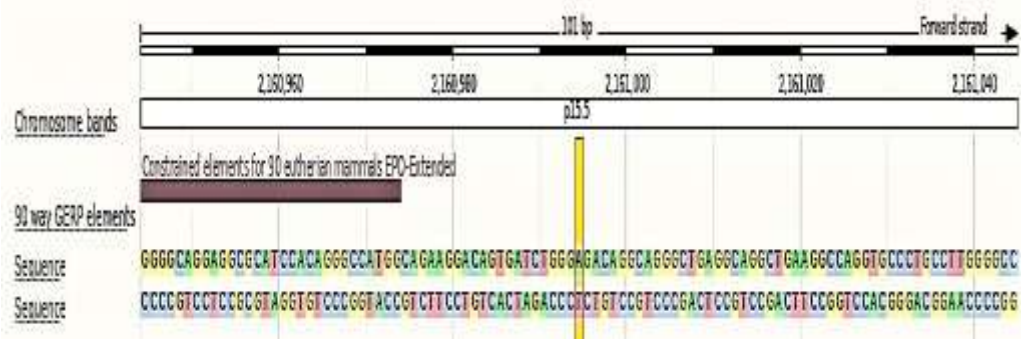

Figure (3): chromosomal position of rs689 variant (48)

Insulin gene mutations:

A mutation refers to an alteration in the standard deoxyribonucleic a` sequence at a specific gene locus. Mutations may be inherited from parents (germline mutations) or acquired throughout an individual's lifetime (somatic mutations). There exist several principal categories of deoxyribonucleic

a` mutations. A point mutation transpires if just one nucleotide is added, removed, or substituted. In addition to point mutations, all of the components of a chromosome may be modified. Polymorphisms may consist of one or multiple nucleotide alterations, similar to mutations. The SNP exemplifies the most frequent polymorphism.(49)

Types of insulin gene mutations:

INS mutations impacting insulin gene translation & transcription:

Insulin biosynthesis within pancreatic beta-cells is meticulously controlled at transcriptional & translational concentrations. Following glucose stimulation, preproinsulin biosynthesis may increase by as many as thirty-fold within one hour, signifying specific regulatory mechanisms. (50)

Pathological & physiological significance of regulatory component in untranslated region of human insulin gene is genetically evidenced by recently identified mutations in the insulin gene that influence its transcription & translation. (51)

Five of these mutations are found in region of insulin promoter, & they may result in the deletion of promoter region that is controlled by MAFA & NEUROD1 or in the disruption of binding sites for supplementary deoxyribonucleic a` binding proteins. These mutations are responsible for a ninety percent decrease in promoter activity (38).

Insulin gene mutations impacting proinsulin folding in ER:

Over seventy percent of autosomal dominant insulin gene mutations are expected to impact on proinsulin normal folding pathway in endoplasmic reticulum. Experimentally, approximately fifty percent of these mutants were validated to induce proinsulin misfolding in endoplasmic reticulum. (52)

Preproinsulin-C96Y mutation is the most extensively researched INS mutation of this type. In vitro & in vivo investigations indicate that the C96Y mutation results in proinsulin misfolding in endoplasmic reticulum, causes endoplasmic reticulum stress, & eventually results in beta cell apoptosis (53).

Mutations impacting insulin binding to the insulin receptor:

The defective binding of released mutant insulins to insulin receptors significantly impairs insulin clearance, leading to a longer half-life of mutant insulins in circulation & a higher ratio of circulating insulin to C-peptide. Specifically, the results offer conclusive proof that the primary mechanism of insulin clearance in vivo is receptor-mediated uptake, which ultimately leads to insulin physiological degradation (54).

Insulin gene recessive mutations caused decreased insulin biosynthesis via several mechanisms, involving altered start codon, gene deletion, modified polyadenylation signal affecting mRNA stability, & promoter mutations (55)

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