REVIEW ARTICLE

Diabetes: discovery of insulin, genetic, epigenetic and viral infection mediated regulation

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Abstract

Diabetes mellitus, commonly referred to as diabetes, is a combination of many metabolic diseases. Insulin deficiency in our body is the main cause of diabetes. Insulin is one of the most well studied proteins, yet the genesis of its discovery was not getting much attention so far. Nevertheless, the history of the discovery of insulin is an exemplary of solving observational and scientific riddles, drudgery, patience and even professional turmoil. It is an inspiration for all medical personnel and scientists who are practising in the field of molecular medicine. Additionally, the genetic and epigenetic regulation of different types of diabetes needs to be addressed because of the widespread nature of the disease. Diabetes not only involves genetic predisposition but environmental factors, lifestyle etc. can be the major contributor for its inception. Nonetheless, viral infections at an early age are also found to trigger the onset of type I diabetes. In this review article, the history of the discovery of insulin is detailed along with the justification for the genetic and epigenetic regulatory mechanisms of diabetes and explained how viral infections can also trigger the onset of diabetes.

Keywords Autoimmunity · Diabetes · Enterovirus · Histone · Hyperglycemia · Insulin · Methylation

Abbreviations

ACE2	Angiotensin converting enzyme 2
ADH	Antidiuretic hormone
AIRE	Autoimmune regulator
APS1	Autoimmune polyglandular syndrome type 1
BACH2	Basic leucine zipper transcription factor 2
CGM	Continuous Glucose Monitoring System
CVB	Coxsackievirus B
DNMT	DNA methyl-transferases
DI	Diabetes insipidus
EGWAS	Epigenome-wide association study
ER	Endoplasmic reticulum
EV	Enteroviral
GLIS3	Gli similar 3 protein
GLUT4	Glucose transporter type 4
GWAS	Genome wide association studies
HAT	Histone-acetyltransferases
Hb	Haemoglobin
HDAC	Histone-deacetylases
HLA-I	Human leukocyte antigen-I
IFIH1	Interferon induced helicase 1;
IL2RA	Interleukin-2 receptor subunit alpha
INS	Insulin gene
IRS-1	Insulin receptor substrate 1
IUGR	Intrauterine growth retardation
LSD1	Lysine specific demethylase-1
LYP	Lymphoid specific phosphatase
MDA5	Melanoma differentiation-associated protein 5
MEF2	Myocyte enhancing factor 2
MHC	Major histocompatibility complex
MET	Metformin
miRNA	Micro-RNA
MxA	Myxovirus resistance protein
MyoD	Myoblast determination protein;
NGS	Next generation sequencing
NK	Natural killer
OCT	Organic cation transporter family
PI3K	Phosphoinositide 3-kinase

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PTPN22	Protein tyrosine phosphatase non-receptor type 22
RNase, RIG-I	Retinoic acid inducible gene-I
RNA	Polymerase
SARS-COV-2	Severe acute respiratory syndrome-coronavirus-2;
SUF	Sulfonylureas
T1D	Type I diabetes
T2D	Type II diabetes
TDG	Thymine DNA glycosylase
TEDDY	The environmental determinants of diabetes in the young
TET	Ten-eleven translocation
TZD	Thiazolidinediones
UBASH3A	Ubiquitin associated SH3 domain containing protein A
UPR	Unfolding protein response
USF1	Upstream transcription factor 1
VNTR	Variable number tandem repeats

Introduction

Looking at the past, it is very easy to understand that since the beginning of creation, man would have liked to make his life continually easier. Perhaps the emergence of science is the result of different arguments and thoughts and consciousness in the pursuit of that desire. In the present age, science has given us the gift of internet, smart phones, e-mail, and many more conveniences. Just as it is meaningless to live in this age without use of many of these things, similarly, it is difficult for a diabetic individual to live a simple and beautiful life without the wonderful invention of the twentieth century, "Insulin".

Diabetes mellitus, commonly referred to as diabetes, is not a disease confined to a definitive boundary, but a combination of many metabolic diseases. Insulin deficiency in our body is the main cause of diabetes. Insulin is secreted from the pancreas to help the cells of the body to take up glucose (sugar) from the blood, which normal cells can utilize to generate energy. The failure of the pancreas to generate enough insulin or the body's inability to use insulin properly, either of these or both in combinations can cause diabetes. The disease not only increases the amount of glucose level in the blood (hyperglycemia) but also abnormalities occur in the metabolism of proteins and fats [51]. Prolonged uncontrolled hyperglycemia initially causes changes in blood vessels and abnormal blood flow. As a result, different cells in the body undergo various changes; several complications arise in the body and the advancement of the diabetic condition continues. It should be noted here that the effects of this deadly disease are not limited to any particular organ; once established, it damages the function of most important organs of the body and without proper treatment those organs can become permanently paralyzed [50]. Notable among these are heart disease, stroke, diabetic retinopathy, kidney failure and anaemia [20, 21]. This review briefly describes the various harmful aspects of diabetes, the ground breaking discovery of insulin, the role of insulin in the treatment of diabetes, the genetic and epigenetic aspects of the disease and the role of viral infections in triggering its onset.

History of the discovery of insulin

During the early years of reason, physicians would describe diseases in their own way and treat patients based on their knowledge of the surrounding ecology. Day after day, an almost sugar-free diet, sometimes 450 cal or less per day (although more calories are needed to survive) in many cases pushed diabetic patients to death [37]. The discovery of insulin has undoubtedly put an end to thousands of years of extreme frustration and failure. Diabetes is one of the longest studied diseases in medical science. Probably, this disease was first mentioned in ancient Egyptian medicine since 1552 BC [120]. The word 'diabetes' was probably used first by the Greek physician Demetrios, meaning "siphon." Much later, in 1674, the British physician Thomas Wills named the disease "diabetes mellitus" to distinguish it from "diabetes insipidus" [82]. In 1776, Matthew Dobson, curious to see why some ants are being attracted to urine, turned his attention to this mystery. He later discovered that diabetic patients had high levels of sugar in their urine, so it tasted sweet, known as glycosuria [77].

During the period known as the "Pre-Insulin Age", or the "Age of Failure, and Depression," a list of the strangest foods was recommended to control the disease. Sugar-free diets, carbohydrate-controlled low-fat diets and starvation diets were particularly notable among the recommended food lists. However, no such treatment or diet has actually been of any use in curing diabetes [123].

Challenging path of insulin discovery

During the period between 335 and 280 BC, the Greek anatomist and surgeon Herophilus was the first to identify the pancreas gland in the human body, although it was named much later [120]. The endocrine gland was discovered by the German physician Paul Langerhans in 1869, which was later renamed as 'Isles of Langerhans', meaning the island of Langerhans. Langerhans first proposed presence of two types of cells in the pancreas, but he could not reveal the exact location and activity of those cells [73]. Later, in 1875, the German physiologist and histologist Rudolf Heidenhein, based on the experimental result, proposed that changes occur in the physiological structure of that gland

when something is secreted. In 1884, Vaillard and Arnozan examined the lining of the pancreas and found that the pancreas was damaged after duct ligation, but there was no effect on the islets of Langerhans [9].

In 1889, two German physicians, Joseph von Mering and Oscar Minkowski, dissected out the pancreas of a dog and made it artificially diabetic. The next day they noticed that the dog had been micturating all day and the urine contained a lot of sugar. Thus, it was established that diabetes is caused by the absence of something within the pancreas [14]. Thereafter, scientists around the world began searching for the substance within the pancreas. While conducting experiments in this area, it was noted that the diabetic patients were not recovered after consuming pancreatic extract in various ways. On the contrary, poisoning effect was noted in some cases. In the midst of such an extreme despair, researchers revealed that the islets of Langerhans are performing a specific function that was completely different from the rest of the pancreas [25]. In 1906, pathologist Dewitt tied a cat's pancreatic duct to extract beneficial diabetic insulates from the islets of Langerhans. Although the insulate was not very effective, yet it was able to maintain glycolytic activity consistently [33]. Subsequently, in 1908, application of pancreatic alcohol extract to diabetic patients showed good results [8]. After reviewing the results of all these experiments, scientists from different countries univocally concluded in 1912 that even if the exocrine pancreas is damaged, hyperglycemia and glycosuria are escapable, but it is inevitable if the islet of Langerhans is destroyed. Thus, considering the importance of the islets of Langerhans, an attempt was made to separate it from the rest of the pancreas. Unfortunately, despite the tireless efforts of scientists in many parts of the world, the two entities were not yet successfully separated.

Eugene Gley, a French physiologist and endocrinologist, was inspired by the hypothesis that "Islets of Langerhans are able to resist glucose in the urine" proposed by the French histologist Gustav-Edward Lagus. He applied pancreatic extracts from a dog to a diabetic dog and noticed that the dog's hyperglycemia, glycosuria and other symptoms of diabetes were reduced significantly. The next question was, did the improvement in the diabetic dog come from the secretion of the islets of Langerhans or from the rest of the pancreas? To prove that, he injected islets of Langerhans tissue extract to a diabetic dog; as a result the dog was found to be in much better condition and its glycosuria and other symptoms of diabetes were significantly improved. After 25 years, Banting and Best repeated the experiment and insulin was discovered. However, at the end of the experiment Gley wrote a report in 1905, sent the report in a sealed envelope to the Biological Society of France and instructed not to open it without his permission, even under pressure of higher authorities.

Gley never performed this test again after 1890. When Banting and Best announced their discovery to the International forum in 1921, Gley ordered the envelope to be opened and realized that he had unknowingly invented insulin [120].

In the final step of the discovery of insulin, the credit actually conferred to four scientists. They were Banting, Best, McLeod and Collip. On October 20, 1920, as Banting was preparing to give a lecture on the 'Role of the pancreas in the carbohydrate metabolism', when he came across a research paper of Moses Baron in the November issue of the Journal of Surgery, Gynaecology and Obstetrics published by the University of Minnesota. During routine autopsy, Baron noticed a sporadic occurrence of stones in the pancreas. Even occasional was the fact that the stone blocked all the major ducts of the pancreas. Baron observed that the islets of Langerhans were not affected in any way, even though all of the acinar cells had been destroyed by the stone. After literature survey, he also realized that this phenomenon is comparable to the experimental results of tying the flow of pancreatic ducts. Studying the research of Baron with great care, Banting after reviewing the research done by Baron realized that when the ducts are open, i.e. under normal conditions, the digestive fluid of the pancreas absorbs the fluid from the islets of Langerhans; however this is not possible if they are closed. Banting, therefore, concluded that the destruction of various pancreatic cells starts after a while when the pancreatic duct was closed. Nonetheless, insulin was found in the fluid secreted from the remaining portion of what remained i.e., islets of Langerhans. Banting knew very little about the prior efforts made by different scientist to discover insulin and their failure and illusion. Such ignorance helped him to remain committed to his ideas [10, 124].

F. R. Miller, the Head of the Department of Physiology, who knew about Banting's hypothesis, advised him to go to John James Richard McLeod and express his new ideas. McLeod was a professor of physiology at the University of Toronto. Moreover, he was also a leading scientist in the study of sugar metabolism. Banting first met McLeod on November 7, 1920, but after talking to Banting, McLeod realized that Banting was of limited merit; he did not know much about the proceedings in the world of science and was only a scholar of biblical knowledge. He discouraged Banting, informing him that many eminent scientists could not isolate this hormone (insulin) despite many attempts. Banting returned back disappointedly, but few days later he met McLeod again. Eventually McLeod agreed, perhaps realizing Banting's huge past experience of surgery in the war camp during World War I. Therefore, Banting could be able to excise much efficiently, the duct of the pancreas of a normal dog replacing it to another diabetic dog. Moreover, many former researchers tried but all of them failed to

observe the end result i.e., when all digestive cells in the pancreas were damaged [56, 116, 124].

During this time, two assistant students, Charles Herbert Best and Edward Clark Noble, joined his laboratory as demonstrators in the hope of obtaining a Master of Arts (MA) degree. McLeod introduced them to Banting and instructed them to help Banting to prove his hypothesis [80, 124]. Banting and Best did a great job together, both of whom were aware and respectful of the techniques of the two and an effective bond was established [108].

Work began on May 17, 1921. Insulin was discovered within a few days in the Department of Physiology at the University of Toronto. Under McLeod's guidance, the two demonstrators began their research with great care. McLeod went on a holiday to Scotland on 14th June, informing them of all the means of communication. At first, they ligated an already dissected dog's pancreas and stored it in the cold. After the duct system of the pancreas was completely destroyed, they placed it inside a diabetic dog through surgery. As a result, the dog recovered [112].

When McLeod returned from vacation on September 21, he could not believe that the research had progressed so far. He questioned the accuracy of their information. Banting was always in a bad mood and used to talk to others with disrespect. So, McLeod was utterly humiliated by his behaviour and could no longer restrain himself. As a result, various bitter arguments started. In fact, McLeod wanted the two of them to repeat all the work done; they started insulin purification so that the results could be confirmed. He also explained to them a special test to prevent the blood sugar from falling due to the effects of dilution. Banting then wanted a separate room to continue his research work and a helper to look after the dogs. Moreover, he also requested a certain amount of salary for himself. When McLeod refused, Banting threatened to give up the job and move to the Mayo Clinic or the Rockefeller Institute. At first, McLeod did not pay much attention to his departure, but after a couple of days, he had realized his importance for continuing the work, and finally accepted Banting's terms. Banting then asked McLeod if biochemist J. B. Collip could join their team. McLeod advised not to expand the team at that time. Finally, Banting resumed his work with Best. The repeat experiments were done precisely and they got the same result. McLeod was convinced with the result, yet realized that there was still much work needed to be done although Banting wanted to move forward for the clinical tests. At this point of time, McLeod accepted Banting's request to include Collip in their research team. The responsibility given to Collip was to purify the insulin-carrying extract from the dog's ductless pancreas as much as possible so that the pure extract could be applied to the diabetic patient as needed. It is worth mentioning here that Collip was experienced in healing animals by injecting fluid from different glands [124, 125].

Finally on January 11, 1922, Banting, Best, McLeod and Collip, all were ready to inject insulin into the body of a 14-year-old diabetic patient, named Thompson. Unfortunately, there was no permission for Banting, Best or Collip to be present in person at the Toronto Medical School, where 15 ml of pancreatic extract was injected to Thompson's body. However, one of the symptoms of diabetes, i.e., ketoacidosis (increase in the level of ketone bodies in the blood due to diabetes) did not change. The level of glycemia and glycosuria decreased a little. Conversely, various toxicities (e.g., sterile abscesses) also occurred at the injection site. Thus, the first clinical trial failed. Everyone was very disappointed! Collip could not accept the failure and worked hard to purify the insulin extract, the treatment began again on January 23, 1922 by injecting the purified extract to Thompson's body. He began to improve as a result of daily injections. The ketone bodies disappeared from the urine, the blood sugar level dropped and he started to look much brighter. His ability to work also began to increase. In fact, it was the first successful experiment on diabetic humans based on the internal secretions of the pancreas. Thompson was the first person in the world to return to normal life with diabetes. The news spread like wildfire all over the world. Banting and McLeod were awarded the Nobel Prize in 1923, the very next year, in recognition of such research for the welfare of the human race. Unfortunately, Best and Collip were deprived for this award. Banting could not accept the omission of Best; he immediately announced that he would give half of his prize money to Best. Likewise, McLeod paid half of his reward money to Collip [10, 108].

Commercial production and beginning of chemical synthesis of insulin

Subsequent to the discovery of insulin, attempts were made to produce insulin commercially around the globe. The University of Toronto licensed pharmaceutical companies to produce insulin without royalties. Within a year of the first injection, people around the world began receiving insulin. Insulin took a special place among the earliest notable proteins. Abel generated a pure crystal of this protein in the year 1926 [49]. With the help of X-ray crystallography, the scientists were able to know the three-dimensional shape of insulin from that crystal and how insulin works with other molecules in the body. In 1955, Frederick Sanger successfully determined the full amino acid sequence of this protein and was awarded the Nobel Prize in 1959 in recognition of his work (Table 1). In fact, insulin was the first protein to be chemically synthesized in a laboratory in 1963 [106]. Notably, even ninety years after the discovery, diabetics still rely primarily on insulin derived from the pancreas of other animals. Although insulin of other animals work well on the whole, their composition is slightly different from that

Table 1 Timetable of events related to insulin discovery

Year	Research team	Discovery
1776	Matthew Dobson	Diabetic patients exhibit glycosuria
1869	Paul Langerhans	Isles of Langerhans in pancreas
1875	Rudolf Heidenhein	Physiological structure of pancreas change after secretory activity
1884	Vaillard & Arnozan	Islets of Langerhans unaffected after duct ligation
1889	Joseph von Mering & Oscar Minkowski	Diabetes is caused by the absence of something within the pancreas
1890	Eugene Gley	Islets of Langerhans tissue extract reduces hyperglycemic complications
1920	Moses Baron	Islets of Langerhans not affected due to duct blockade by pancreatic stone
1921	Frederick Banting, Charles Best, James Collip	Discovery and purification of insulin
1922	Frederick Banting, John McLeod, Charles Best, James Collip	Insulin extract successfully reduces diabetic complications in a 14 year old patient
1926	John Abel	Pure crystal of insulin
1955	Frederick Sanger	Full amino acid sequence of insulin

of human insulin, so occasional side effects (such as rashes) do occur.

Biotechnological interventions to produce human insulin

In 1978, human insulin was produced as the first protein with the help of biotechnology. Subsequently, in 1997, the FDA (Food and Drug Administration) allowed the use of modified insulin (named insulin lispro or Humolog) that is specifically formulated to work immediately after injection.

Modified insulin gene developed in the laboratory, inserted into a loop of bacterial DNA called plasmid and transfected into a bacterium generates the recombinant bacterium. Such recombinant bacteria placed in fermentation tanks produce insulin using the inserted gene. This insulin is harvested and purified for medical use. Insulin preparations using recombinant DNA technology began in the 1980s. It was one of the first instances of generation of a substance for medicinal purpose using technological innovation. Insulin aspart or NovoRapid is generated in *Saccharomyces cerevisiae* via recombinant DNA technology [119].

The insulin analogues later approved for human use were aspart insulin (NovoRapid), glargine insulin (Lantus), glulisine insulin (Apidra), detemir insulin (Levemir) and inhalable insulin, Exubera & Afrezza, in 2000, 2000, 2004, 2005, 2006 and 2014 respectively [104]. "Degludec Insulin" was withdrawn from the market in 2013 for severe side-effects with other drugs, but in 2015 it was again allowed to use with some changes in its composition [39].

In 2019, a team of researchers at MIT, US have discovered an insulin capsule that can be used like any other capsule [43]. If it gets FDA approval, type 1 diabetic patients will be able to take this capsule every day instead of injections.

Genetic regulations of diabetes

Type I diabetes

Type I Diabetes (T1D) is associated with pancreatic β-cell destruction mediated insulin deficiency [102]. T1D is associated with some modes of autoimmune destruction mediated diabetes [55]. Autoimmune diabetes is sometimes associated with genetic mutation mediated alterations in immune functions. For example, autoimmune polyglandular syndrome type 1 (APS1) is initiated by the mutation of the autoimmune regulator (AIRE) gene and is symptomatically linked with T1D [1, 55]. On the other hand, mutation of the STAT3 gene may lead to polyautoimmunopathy and is associated with autoimmune neonatal diabetes [43].

T1D exhibits 1.3–4% risk in the children of female diabetic patients and a 6–9% risk in the children of male diabetic patients [36, 88]. The risk of T1D is 70% higher in identical twins than non-identical ones [103]. In T1D, the rate of affected children varies greatly; 0.1 per 100,000/year in China & Venezuela and 40.9 per 100,000/year in Finland [34]. In Sardinia, a high rate of occurance of such phenomena has been reported, an observation discordant in respect to the whole of Italy. Several European countries and different parts of North America have been reported to show high or moderate rate of such incidence. In Asia, it is considerably low. Though T1D can be elicited at any stage of life, but its exhibits elevated rate of onset from birth to 14 years of age. The occurrence of this disease is increased in low income countries. Environmental factors play an important role in islet autoimmunity. Therefore, improvement of living standards enhances the chance of development of autoimmunity. The incidence of this disease is associated with seasonal changes with highest incidence in winter and autumn [85].

Chromosome 6p21 bears the HLA region which imparts 50% of the familial accumulation of T1D. Other than the HLA region, insulin gene (INS) exhibits a strong association with T1D. Chromosome 11p15 bears INS and the region exhibits polymorphisms by virtue of 3 variable number tandem repeats (VNTR). These polymorphisms control the level of insulin mRNA in the thymus and influences immune tolerance towards insulin [100, 118].

Several genetic polymorphisms are linked with T1D. The protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene on chromosome 1p13 exhibits polymorphisms and encodes lymphoid specific phosphatase (LYP) [93]. LYP suppresses activation of T cells and is linked with T1D [68]. On the other hand, the interferon induced helicase 1 (IFIH1), interleukin-2 receptor subunit alpha (IL2RA), ubiquitin associated SH3 domain containing protein A (UBASH3A) and basic leucine zipper transcription factor 2 (BACH2) are important genetic loci associated with T1D [23, 24, 114, 121]. Additionally, the Gli similar 3 protein (GLIS3) genetic locus is also linked to neonatal diabetes and T1D [11]. It is associated with the generation and apoptosis of pancreatic β cells and the expression of INS [91].

Next Generation Sequencing (NGS) helps in the identification of most of the genetic variants observed in the genome of an individual, irrespective of their respective frequencies. It is a more powerful tool than Genome Wide Association Studies (GWAS). Several studies related to NGS applications in T1D have been carried. For example, (1) NGS has led to the identification of HLA-DRB 3,4,5 to be associated with increased risk of T1D in children, (2) NGS has identified HNF1B and K-ATP channel genetic variants to be more involved with monogenic diabetes [35, 134].

Type II diabetes

Type II Diabetes (T2D) is associated with a reduction in insulin sensitivity or insulin resistance mediated hyperglycemic condition. In T2D, detection of onset of the disease is difficult. Lack of noticeable acute metabolic disturbance (as observed in type 1 diabetes) makes the detection of early onset of the disease quite problematic, thereby resulting in about one half of the population remaining undiagnosed. In rural areas, the number of affected individuals is relatively low compare to urban populations with western lifestyles. According to thrifty gene hypothesis, increased chances of deposition of fat and T2D are observed in individuals migrating from a region of food scarcity to an area of food abundance. In UK, the number of diabetic South Asian and African Caribbeans is high in number compared to European populations [44]. There is no significant sexual biasness in affected individuals and the chance increases along with advancement of age [45].

Applications of NGS to T2D patient samples have led to various advances in research. Some of them are: (1) mapping of rare as well as common genetic variants (e.g., identification of COBLL1 and MACF1 from Danish population studies), (2) identification of epigenetic markers (e.g., TCF7L2 as blood biomarker), (3) RNA-Seq mediated transcriptional profiling of cellular and tissue samples (e.g., identification of miR-375 RNA affecting genes of pancreatic islets), etc. [4, 41, 89, 126].

For the treatment of T2D, metformin, sulfonylureas/glinides, thiazolidinediones and GLP-1 receptor agonists/DPP-4 inhibitors are used. Genome wide association studies (GWAS) have provided strong connection of gene-drug interactions. The variants of organic cation transporter family (OCTs) encoded by SLC22A1 of chromosome 6q25.3, ATM of chromosome 11q22.3, and SLC2A2 loci of chromosome 3q26.2 are associated with Metformin (MET) response; CYP2C9 of chromosome 10q23.33, TCF7L2 of chromosome 10q25.2/10q25.3, ABCC8 of chromosome 11p15.1, KCNJ11 of chromosome 11p15.1 and IRS1 loci of chromosome 2q36.3 are associated with sulfonylureas/glinides (SUF) response; PPARG locus is associated with thiazolidinediones (TZDs) response; and GLP1R locus is associated with GLP-1 receptor agonists/DPP-4 inhibitors response [81]. Such pharmacogenomic studies help in the identification of drug responses associated with allelic variants and holds immense potential in catalyzing tailored therapies.

Homozygotic carriers of a loss of function mutation of TBC1D4 exhibits a tenfold higher risk of T2D among Inuit populations of Greenland. The mutant allele carriers bear low concentration of glucose transporter type 4 (GLUT4) in their skeletal muscles than the non-carriers [72, 86]. Thus, inadequate GLUT4 mediated glucose uptake during postprandial hyperglycaemia increases the risk of T2D. On the other hand, exome sequencing has led to the detection of a loss of function mutation in ADCY3 which is associated with obesity and diabetes in the same Inuit population [54]. Several T2D susceptibility variants observed in various populations include that of GRB10, BCL2, FAM19A2, NAT2, PPARγ, IRS-1, TCF7L2, TCERG1L, SC4MOL, ARL15 and PPP1R3B [5, 17, 26, 29, 61, 65, 67, 78, 99, 111, 122].

Machine learning techniques can be used for predicting disease susceptibility among populations. In a recent study, decision tree (WEKA), random forest (WEKA) and neural network (MATLAB) techniques were used for the prediction of diabetes mellitus in a Chinese population. There were 14 chosen attributes: age, breathe, pulse rate, left systolic pressure (LSP), height, right systolic pressure (RSP), left diastolic pressure (LDP), weight, right diastolic pressure (RDP), waistline, physique index, low density lipoprotein (LDL), fasting glucose and high density lipoprotein (HDL). It was observed that fasting glucose exhibited better performance

in test results. Therefore, it is an important index for prediction. However, due to the nature of the data set, the type of diabetes could not be predicted [136]. Further advancement of research in this field holds potential in unveiling methods for the accurate prediction of diabetic onset (T1D or T2D) based on data of genetic susceptibility markers.

Epigenetic influence in diabetes

Epigenetics refers to the heritable changes of gene expression under environmental influence without altering DNA sequence [15]. Environmental factors control the activation or inhibition of a particular gene expression by regulating transcription factor accessibility to DNA. Among epigenetic modifications, DNA methylation, histone modifications and altered microRNA mediated genetic expression trigger the onset of several autoimmune disorders including T1D [68]. The main environmental factors responsible for onset of T2D are intra-utero environments, advancement of age, low birth weight, obesity of mother that ultimately cause intrauterine growth retardation (IUGR) of child [63]. The impacts of IUGR on adulthood lead to pancreatic abnormalities by interfering in cells proliferation, differentiation, and maturation that cause onset of T2D.

DNA modifications in T1D

DNA methylation

During DNA methylation, DNA methyl-transferases (DNMT) catalyse the addition of methyl group on the fifth carbon cytosine in the CpG island to form 5-methylcytosine [66]. Basically, DNMT1, DNMT3a, and DNMT3b are responsible for this conversion [31]. DNMT1 causes methylation of newly synthesized un-methylated daughter strands during DNA replication to keep methylation pattern of genome in check. On the other hand, DNMT3a and DNMT3b are essential for de novo DNA methylation process. On the contrary, in the process of DNA demethylation, oxidation of 5-mC and thymine DNA glycosylase (TDG) mediated removal of modified base generates cytosine occurs by replacing 5-mC. Additionally, a family of ten-eleven translocation (TET) methyl-cytosine dioxygenases are also involved in the process of demethylation [69]. Altered methylation status of DNA changes the expression profile of genes related to insulin secretion, beta cell survival, and autoimmunity and therefore trigger the onset of T1D [137]. In a particular study, genome wide methylation profiling of monozygotic (MZ) twins showed discordancy of T1D due to T1D related methylation of genes regulating inflammation, immunity, and apoptosis. Moreover, there were 88 CpG sites showing differential methylation in T1D-discordant MZ twin

pairs. In pancreatic β cells and thymic epithelial cells, methylation of INS gene promoter is associated with T1D onset [47, 109]. Heavy methylation at CpG–69, –102, –180, –206 and low methylation at CpG–19, –135, and –234 in INS gene has been found in patients with T1D compared to healthy controls. Pro-inflammatory cytokine mediated methyl transferase activation causes methylation of Ins1 exon-2 and Ins2 exon-1 which control the expression of INS gene. Besides INS gene, epigenetic modulation of Interleukin 2 receptor a-chain gene (IL2RA) also initiates T1D development. High expression of Interleukin 2 receptor on regulatory T cell surface suppresses the auto-reactive T cells activity. High methylation of IL2RA CpGs–373 and –456 in T1D patients generates autoimmunity [13]. Various complications associated with T1D like diabetic nephropathy are also regulated by epigenetic modulation. In T1D patients, methylation CpG site near transcription start site of UNC13B gene triggers the onset of diabetic nephropathy [12].

Micro-RNA modifications

The micro-RNAs (miRNAs) are single stranded non-coding RNA molecules that act as RNA silencer and thereby act as post-transcriptional regulator [115]. Inside the nucleus, in the presence of RNA polymerase (RNase) II and III, primary miRNA is transcribed and processed to precursor miRNA with the help of Drosha/DGCR8. After being released in the cytoplasm, it is finally converted to mature miRNA by RNase III dicer complex [22]. Mature miRNAs bind to 3′ UTR of targeted mRNA, and repress protein production by destabilizing target mRNA [40]. miRNAs play an important role in controlling cellular processes like proliferation, differentiation, glucose homeostasis, apoptosis, carcinogenesis, inflammation etc. The association of miRNAs with the production and secretion of insulin is well documented [57]. In T1D, altered miRNA levels affect insulin secretion, immune-regulation and the mitogen-activated protein kinase (MAPK) signaling pathway [6]. During proliferation of β-cells, some of the miRNAs show positive impact while others exhibit negative impact. miR-375 targets various growth inhibiting genes to control β-cell proliferation [76]. miR-375 knock out mice exhibits diminished β-cell mass as miR-375 inhibits the activity of Cadm1 which represses G1/S transition and cellular growth [98]. Similarly, miR-181a exhibits protective effects by inducing β-cell proliferation [133]. On the other hand, increased expression of miR-24 in db/db mice is associated with the aging of the mice [135]. miR-29a inhibits proliferation of INS-1E cells (pancreatic islet β cells) and diminished insulin secretion from β-cell [7]. Although miR-29 has an important role in β-cell proliferation, it can negatively control insulin secretion by targeting Stx-1a which is associated with insulin exocytosis [52]. miR-155-5p upregulation in human islet derived exosomes targets mRNA of the

transcriptional and immune response regulator gene. This miR-155-5p also evokes inflammatory response in T1D by interacting with toll-like receptors, resulting in the activation of the NF-kB pathway [48]. miR-146a-5p negatively regulates IL-6 activity whereas in T1D, low level of miR-146a-5p expression is associated with increased IL-6 production. Therefore, miR-146a-5p can control inflammation by negative feedback effect on NF- kB and its low level activity in T1D triggers inflammation [92]. Furthermore, in T1D patients, upregulated expression of miR-23b, miR-98, and miR-590-5p in cytotoxic CD8+T cells suppress the function of various apoptotic gene (like TRAIL, FAS) and facilities survival and enhanced proliferation of auto-reactive T lymphocytes [28].

Circulatory miRNA can be used as a potential biomarker for early detection of T1D. Increased expression of circulating miR-125b-5p and miR-365a-3p is associated with increased HbA1c level (glycated haemoglobin) whereas urinary miR-377 is positively correlated with upsurge HbA1c level and urinary albumin creatinine ratio of T1D patients [38].

Histone modifications

Histones modifications refer to post translational modification of histone protein by various processes like methylation, acetylation, phosphorylation, ubiquitination, and sumoylation. In histone methylation, methyl group addition to arginine or lysine residues leads to the activation or suppression of transcription based on the degree of modification whereas, in histone demethylation, lysine specific demethylase-1 (LSD1) demethylated mono- and di- methylated lysine, specially H3K3 and H3K4 region [68, 107]. The histone acetylation and de-acetylation indicate addition or removal of acetyl group with the help of histone-acetyltransferases (HATs) and histone-deacetylases (HDACs), respectively. Histone acetylation increases the accessibility of transcription factor to the DNA by opening the chromatin structure via reduction of electrostatic attraction between DNA and histone [53].

Modification of histone protein alters the chromatin structure that causes the onset of various pathophysiological conditions including T1D. In T1D patients, increased H3 lysine-9 di-methylation (H3K9me2) was found in CLTA4 gene and other genes related to auto-immunity and inflammation. Moreover, huge variation in H3 lysine-9 acetylation (H3K9Ac) in the upstream regions of HLA-DRB1 and HLA-DQB1, is intensely associated with T1D [84]. In T1D patients, increased level of H4 acetylation compared to T1D patients with cardiovascular complications indicates that histone acetylation may protect against T1D associated complication development [18].

DNA modifications in T2D

DNA methylation

The process of DNA methylation in the islets depends on methylation of lysine 9 on H3 (H3K9) by the enzyme DNA methyl-transferase [110]. This causes the epigenetic change in IUGR islet where HDAC/mSin3A complex interaction with Pdx1 leads to deacetylation and inhibits Pdx1 transcription and resulting in pancreatic agenesis [62]. Although minor Pdx1 protein level depletion does not affect usual β cell mass, it impairs β cell insulin secretion activity. Epigenome-wide association study (EGWAS) shows that methylation on CpG site in ABCG1 gene present on 21st chromosome is linked with insulin resistance. This methylation on ABCG1 is associated with fasting insulin and can therefore be used as a disease marker [58]. EGWAS analysis on Indian populations suffering from T2DM shows that methylation of ABCG1, PHOSPHO1, SOCS3, SREBF1, and TXNIP is associated with early incidence of T2DM [16]. DNA methylation at the ABCG1 locus cg06500161 of DNA is positively correlated with HbA1c, fasting insulin, and triglyceride levels in the blood of diabetic twin among monozygotic twins which are discordant for T2D. DNA methylation at PHOSPHO1 locus cg02650017 is positively associated with HDL levels in diabetic in comparison to non-diabetic monozygotic twins [27]. In Mexican American populations, methylation of TXNIP, ABCG1 and SAMD12 is responsible for heritability of T2D [70].

Modification of histone protein activity in IUGR rat muscle indicates insulin resistance [62, 94]. Under normal conditions, transport of glucose from blood to cell followed passive diffusion with the help of GLUT4 mainly in adipose tissue, skeletal muscles, and cardiac muscle [64]. The expression of GLUT4 is regulated by myoblast determination protein (MyoD) and myocyte enhancing factor 2 (MEF2) factors [87]. Upregulated expression of MEF2D and downregulated expression of both MYoD and MEF2A cause repressed GLUT4 expression. Another pathway that controls IUGR GLUT4 transcription was H3K14 (histone 3 lysine 14) deacetylation. HDAC1-HDAC4 mediated deacetylation of H3K14 facilitates the recruitment of the suppressor of variegation 3–9 homolog 1 (Suv39H1) methylase that causes H3K9 dimethylation and increased attachment of heterochromatin protein 1. This leads to IUGR GLUT4 gene repression [101]. These indicate perinatal nutrition deficiency mediated IUGR leads to histone modification that eventually decreases GLUT4 expression and glucose transportation inside the cells. Figure 1 represents various genetics and epigenetics reasons of Type 1 and Type 2 diabetes.
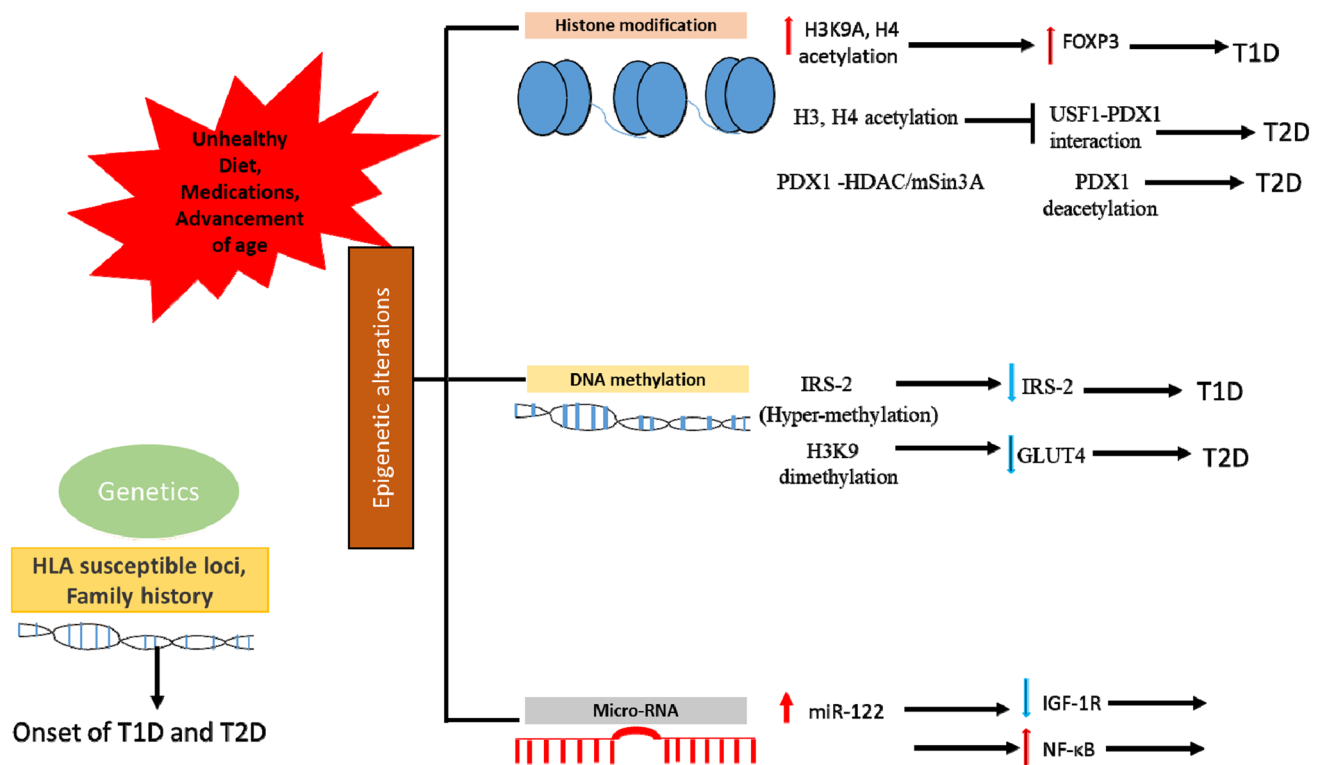
Fig. 1 Flow diagram depicting various genetics and epigenetics reasons of Type 1 and Type 2 diabetes

Micro-RNA modifications

During T2DM, miRNAs effectively control insulin signalling and insulin resistance (IR) [41]. Interaction between ligand and receptor is crucial for insulin signalling. Elevated expression of miR-195 and miR-15b causes downregulation of insulin receptor by binding to its 3′-UTR site and resulting in signalling disruption [129, 131]. Increased expression of miR-29a suppresses insulin receptor substrate 1 (IRS-1) by directly interacting with its 3′-UTR site [130]. Overexpression of miR-103/107 interferes with IRS-1 and caveolae (Cav-1) interaction and decreases IRS-1 stability [117]. In IRS-1-deficient condition, IRS-2 acts as an alternative substrate and interaction of its 3′-UTR with miR-135a negative impacts insulin signalling [2].

To predict the early onset of T2DM, the presence of miRNAs in the circulation is a reliable biomarker. It has been found that plasma miR-126 is associated with T2DM. It is the only mRNA whose activity is decreases in T2DM patients [132]. Moreover, on comparison of miRNA expression profile between pre-diabetic and T2DM individuals, miR-320b, miR-1249, and miR-572 have been found to be potential biomarkers for early detection of T2DM [128]. Advancement of T2DM triggers the onset of various health complications. Among them, in diabetic nephropathy (DN), expression profile of miR-29a/b/c, miR-21, and miR-192 is

upregulated [19]. During DN, miR-21 causes PTEN suppression, while miR-29c inhibits SPRY1 expression that regulates mesangial matrix accumulation and albuminuria in diabetic murine models [74, 79]. Interestingly, another type of miRNA that may serve as a candidate marker for DN are urinary miRNAs. Aberrant urinary miR-320c, isolated from urinary exosomes, may impact the TGF-β signalling pathway by targeting THBS1 and can be used as a novel marker for disease progression in DN [30].

Histone modification

Pdx1, a homeodomain-containing transcription factor, plays a key role in early generation of both exocrine and endocrine pancreas and on β cell development in later stages. In IUGR rats, there was the repression of Pdx1 expression gradually after birth due to epigenetic alteration. In IUGR rat, isolated β cells from pancreas showed the reduced level of histone acetylation in H3 and H4 at Pdx1 promoter [96]. Decreased acetylation of H3, and H4 hinder the interaction of upstream transcription factor 1 (USF1) to the promoter region of PDX1. USF1 is crucial for PDX1 transcription and reduced USF1 PDX1 interaction causing transcriptional silencing of PDX1 [113]. In IGUR rat, increased histone deacetylation along with decreased trimethylation of H3K4 and increased H3K9 dimethylation caused chromatin gene repression [96].

These factors altogether disrupt glucose homeostasis and upsurge oxidative stress in IUGR.

Viral infection and type I diabetes

Viral infection leads to the activation of antigen presentation and release of inflammatory cytokines. Molecular mimicry, inflammatory cytokines, repeated viral infections, induction of Class I Major Histocompatibility Complex (MHC) and the localized release of interferons induce the autoreactive effector T cell mediated destruction of pancreatic β cells and cause enhancement of T1D. On the other hand, sequestering of effector T cells or their elimination by inflammatory cytokines from the pancreatic islets during acute or chronic infections and repeated infection mediated accumulation and viral persistence directed induction of protective regulatory T cells prevent the destruction of pancreatic β cells and cause the abrogation of T1D. Thus, viral infections at an early age can trigger the onset of T1D (Fig. 2) [42].

Viral infection of pancreatic β cells mediated inflammation and dysglycemia can elicit oxidative and endoplasmic reticulum (ER) stress mediated changes in cellular proteins through carbonylation, alternative splicing, deamidation, initiation of defective ribosomes, phosphorylation, citrullination, sumoylation etc. This promotes the generation of neo-epitopes which elevate the immunogenicity of the β cells and the responses of autoreactive T and B cells [97].

Enteroviral (EV) infection can lead to destruction of pancreatic β cells by directly killing them or by eliciting inflammatory response in the pancreatic islets to attract autoreactive T cells at the site of inflammation [105]. The viral persistence and high viral load mediated responses include upregulation of melanoma differentiation-associated protein 5 (MDA 5), myxovirus resistance protein (MxA), human leukocyte antigen-I (HLA-I), retinoic acid inducible gene-I (RIG-I) etc. EV infection leads to the release of interferons and the induction of ER stress and unfolding protein response (UPR) activity. Inflammatory cytokines and EV antigens lead to the development of adaptive autoimmunity, which in turn, further augments destruction of pancreatic β cells [3]. This promotes T1D.

Viruses associated with T1D include enteroviruses like coxsackievirus B (CVB), rotavirus, mumps virus, cytomegalovirus and rubella virus with varying degrees of pathogenicity [32, 46, 59, 60, 83, 95].

TEDDY (The Environmental Determinants of Diabetes in the Young) analyses assess the effect of environmental factors like viral infections in the onset of autoimmune diabetes. It demands monitoring of children during any viral infection for higher genetic risk of T1D [42].

SARS-COV-2 infection has the potential to destroy pancreatic β cells, reduce insulin secretion and induce T1D. On the other hand, treatment of SARS-COV-2 infected patients, suffering from hyperglycemia, with steroids, induces hyperinflammation, insulin resistance (T2D) and severity of COVID-19 symptoms [71]. SARS-COV-2 infection increases the level of inflammatory cytokines, lipopolysaccharides and Natural Killer (NK) cells, which lead to lung fibrosis and acute lung damage. It also induces
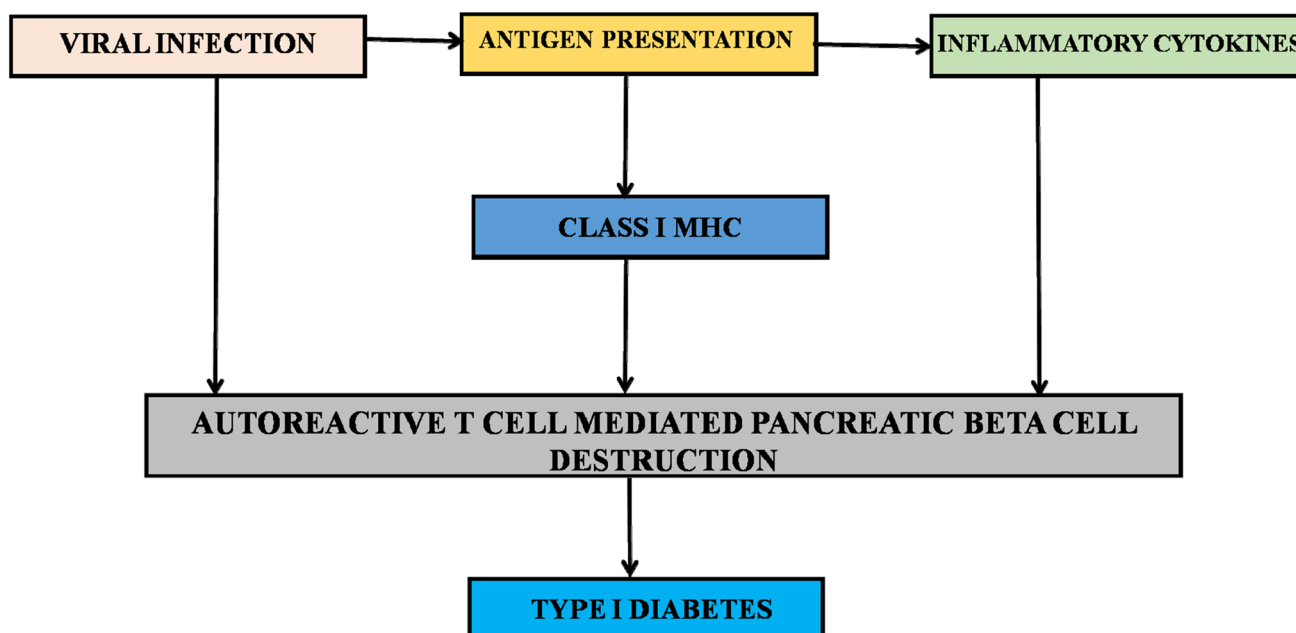

Fig. 2 Flow diagram showing different means of viral infection may lead to Type I diabetes

oxidative stress mediated hyperglycemia and angiotensin II mediated insulin resistance [75]. SARS-CoV-2 enters the host cell through the angiotensin converting enzyme 2 (ACE2) receptor, thereby disrupting ACE2 mediated catalysis of the conversion of angiotensin II to angiotensin (1–7) [90]. The elevated level of angiotensin II inhibits the insulin dependent activation of phosphoinositide 3-kinase (PI3K) pathway and disrupts translocation of Glut-4 in insulin-sensitive tissues, thereby resulting in systemic insulin resistance [127]. The hyperglycemic condition leads to further progression of lung pathophysiology.

Conclusion

The history of the discovery of insulin is a fascinating story of sacrifice, patience, toil, solving complex observational scientific riddles and occupational complexities. It is an inspiration for future researchers in the field of protein biology, drug discovery and other aspects of pharmacology. Based on the present global situation in relation to the disease, the genetic and epigenetic regulations of diabetes need to be studied with utmost care and importance. While genetic predisposition analyses cater to a deterministic path, the epigenetic studies address the stochastic and self-controllable triggers of the disease. Since genetic regulation is one of the major contributors for T1D, lifestyle itself is a major factor for T2D and the medics need to address the patients accordingly for a proper cure. Recent studies have also revealed that viral infections at an early stage can also contribute to the onset of the disease in some individuals. More extensive research in the discussed fields is still awaiting.

Finally, let's go back to that old saying in a new way and say that there is no substitute for research on various topics, whether it is diabetes or something else, for us to live a beautiful and healthy life. As new problems come along, new research paths open up with the blessings of logic and science. The pursuit of science with relentless effort and endless patience, which people could not have imagined before, will easily come under our control and make the path of the next generation easier and more beautiful.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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