

Chapter 1

Etiology of diabetes mellitus

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Introduction

The defining characteristics of diabetes, irrespective of the precise etiology, relate to the presence of hyperglycemia. The American Diabetes Association (ADA) has set forth specific criteria for the definition of diabetes. In the ADA guidelines, the following are necessary for the diagnosis of diabetes: (1) hemoglobin A1c (HbA1c) equal to or greater than 6.5% OR (2) fasting plasma glucose equal to or greater than 126 mg/dl OR (3) two-hour plasma glucose equal to or greater than 200 mg/dl during an oral glucose tolerance test (OGTT) (glucose load containing 75 grams anhydrous glucose dissolved in water) OR (4) in a patient with classic symptoms of diabetes or during a hyperglycemic crisis, a random glucose of equal to or greater than 200 mg/dl suffices to diagnose diabetes [1].

In this chapter, we will review the major types of diabetes and the etiologic factors that are known to or are speculated to contribute to these disorders. Furthermore, we will take the opportunity to present an overview of emerging theories underlying the pathogenesis of type 1 and type 2 diabetes. Types 1 and 2 diabetes constitute the vast majority of diabetes cases. Interestingly, both of these types of diabetes are on the rise worldwide [2, 3]. In addition to types 1 and 2 diabetes, we will also discuss gestational diabetes. Often a harbinger to the ultimate development of frank type 2 diabetes in the mother, this form of diabetes is potentially dangerous to both the mother and the developing fetus. Finally, we will discuss the syndromes known as MODY or maturity onset diabetes of the young. The disorders underlying MODY have very strong genetic components and are due to mutations in multiple distinct genes.

The greatest long-term danger of diabetes, irrespective of the etiology, lies in the potential for complications. The complications of the disease are insidious, deadly, and difficult to treat or reverse; hence, there is great urgency to identify specific means to prevent or mitigate these most common types of diabetes.

Type 1 diabetes

Type 1 diabetes accounts for approximately 5–10% of all cases of diabetes [1]. The countries with the highest incidence of type 1 diabetes include Finland and Sardinia [4]. Type 1 diabetes is usually diagnosed in childhood, hence the original classification “juvenile onset diabetes.” Indeed, type 1 diabetes accounts for more than 90% of diabetes diagnosed in children and adolescents. Given that the disease is often diagnosed in adults, however, even into advanced age, the term “type 1 diabetes” has been adopted to more accurately reflect the diversity of affected ages. In type 1 diabetes, the primary etiology is due to a cellular-mediated autoimmune-mediated destruction of the β cells of the pancreas. Traditionally, in subjects with type 1 diabetes, autoantibodies may be detected that reflect the underlying attack against these cells [5]. These include autoantibodies to insulin, to GAD65, and to IA-2 and IA-2 β (the latter two are tyrosine phosphatases). These antibodies are often detected up to years before the diagnosis of type 1 diabetes [6]. In most subjects with type 1 diabetes, one or more of these antibodies is evident. Indeed, in vulnerable subjects, such as first-degree relatives of affected individuals, the presence of these autoantibodies is often, but not always, a harbinger of the eventual diagnosis of diabetes. Hence, these antibody profiles may be used to predict the risk of diabetes in the siblings and relatives of affected subjects with type 1 diabetes [6].

Genetics of type 1 diabetes

More than forty years ago, type 1 diabetes was found to have very strong links to the human leukocyte antigen (HLA)-encoding genes [7]. The largest study to address this issue was known as the Type 1 Diabetes Genetics Consortium (T1DGC). This group was composed of an international collaboration and amassed more than 14,000 samples [8]. By far, the greatest association to type 1 diabetes was found in the HLA, particularly in the HLA DR-DQ haplotypes. Furthermore, other genes found to have strong genetic association were in polymorphisms identified in the insulin gene [9]. The researchers of T1DGC earlier reported that beyond these two associations, two other loci were found to have odds ratios (ORs) greater than 1.5, and included *PTPN22* and *IL2RA* [9]. However, the ORs for these genes were relatively much lower than that of the HLA region, consistent therefore with the overall strong role of the HLA in the susceptibility to type 1 diabetes.

A number of groups have published the results of genome wide association studies (GWAS) in type 1 diabetes and identified more than 40 potential susceptibility loci in the disease [11]. Candidate genes identified in this approach included those encoding *IL10*, *IL19*, *IL20*, *GLIS3*, *CD69*, and *IL27*; these are all genes strongly linked to the immune/inflammatory response [10]. In their report, Bergholdt and colleagues integrated the data from these GWAS studies and translated them to a more functional level, that is protein-protein interactions and, finally, they tested their relevance in human islets and in a β cell line, INS-1 cells (rat insulimona-derived cells) [11]. First, they performed a meta-analysis of the type 1 diabetes genome wide Association studies that were available. From these, they identified 44 type 1 diabetes non-major histocompatibility complex (MHC) low density (LD) regions with significance; these regions contained more than 395 candidate genes. They then performed network analysis studies with the intention to more deeply

Table 1.1 Examples of non-HLA type 1 diabetes-associated loci.

Locus	Description	Comments
<i>PTPN22</i>	Protein tyrosine phosphatase, non-receptor type 22	Modulation of T and B cell function
<i>INS</i>	Insulin	Deficient in type 1 diabetes
<i>IL2RA</i>	Interleukin-2 receptor, α	T lymphocyte function
<i>IL10</i>	Interleukin-10	Immunoregulation Inflammation
<i>IL19</i>	Interleukin 19	Immunity/inflammation
<i>GLIS3</i>	Gli-similar 3 protein	Pancreatic β cell generation Insulin gene expression Modulation of pancreatic β cell apoptosis
<i>TRAF3IP2</i>	TRAF3 interacting protein 2	Implicated in IL17 signaling Interacts with members of Rel/NF- κ B transcription factor family
<i>PLCG2</i>	Phospholipase C, γ 2	Leukocyte signal transduction NK cell cytotoxicity
<i>CCR5</i>	CC-chemokine receptor 5	Major co-receptor for HIV entry into cells Immune cell recruitment
<i>MYO1B</i>	Myosin 1B	Cell membrane trafficking and dynamics

probe network connections and protein-protein interactions. From this work, 17 protein networks were identified (which contained 235 nodes) containing at least two genes from different type 1 diabetes LD regions [11].

To follow up on these findings, human islets were exposed to pro-inflammatory cytokines and comparisons were made between the treated and untreated human islets (retrieved from eight donors). From this, the following genes were found to be significantly impacted by the cytokine stimulation in the human islets: *IL17RD*, *CD83*, *IFNGR1*, *TRAF3IP2*, *IL27RA*, *PLCG2*, *MYO1B*, and *CXCR7*. Interestingly, the study design suggested that perhaps these traditionally inflammation-associated factors were being produced by pancreatic β cells and not necessarily solely by immune cells. To test this specific point, rat INS-1 cells were treated with cytokines and the above eight genes were examined. Indeed, all but *IL27ra* were identified in the stimulated INS-1 cells [11]. In the case of cultured INS-1 cells, no immune cells are present, therefore suggesting the interesting possibility that these factors may be produced both by islet β cells themselves as well, likely, by infiltrating inflammatory cells. Examples of non-HLA genes linked to type 1 diabetes are illustrated in Table 1.1.

Pathogenesis of type 1 diabetes

There is strong evidence that links the pathogenesis of type 1 diabetes to immune-mediated mechanisms of β cell destruction, including the detection of insulinitis, the presence of islet cell autoantibodies, activated β cell-specific T lymphocytes and, as considered above, association of the disease with a restricted set of class II major histocompatibility alleles [12]. Importantly, the rate of the development of type 1 diabetes after the appearance of autoantibodies may be quite variable, reflecting perhaps the contribution of protective mechanisms (such as CD4+ –T regulatory cells and other regulatory cells such as invariant

natural killer T [NKT] cells). Such protective factors may differ among individuals, thereby possibly accounting for the variable progression of damaging autoimmunity and the appearance of diabetes. The diagnosis of type 1 diabetes, often made by the appearance of diabetic ketoacidosis [13], is linked to the absence or near absence of plasma C-peptide (N-terminus fragment of insulin that is used to monitor the ability to produce insulin) [14]. It has been suggested that particularly in adults, residual β cell function may be retained for years after the appearance of autoantibodies without manifestation of ketoacidosis. In the sections to follow, we consider some of the specific factors that have been linked to the pathogenesis of type 1 diabetes.

Type 1 diabetes and the environment: infectious agents

As discussed above, the incidence of type 1 diabetes is on the rise at a rate of 3–5% per year that is doubling every 20 years. This is occurring particularly in very young children and is present more often in subjects bearing the low risk alleles [15, 16]. What accounts for these findings? Certainly, genetic risk cannot explain the overall rise in this disorder over relatively short time periods, thereby placing a spotlight on so-called “environmental” factors. For example, it has been suggested that acute infections such as those that are bacterial or viral in nature may precipitate the disease. After such an acute onset, subjects may often enter so-called “honeymoon” periods during which time hyperglycemia abates and the subjects do not require insulin for survival. Examples of viruses linked to type 1 diabetes include cytomegalovirus, coxsackie B, mumps, rubella, Epstein-Barr virus, rotavirus, and varicella zoster virus [17]. An intriguing example of an association between an environmental trigger and type 1 diabetes was speculated to have occurred in Philadelphia in 1993. During the first six months of that year, a substantial rise in the incidence of type 1 diabetes among children was observed. It had been noted that in the two years prior to this event, an outbreak of measles had occurred in the same location, thereby raising the hypothesis that the viral infection stimulated factors that caused type 1 diabetes to emerge in vulnerable children [18].

Type 1 diabetes: the microbiome

In the human intestine, it is estimated that more than 100 trillion bacteria reside and colonize the organ [19]. Far from being a passive factor in the host, these bacteria critically interface with the immune and metabolic systems. Studies have suggested that specific classes of bacteria may exert effects on the immune system. For example, Bacteroidetes were shown to reduce intestinal inflammation [20]. Segmented filamentous bacteria were suggested to induce Th17 immune responses [21]. Th17 immune responses are usually linked to the clearance of extracellular pathogens during periods of infection; Th17 T cells produce major cytokines that induce inflammation such as IL6 and IL8 [22].

In animal models, interference with the normal gut microbiota has impacted the incidence of type 1 diabetes. For example, raising two major mouse and rat models of type 1 diabetes in germ-free or altered flora environments resulted in the animals developing insulinitis and type 1 diabetes at accelerated rates [23, 24]. In contrast, feeding type 1 diabetic-vulnerable animals antibiotics significantly delayed or prevented type 1 diabetes [25]. Based on these considerations, the hunt is on to identify

the specific phyla of bacteria that display adaptive/anti-type 1 diabetes impact. So called “probiotics” might one day be identified as treatments to alter the course of type 1 diabetes development, such as the protective effects shown by treatment of type-1-diabetes-vulnerable rats with *Lactobacillus johnsonii* [26].

In the context of the microbiome, it is interesting that type 1 diabetes may appear more frequently in individuals born by Cesarean section vs. natural deliveries [27]. It was shown that in the earliest time of life, the gut microbiome constituents differ in these two states with skin vs. vaginal microbes, respectively, reflecting the major microbiota in subjects born by these two methods. Hence, via Cesarean birth, there is a delay in the colonization of the gut with organisms such as *Bacteroides*, *Bifidobacterium*, and *Lactobacillus*; the extent to which this might account for increased type 1 diabetes is not clear [28]. The possibility that the distinct phyla of bacteria may influence the types of immune/inflammatory cells in the gut is under consideration as a contributing factor in type 1 diabetes. In this context, type 1 diabetes manifests with an increased number of intestinal inflammatory cells in parallel with reduced numbers of FoxP3+CD4+CD25+ T lymphocytes [28]. Hence, it is possible that alteration of the gut microbiota might lead to alterations in immune cell patterns in the gut.

In studies in Finnish subjects with type 1 diabetes, experimental analyses have shown that within the gut microbiome, there is a change in the ratio of two key phyla of bacteria—an increased percentage of Bacteroidetes in parallel with a lower percentage of Firmicutes [29]. Whether this association is linked mechanistically to type 1 diabetes has yet to be clarified. 16S sequencing and metagenomics are current strategies under way to determine if there are actual mechanistic links between alterations in the gut microbiome and the susceptibility to type 1 diabetes.

Type 1 diabetes: vitamin D

Vitamin D, or 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), has been linked at multiple levels to the pathogenesis of type 1 diabetes. Most importantly, vitamin D plays immunomodulatory roles in cells that express the vitamin D receptor (VDR). Included among such cells are antigen presenting cells, activated T cells, and pancreatic islet β cells [30]. Studies have shown that administration of vitamin D or analogues may exert protection against type 1 diabetes in non-obese diabetic (NOD) mice [31]. Experimental studies to discern the underlying mechanisms showed that administration of 1,25(OH)₂D₃ reduced inflammatory cytokine (such as IL6) production in parallel with increased regulatory T cells. On the contrary, mice deficient in 1,25(OH)₂D₃ were shown to be at higher risk of developing type 1 diabetes [32].

What is the evidence in human subjects linking vitamin D to type 1 diabetes? Insights into this question became evident in the study of vitamin D receptor (VDR) polymorphisms. The gene encoding the VDR is located on chromosome 12q12-q14 in the human and single nucleotide polymorphisms (SNPs) have been shown to alter the function of the receptor. The results of studies examining these SNPs have yielded contrary data but the largest meta-analysis to date showed that one of the VDR polymorphisms, *BsmI*, was associated with significantly increased risk of T1D but other SNPs, including *FokI*, *Apal*, and *TaqI*, did not display a significant association with T1D [33]. It remains possible that the

VDR locus is not itself the disease affecting locus; rather, the *VDR* may in fact be a marker locus in linkage equilibrium with the true disease locus. Certainly greater functional studies on the SNPs and vitamin D actions are essential to mechanistically link the SNPs to pathological function of the receptor and associations with the pathogenesis of T1D.

What about the levels of vitamin D? Multiple studies in different countries have addressed this question and suggest that lower levels of vitamin D might be related to type 1 diabetes. For example, studies in Switzerland, Qatar, North India, the northeastern United States, and Sweden suggested that levels of vitamin D were lower in type 1 diabetic subjects vs. control subjects. In contrast, in the sun-enriched state of Florida no differences in vitamin D levels were noted between type 1 diabetic subjects and their unaffected first degree relatives and control subjects [30].

Interestingly, support for the North to South incidence of type 1 diabetes emanates from the fact that sun exposure, which is strongly linked to latitude, has possible relationships with type 1 diabetes. Specifically, a number of observational studies have suggested increased type 1 diabetes prevalence in the northern, less sun-exposed latitudes vs. more sun exposed regions. In the EURODIAB study, the incidence of diabetes was found to be higher in the northern region study centers vs. the southern centers, with the exception of Sardinia. Sardinia is considered to be in the southern region but it reported higher rates than those observed in neighboring southern region sites [34, 35]. Not taken into account in these studies are the genetic variations and other vulnerabilities and associations with type 1 diabetes, such as affluence (the latter associated with type 1 diabetes) [36].

The above considerations suggest that supplementation with vitamin D might be protective in type 1 diabetes. When a meta-analysis of multiple observational studies was performed, the results suggested that the incidence of type 1 diabetes was reduced by up to 29% in subjects given supplementation with vitamin D [37]. It is notable, however, that in these studies, concerns regarding many factors, such as reporting of vitamin D levels, doses of vitamin given, and the absence of documentation of vitamin consumption, as examples, limited the overall interpretability of these studies. Hence, a prospective randomized clinical trial is definitely needed to rigorously address these questions and establish possible causality between vitamin D and type 1 diabetes. At this time, no specific answer is available to unequivocally address this issue. Despite these caveats, however, it is essential to address this issue as supplementation with vitamin D should be feasible.

Type 1 diabetes and insulin resistance

In the sections above, we discussed some of the major factors impacting the etiology of type 1 diabetes. Of late, the issue of “double diabetes” has emerged; this term, first employed to describe this concept in 1991, suggests that there is an emergence of insulin resistance in subjects with type 1 diabetes [38, 39]. For example, in type 1 diabetic subjects with obesity or in whom even very high levels of exogenous insulin did not achieve euglycemia, insulin resistance was speculated to be present [38, 39]. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study suggested that a family history of type 2 diabetes significantly predicted excess weight gain in type 1 diabetic subjects [40]. Thus, the degree of peripheral insulin resistance might result from genetic and/or environmental factors (such as energy intake and physical activity).

In fact, in the Pittsburgh cohort of the Epidemiology of Diabetes Complications (EDC) study, there is evidence that the prevalence of obesity has risen significantly in type 1 diabetic subjects, similar to the findings reported in the general population. From 1987 to 2007, this study showed that the prevalence of obesity rose seven-fold and that the prevalence of overweight rose 47% [41]. Although some of these changes might be attributable to insufficient glycemic control in the past decades, the overall premise is that the general increase in obesity/overweight has also impacted the type 1 diabetic subject population.

Finally, it is plausible that the development of insulin resistance might be accounted for, in part, by the route of administration of therapeutic exogenous insulin. When insulin is administered by the subcutaneous route, this has been associated with relative peripheral hyperinsulinemia together with hepatic hypoinsulinemia. Such a regimen might ultimately lead to reductions in peripheral insulin-mediated glucose uptake and increased hepatic glucose production [42]. It remains to be seen which factors may underlie the observed insulin resistance in type 1 diabetes and how these might best be managed in type 1 diabetes.

Type 1 diabetes: summary

In summary, the incidence of type 1 diabetes is on the rise. As Figure 1.1 illustrates, there are multiple contributing factors. Although genetic factors are a major underlying cause, emerging evidence suggests that subjects with traditionally lower genetic risk alleles are being diagnosed with type 1 diabetes. These considerations strongly implicate so-called “environmental” factors in the multiple steps beyond genetic risk that are required before frank type 1 diabetes results. Insights into the interactions between the host and microbiome with respect to modification of genetic risk highlight the complexity of the factors that may significantly modify type 1 diabetes risk.

Type 2 diabetes

Type 2 diabetes is the most prevalent form of diabetes, accounting for up to 90–95% of diagnosed cases of diabetes, and is on the rise [1]. The International Diabetes Foundation (IDF) reported that in the age range of 20–79 years, approximately 285 million adults suffer from diabetes, a number which is expected to rise to approximately 438 million in the year 2030 [2]. In fact, about 90–95% of these cases will be in the type 2 diabetes classification. Older nomenclature referred to this form of diabetes as “non-insulin dependent” or “adult-onset diabetes.” In this form of diabetes, at least early in the course of the disease, subjects display insulin resistance with a “relative” deficiency of insulin. However, in the later stages of disease, some subjects are not able to produce sufficient amounts of insulin to compensate for the hyperglycemic stress [1]. This reflects underlying dysfunction of the pancreatic β cell. In type 2 diabetes, ketoacidosis seldom occurs; where it does occur, it may be precipitated by events such as infections. In cases in which very high levels of glucose are present, subjects may present with coma [43].

In general, the risk of developing type 2 diabetes rises with age and is associated with obesity and diminishing physical activity. Type 2 diabetes occurs more frequently in women who displayed gestational diabetes (GDM) during their pregnancies. Further,

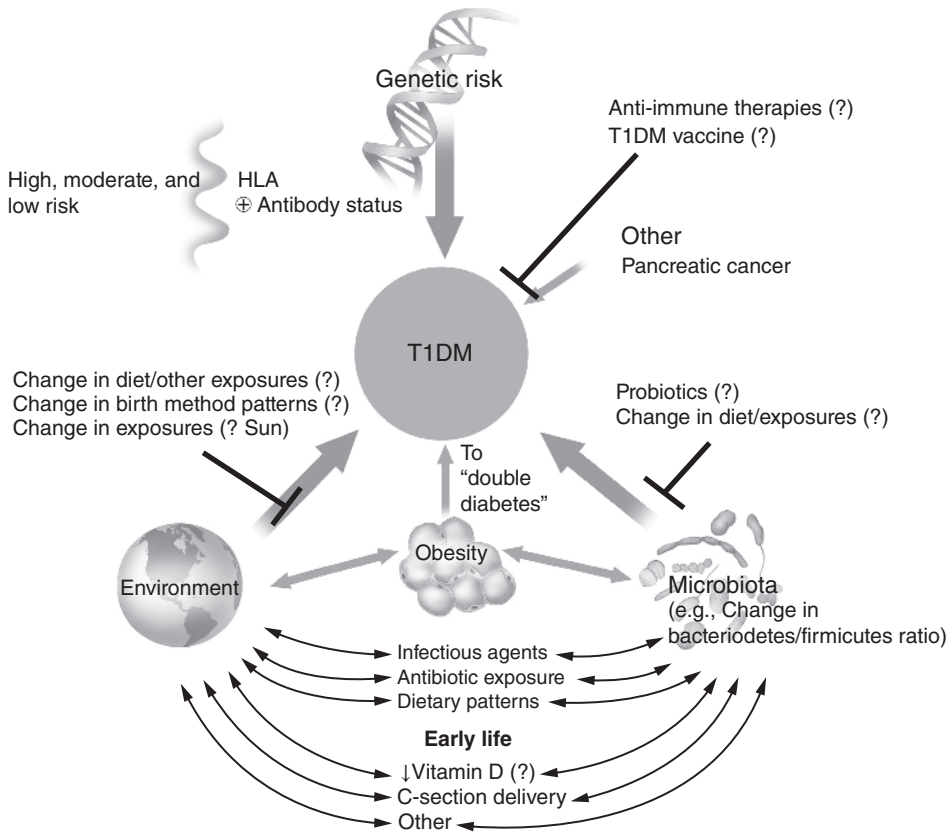


Figure 1.1 Contributory factors to the development of type 1 diabetes. Multiple factors, from genetic risk to environmental influences and perhaps the interface with the gut microbiome, contribute to the pathogenesis of type 1 diabetes. Given that in the vast majority of subjects, genetic risk/antibody status may be discerned and tracked, novel interventions hold promise, ultimately, for the prevention of type 1 diabetes. Dangers in type 1 diabetes, however, include the possible influence of obesity on the rise in “double diabetes.” Efforts to minimize possible contributory risks for type 1 diabetes are essential.

epidemiological evidence suggests that the incidence of type 2 diabetes is rising in childhood and adolescence, presumably due to increased obesity and reduced physical activity [44]. In type 2 diabetes, there is a very strong association with genetic factors. Many studies have addressed this issue and will be considered in the sections that follow.

Genetics of type 2 diabetes

The genetics of type 2 diabetes must take into account two key underlying etiologies of the disease, that is, β cell function (insulin secretion) and insulin resistance [1]. In the pre-GWAS era, the strong genetic contribution to type 2 diabetes was determined via family and twin studies [45]. From these efforts, a major gene found to be linked to type 2 diabetes included *CAPN10* (first described in a Mexican-American population) [46].

Others found that regions within chromosomes 5 and 10 were linked to type 2 diabetes, including within the latter, the *TCF7L2* gene [47, 48]. Multiple independent studies confirmed that SNPs in *TCF7L2* were linked to type 2 diabetes. The association of this gene with type 2 diabetes was confirmed in multiple distinct populations [49, 50]. Candidate gene approaches also identified *PPARG* and *KCNJ11* (the latter a potassium inwardly rectifying channel subfamily J member 11) as susceptibility genes for type 2 diabetes [51, 52]. It was not until the GWAS era that more modern and effective approaches were used to identify susceptibility genes in type 2 diabetes.

The first reported GWAS in type 2 diabetes was performed in a French cohort and was composed of 661 cases and 614 control subjects. The number of SNP loci covered in this study was 392,935. From this study, the following genes were identified as association signals in type 2 diabetes: *SLC30A8*, *HHEX*, *LOC387761*, and *EXT2*, and the study validated the association of the disease with *TCF7L2* [53]. Following this study, the Icelandic company deCODE Genetics and its colleagues confirmed the association of type 2 diabetes with *SLC30A8* and *HHEX* and added *CDKALI* [54]. Following this, three collaborating groups (Wellcome Trust Case Control Consortium/United Kingdom type 2 Diabetes Genetic consortium, the Finland-United States Investigation of NIDDM [FUSION] and the Diabetes Genetics Initiative [DGI]) published the findings confirming the association of type 2 diabetes with *SCL30A8* and *HHEX* and added newly discovered associations with *CDKALI*, *IGF2BP2*, and *CDKN2A/B* [55–57].

Following these discoveries, the need to increase sample size led to the above groups combining efforts to form the Diabetes Genetics Replication and meta-analysis, or DIAGRAM, consortium. Upon testing of an additional 4,549 cases and 5,579 controls, an additional five loci were discovered including *JAZF1*, *CDC123/CAMK1D*, *TSPAN/LGR5*, *THADA*, and *ADAMSTS9* [58]. By the continued addition of new subjects into these studies, an additional 12 new loci were reported in 2010 [59].

What has emerged from these studies is that many of the type 2 diabetes susceptibility loci are linked to insulin secretion based on human studies examining these loci with functional indices [45]. Hence, it is plausible that pancreatic β cell dysfunction may be a major factor linked to the susceptibility to type 2 diabetes. Examples of genes linked to type 1 diabetes are illustrated in Table 1.2.

The limitations of GWAS have been uncovered by results in a European twin study in which it was found that only approximately 10% of the known type 2 diabetes heritability might be explained by the loci identified in the GWAS [45]. To the extent that SNPs that might be important clues for type 2 diabetes but not be included in the screening modalities will influence missing heritability. In addition, it is quite possible that low-frequency risk variants may indeed possess large effects. Therefore, the next steps include next-generation sequencing strategies such as genome-wide (exome) sequencing [60]. It is hoped that such strategies, as well as utilization of other genetic tools (such as analysis of small RNAs and epigenetics analyses), will fill in the gaps of the missing heritability.

Pathogenesis of type 2 diabetes

In the sections to follow, we will consider the major factors speculated to contribute to the pathogenesis of type 2 diabetes.

Table 1.2 Examples of type 2 diabetes-associated loci.

Locus	Description	Comments
<i>TCF7L2</i>	Transcription factor 7-like 2	Wnt signaling and regulation of glucose metabolism
<i>PPARG</i>	Peroxisome proliferator activated receptor γ	Regulation of lipid and glucose homeostasis, anti-inflammation, and fatty acid oxidation
<i>KCNJ11</i>	Potassium inwardly rectifying Channel J, member 11	Roles in insulin secretion
<i>IGF2BP2</i>	Insulin-like growth factor-2 mRNA binding protein	Binds mRNA encoding IGF2
<i>WFS1</i>	Wolfram syndrome 1	Rare recessive neurodegenerative disorder, one component of which is diabetes
<i>CDKAL1</i>	CDK5 regulatory subunit associated protein1-like 1	Glucose homeostasis; likely roles in insulin secretion and sensitivity
<i>SLC30A8</i>	Soluble carrier family 30 (zinc transporter), member 8	Putative roles in insulin secretion
<i>HHEX</i>	Hematopoietically expressed homeobox	Putative roles in insulin secretion
<i>FTO</i>	Fat mass and obesity associated gene	Roles in methylation, associated with obesity and energy metabolism
<i>HNF1B</i>	Hepatocyte nuclear factor-1beta	Roles in pancreatic exocrine function; related to MODY (maturity onset diabetes of the young)

Type 2 diabetes and obesity

Obesity is considered a major risk factor for the development of type 2 diabetes. How does obesity mediate insulin resistance and diabetes? This is an intensely active area of investigation stimulated by the pioneering studies of Hotamisligil and Spiegelman. They set the stage for linking adipose tissue “inflammation” to insulin resistance in obesity. In 1993, they showed that tumor necrosis factor (TNF)- α mRNA was highly expressed in the adipose tissue of at least four different rodent models of obesity with consequent diabetes and that when TNF- α was neutralized in obese *fa/fa* rats, insulin sensitivity was improved, as evidenced by increased peripheral uptake of glucose [61]. In 2003, Weisberg and Ferrante showed that obesity in human subjects and in animal models was associated with increased infiltration and/or retention of macrophages in the perigonadal, perirenal, mesenteric, and subcutaneous adipose tissue [62]. Ferrante’s later work linked CCR2 and its chemoattractant functions to the increased infiltration of macrophages to adipose tissue in high fat feeding in mice [63]. Further work on the macrophage populations by Olefsky and colleagues suggested that expression of CD11c was a key contributor to obesity-associated insulin resistance [64]. Other studies have suggested that macrophage populations cause increased activation of NF- κ B and JNK MAP kinase signaling pathways, both linked to insulin resistance [65, 66]. Various genetic modification studies in mice suggest

that these pathways are required for the link between high fat feeding/obesity and the development of insulin resistance. Taken together, these seminal findings suggest that in obesity, inflammatory cells and their inflammatory mediators contribute to metabolic dysfunction, insulin resistance, and the ultimate development of type 2 diabetes, and that targeting these pathways may be beneficial in suppression of the adverse effects of obesity [67, 68].

Type 2 diabetes and the microbiome

As in type 1 diabetes, emerging evidence suggests links of the gut microbiome to type 2 diabetes [69]. Jumpertz and colleagues studied the effects of altering energy balance in human subjects on gut microbiota profiles; these studies were performed in 12 lean and 9 obese subjects who consumed two calorically different diets. Simultaneous monitoring of the gut microbiota was performed, together with pyrosequencing of 16S rRNA in feces and monitoring of stool calories by bomb calorimetry. These findings revealed that changes in the diet (nutrient load) altered the bacterial composition of the microbiome rapidly [70]. Specifically, increased proportions of Firmicutes and reductions in Bacteroidetes taxa were linked to increased energy harvest [70]. Such data directly link gut microbiota and nutrient absorption in the human subject. Interestingly, the ratio of Bacteroidetes and Firmicutes is also altered in animal models when the animals are subjected to dietary modulation [71]. Importantly, the specific mechanisms by which these distinct taxa exert these effects have yet to be identified.

It has been shown that the gut microbiome interfaces with the host to exert specific impact on catabolism of dietary toxins, micronutrient synthesis, absorption of minerals and electrolytes, and short chain fatty acid (SCFA) production which affects the growth and differentiation of gut enterocytes and colonocytes, as examples [69]. In germ-free raised mice, studies have revealed that the gut microbiome plays major roles in whole body metabolism including regulation of phosphocholine and glycine levels in the liver [72]. Further, germ-free rats displayed increased concentrations of conjugated bile acids which accumulate in tissues such as the liver and heart [73].

Work by Cho and Blaser revealed that the administration of subtherapeutic doses of antibiotics to young mice resulted in increased adiposity. In parallel, multiple effects on metabolism were noted, including changes in gene expression patterns linked to metabolism of carbohydrates to short chain fatty acids, increased levels of colonic short chain fatty acid levels, and altered hepatic metabolism of lipids and cholesterol. Examination of the taxa revealed that although there was no change in overall bacterial census, an increase in the relative concentrations of Firmicutes vs. Bacteroidetes was noted in the antibiotic-fed mice vs. the controls. These effects were found to parallel the changes in adiposity in the mice [74].

These and other studies reflect and underscore the dynamic nature of the composition of the gut microbiome. Indeed, in human subjects who underwent bariatric surgery, it was shown that the fecal material displayed significant changes in the composition of the microbiome. Specifically, Graessler and colleagues performed metagenomic sequencing and showed that overall the surgery resulted in a reduction in Firmicutes and Bacteroidetes and an increase in Proteobacteria [75]. Overall, establishing causality between the gut

microbiome constituents and obesity has not yet been accomplished; much work is underway to discern the specific means by which these varied taxa of bacteria may impact energy utilization and metabolism in processes linked to obesity.

Type 2 diabetes and vitamin D

As in the case of type 1 diabetes, vitamin D levels have been speculated to contribute to the pathogenesis of type 2 diabetes. Mezza and colleagues reviewed the available literature from human studies linking vitamin D deficiency to type 2 diabetes; their conclusion was that the results are “mixed”; whereas some studies suggested that deficiency of vitamin D was associated with increased type 2 diabetes, others identified no such association [76]. Similar caveats to the reported studies in type 1 diabetes prevailed in this setting as well. Specifically, many of the studies were cross-sectional, they did not take into account dietary factors, the subjects often displayed varied diabetes risk profiles as well as different patterns of serum vitamin D levels, and only single measurements of vitamin D were reported in many of the studies.

Others performed meta-analyses to identify potential relationships between levels of vitamin D and type 2 diabetes as follows: First, Forouhi and colleagues only considered prospective studies and reported a significant inverse association between the incidence of type 2 diabetes and the levels of vitamin D. Causality was not identified by the work of this report [77]. Second, in therapeutic interventions, George and colleagues reviewed the impact of supplementation with vitamin D and suggested that there was no evidence that such treatment was beneficial in terms of prevention of type 2 diabetes or improvement in glycemic control [78].

In addition to potential links to type 2 diabetes, vitamin D levels have also been explored with respect to insulin resistance. *In vitro* studies suggested a potential role of Vitamin D in preventing free fatty acid mediated insulin resistance in C2C12 (skeletal muscle) cells [79]. Several potential molecular mechanisms by which vitamin D may be associated with insulin include the following: (1) vitamin D may influence insulin action by stimulation of the expression of insulin receptors and amplifying glucose transport, and (2) the effects of vitamin D on the intracellular calcium pool may contribute to regulation of peripheral insulin resistance. Further links between vitamin D and type 2 diabetes have been suggested by relationships between vitamin D and insulin secretion [76]. In animal models, vitamin D-deficient diets have been associated with reduced insulin secretion [80].

Interventional studies on administration of vitamin D to insulin resistant/glucose intolerant subjects have yielded conflicting results that have not resolved the issue. At this time, several interventional clinical trials are under way to rigorously test the effects of vitamin D supplements on pancreatic β cell function and insulin resistance in human subjects highly vulnerable to the development of type 2 diabetes, and in other studies, in subjects newly diagnosed with type 2 diabetes or prediabetes syndromes [76].

Hence, although studies to date have not yielded clear results, the underlying concept that vitamin D supplementation may be of utility in type 2 diabetes and prediabetes syndromes remains an unanswered question and one that may be addressed when the results of ongoing trials are finalized and released. Clearly, however, this is an area that requires

further and standardized investigation; it is intriguing to link vitamin D metabolism to the composition and function of the gut microbiome. Indeed, Bargenolts reviewed the links between vitamin D metabolism and the gut microbiome and suggested a two hit model: First, an obesity-provoking diet shifts the microbiome from symbiosis to dysbiosis and the double hit of steatosis (fat accumulation in the various target organs) and inflammation together with the second hit (such as vitamin D deficiency) are necessary to activate signaling pathways that suppress adaptive insulin receptor signaling. Bargenolts hypothesized that alterations in dietary patterns, such as vitamin D supplementation and prebiotics, might improve prediabetes and type 2 diabetes management if initiated early in the process of obesity [81].

Type 2 diabetes and environmental pollution

Multiple epidemiologic studies, performed in such locations as Ontario, Canada; Ruh, Germany; the United States (multiple cohorts); Denmark; Iran; and Taiwan have shown associations between exposure to particulate matter (PM), such as in air pollution, and type 2 diabetes as well as insulin sensitivity. In those studies, varied measures of type 2 diabetes, glycosylated hemoglobin levels, or HOMA-IR were reported—all reflective of significant metabolic dysfunction [82].

Intriguingly, these studies suggest that primary inhalation of these PMs is linked mechanistically to inflammatory signals that are related to metabolism. How is this possible? Rajagoplan and Brooks summarized the work of various authors whose work implicated specific mechanisms by which this might occur [82]. For example, first, it is possible that alveolar macrophages subjected to PM exposure might release pro-inflammatory cytokines that secondarily cause a systemic inflammation, which might contribute to metabolic dysfunction. Second, it is possible that oxidative stress triggered by PM might activate local inflammatory signaling pathways whose products may impact the organism via systemic release. Third, it is plausible that the uptake of the PM by macrophages may cause presentation via dendritic cells to T lymphocytes within the secondary lymphoid organs, thereby triggering an immune/inflammatory-mediated response. Fourth, it is possible that the PM and their components may be able to gain direct access to the circulation and thereby cause inflammation and, potentially, contribute to insulin resistance. Finally, pathways linking the lung to the brain might be directly responsible for inflammation which might contribute to insulin resistance and metabolic dysfunction [82].

Based on these epidemiological and basic research studies, it is possible that strict efforts to combat air pollution and PM may ultimately lead to reduction in type 2 diabetes, prediabetes, and the metabolic dysfunction syndromes in human subjects.

Type 2 diabetes: summary

As Figure 1.2 illustrates, type 2 diabetes is associated with a very strong genetic predisposition based on the results of GWAS that were performed/confirmed in multiple populations. To date, although a number of the linked genes have been identified through earlier candidate and GWAS efforts, it is believed that the great majority have yet to be discovered. Interestingly, many of the genes uncovered by these approaches are linked to

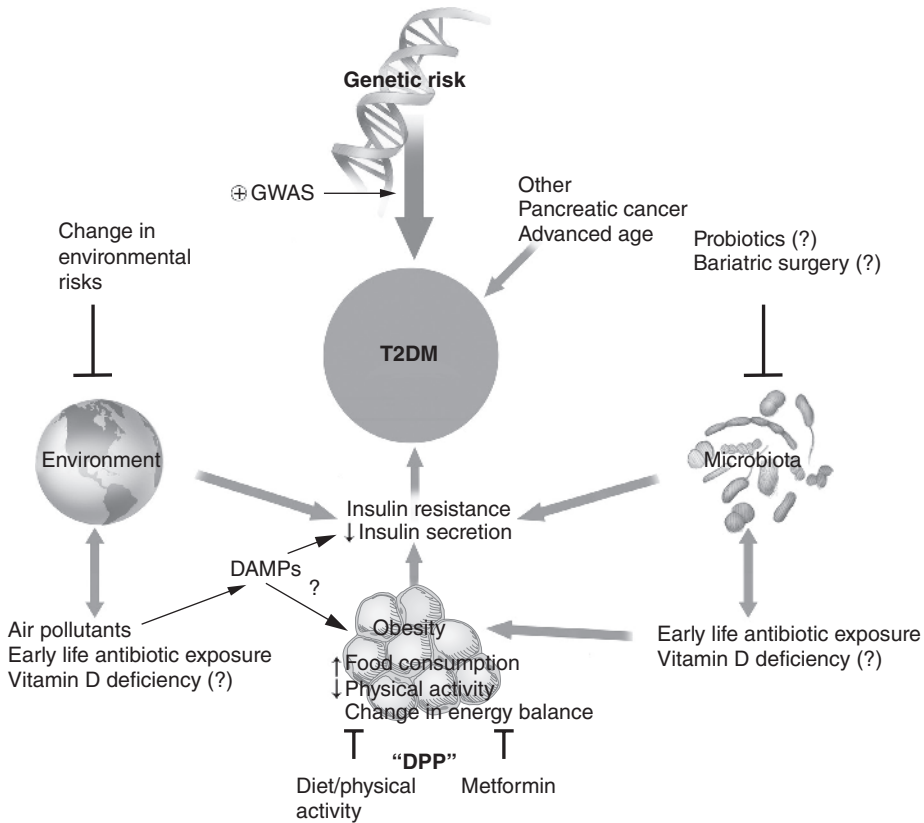


Figure 1.2 Contributory factors to the development of type 2 diabetes. A major cause of type 2 diabetes is accounted for by obesity and the reductions in physical activity. Strong genetic risk along with multiple influences in the environment and in the microbiome may substantially modify the risk of type 2 diabetes. The DPP study showed that in highly vulnerable subjects, metformin or change in diet/physical activity were able to prevent type 2 diabetes vs. placebo control. Efforts to augment protective therapies for type 2 diabetes are essential.

a greater extent to insulin secretion rather than resistance. Obesity, physical activity, and changes in lifestyle are believed to be the cause of the striking increases in type 2 diabetes world-wide. The rapid success of certain forms of bariatric surgery in reversing type 2 diabetes even before significant weight loss suggests that host interfaces with the gastrointestinal tract and other neuro/immune/metabolic systems contribute integrally to type 2 diabetes. Perhaps future studies will uncover roles for the gut microbiome directly in these findings; this remains to be determined.

Taken together, evidence suggests roles for the gut microbiome, vitamin D metabolism, and PM in air pollution in the exacerbation of type 2 diabetes and prediabetes syndromes. The extent to which type 2 diabetes may be reversed by adaptive modulation of body mass, gut bacteria, vitamin D levels, and air pollution remains an open question. However, the identification of putative aggravating factors to this disease hold promise for the ultimate prevention/reversal of type 2 diabetes, at least in certain subjects.

Gestational diabetes

Epidemiology and diagnosis

Oliveira and colleagues reiterated the definition of gestational diabetes (GDM) as follows: “glucose intolerance with onset or first recognition during pregnancy or as carbohydrate intolerance of variable severity diagnosed during pregnancy, which may or may not resolve afterward” [83]. GDM is important to diagnose and treat because it is linked to increased complications for both the mother and the developing child throughout the pregnancy and delivery. It is estimated that one-third of women with GDM remain affected with either type 2 diabetes or altered glucose metabolism post-delivery [1].

The consensus from epidemiological studies is that GDM is on the rise, at least in part due to increased obesity that is observed in women of child-bearing age. Barbour and colleagues reported in 2007 that the incidence of GDM had doubled over the prior six to eight years and that this paralleled the obesity epidemic [84]. Importantly, about 40–60% of pregnant women have no apparent risk factors for GDM, thereby stressing the urgent need to carry out screening on all pregnant women [85]. It is estimated that 15–50% of women afflicted with GDM will ultimately develop diabetes in the decades after pregnancy [86].

The diagnosis of GDM is generally based on the following algorithm: first, a fasting plasma glucose level is determined at the first surveillance visit for pregnancy. A normal value is considered less than 92 mg/dl. This is followed up by a 75-gram oral glucose tolerance test (OGTT) between weeks 24 and 28 of pregnancy. If the first visit fasting plasma glucose exceeds 92 mg/dl, then this suffices for the diagnosis of GDM and follow-up OGTT is not performed. If the initial fasting plasma glucose exceeds 126 mg/dl, this likely indicates that diabetes existed prior to the pregnancy [1, 83, 85]. By World Health Organization criteria, a level of glycosylated hemoglobin equal to or greater than 6.5% suffices for the diagnosis of probably diabetes. Based on the above findings, the 75-gram OGTT may be indicated. This consists of a fast between 8 and 14 hours; following the consumption of 75 grams glucose, plasma glucose is assessed at 1 and 2 hours. GDM is diagnosed when one or more of the values exceeds or is equal to 180 mg/dl or 153 mg/dl at 1 or 2 hours, respectively [1, 83, 85].

Metabolic factors and etiology of GDM

As in other forms of diabetes, the key to hyperglycemia in GDM rests on the forces that modulate insulin sensitivity and the ability of the pancreatic β cell to produce and release insulin. Human pregnancy is naturally characterized by an increase in insulin resistance; in normal pregnancy, both skeletal muscle and adipose tissue develop insulin resistance [87, 88]. In the normal setting, an approximately 50% reduction in insulin-mediated glucose disposal occurs in parallel with a 200–250% increase in insulin secretion, the latter required to maintain normal levels of blood glucose in the mother [88, 89]. Placental-derived hormones are critical in the mechanisms by which euglycemia is maintained [85]. For example, human placental lactogen (hPL) has been shown to increase up to 30 times during pregnancy; its role is to induce release of insulin from the pancreas during the

pregnancy [90]. A second placenta-derived hormone, human placental growth hormone (hPGH), also increases in pregnancy. This hormone, similar in its sequence and effects to human growth hormone, causes a severe decline in peripheral insulin sensitivity during pregnancy [91].

How are these changes manifested at the molecular level? Barbour and colleagues obtained access to skeletal muscle fibers from non-pregnant women, pregnant women without GDM, and pregnant women with GDM and examined the various key components of the insulin signaling pathway. They reported that skeletal muscle IRS-1 was reduced in normal pregnancy and even further reduced in GDM pregnancy vs. non-pregnant controls. Skeletal muscle levels of p85 α of PI3K (which normally blocks the association of PI3-kinase with IRS-1, overall leading to reduced GLUT4 translocation to the plasma membrane and, hence, less insulin-stimulated glucose uptake to the skeletal muscle) is higher in normal and GDM pregnancy vs. non-pregnant controls. However, small but significantly lower levels of p85 α in skeletal muscle were observed in GDM pregnancy vs. normal pregnancy. In adipose tissue, levels of IRS-1 were lower than those observed in the absence of pregnancy or in pregnancy without GDM and the levels of p85 α were higher in the adipose tissue of non-pregnant and normal pregnant subject tissue [85]. Furthermore, in GDM pregnancy, alterations in serine and tyrosine phosphorylation of IR and IRS-1 further suppress insulin signaling. Overall, the effect is to reduce GLUT-4 translocation to the membrane and thereby reduce glucose uptake even further in GDM pregnancy than in normal pregnancy [85].

In addition, inflammatory markers are altered in GDM pregnancy. For example, pregnant women with GDM display higher levels of tumor necrosis factor (TNF)- α in skeletal muscle than in non-GDM pregnancy, which persists even in the post-partum period [92]. Furthermore, levels of adiponectin, a hormone that serves to enhance insulin sensitivity, is reduced in GDM adipose tissue, thereby suggesting it may be linked to the syndrome of insulin resistance in pregnancy and especially in GDM pregnancy [93]. In addition, levels of PPAR-gamma decline to greater degrees in GDM adipose tissue [94]. Such a change favors and increases lipolysis, thereby increasing the release of free fatty acids, molecular mediators that may serve to mediate insulin resistance and hepatic glucose production.

Taken together, these underlying factors serve to significantly increase insulin resistance, particularly in GDM pregnancy. Furthermore, together with impaired β cell function and reduced adaptation of the β cell during pregnancy, multiple factors converge to increase risk and severity of GDM in pregnant women.

Gestational diabetes and potential roles for vitamin D

Alzaim and Wood have reviewed the existing literature for potential roles of vitamin D deficiency in GDM. They summarize the results of five cross-sectional studies which suggested that women with GDM had poorer vitamin D status vs. pregnant women without GDM [95]. However, these authors provided a number of caveats to these five studies, as follows: First, all of the studies were cross-sectional in design. Second, there was inconsistent accounting for such factors as ethnicity, season during pregnancy, physical activity of the subjects, the number of pregnancies (particularly the order of the current pregnancy under study), and body mass index pre-pregnancy. Third, in most of the studies the levels

of vitamin D were measured late in the pregnancy and after GDM had already developed. There were no reports of the vitamin D levels pre-pregnancy; hence, the potential predictive value of the pre- to pregnancy state were not available for consideration [95].

In fact, in only one study by Zhang and colleagues was a prospective cohort study performed among mostly non-Hispanic Caucasian pregnant women in the United States (Tacoma, Washington). In the study, the levels of vitamin D were measured at approximately the 16th week of pregnancy. Overall, the authors concluded that (1) vitamin D deficiency was found in 33% of women who developed GDM vs. 15% in the women who did not develop GDM; (2) at 24–28 weeks gestation, the risk of developing GDM was 2.66-fold higher in vitamin D-deficient women vs. the non-vitamin D-deficient pregnant women; and (3) when the results were limited to non-Hispanic Caucasian women only, the risk of developing GDM was 3.77-fold higher with vitamin D deficiency vs. without vitamin D deficiency [96]. The researchers pointed out that a caveat to this study is that the levels of vitamin D were only measured once during the course of the study; therefore, it remains possible that those levels as reported were not consistent during the entire course of the pregnancy.

At this time, interventional studies on the use of vitamin D in pregnancy are quite limited. In one study by Rudnicki and Pedersen, vitamin D was administered by intravenous route followed by oral supplementation to pregnant women with established GDM. The study showed that after the intravenous dose of vitamin D, fasting serum glucose declined significantly. However, these benefits were not sustained after the patients began to take the oral supplementation [97]. It was speculated that perhaps the oral doses might have been too low or that pharmacologic factors based on the precise form of vitamin D administered to the subjects accounted for the reduced efficacy.

Taken together, the available data strongly suggest that definitive conclusions will be essential to determine if and when to administer vitamin D to pregnant subjects and whether or not specific subsets of pregnant women are most likely to benefit.

GDM: Summary

In summary, epidemiologic evidence indicates a rise in GDM that, perhaps not surprisingly, parallels the increase in obesity and its consequences. Given that GDM exerts potentially damaging effects to both mother and the developing fetus, prevention and therapeutic efforts are essential to ensure safer pregnancies and improved outcome for the fetuses. In this context, the potential benefits of vitamin D supplementation have yet to be conclusively addressed. However, preliminary evidence from cross-sectional studies might suggest a link between deficiency in vitamin D and GDM. Further studies are required to address this question.

Maturity onset diabetes of the young

Maturity onset diabetes of the young (MODY) is a group of monogenic disorders, inherited in an autosomal dominant manner, in which specific genetic mutations causing defects in insulin secretion but not generally with insulin action result in hyperglycemia,

usually before the age of 25 years. MODY is believed to be responsible for approximately 2–5% of all cases of diabetes [1, 98]. To date, mutations in at least six distinct genes have been identified to account for MODY [99].

The most common form of MODY is a mutation in chromosome 12 in the gene *HNF1 α* . This gene encodes for hepatic nuclear factor 1 α [100]. A second form of MODY is associated with mutations in the gene encoding glucokinase; this mutation is located on chromosome 7p [101, 102]. Glucokinase serves to convert glucose to glucose-6-phosphate; the metabolism within this pathway is then responsible for stimulation of insulin secretion. In this setting, higher levels of glucose are thus required for stimulation of insulin secretion. Other forms of MODY result from mutations in the following genes: *HNF4 α* [103], *HNF1 β* [104], *IPF1* (insulin promoter factor) [105], and *NEUROD1* [106]. Beyond these mutations, numerous others have been reported that account for the MODY syndrome; however, they are much rarer.

Because of the age of onset, MODY disorders are often misdiagnosed as type 1 or type 2 diabetes. Key components for diagnosis therefore include diagnosis before age 45 years, the absence of β cell autoimmunity (auto-antibodies), absence of obesity or any features of the metabolic syndrome, sustained production of insulin despite hyperglycemia, and because of the genetic nature of the disease, a strong family history [107]. It is important to note that the presence of MODY does not, of course, exclude obesity. These criteria are meant to inform possible clues to direct the practitioner to a diagnosis of MODY vs. a more typical form of diabetes (type 1 or type 2).

Summary

Other notable causes of diabetes

In addition to the most common causes of diabetes detailed above, it is important to note that there are many other less common causes that require mention, such as those forms of diabetes induced by drugs (e.g., corticosteroids) or as components of distinct autoimmune syndromes. Refer to the review [11] which details the myriad etiologies underlying the most common and the very rare causes of this disease [1]. One seminal link to note is the association between pancreatic cancer and diabetes. Pancreatic cancer is the fourth leading cause of death due to cancer in the United States and the sixth leading cause of cancer death in Europe and Japan [108]. Cigarette smoking remains an extremely strong risk factor; given the decline of smoking in the last decades, the incidence in this form of cancer has declined, but only in countries in which smoking has generally declined as well [108]. Pancreatic cancer remains highly intractable to curative efforts; the five-year survival rate is less than 5%. Diabetes has important “bidirectional links” to pancreatic cancer. Type 2 diabetes has been shown to increase the risk of pancreatic cancer [109]. On the other hand, it has also been noted that new-onset diabetes may be a spotlight that uncovers the presence of undiagnosed pancreatic cancer, especially in patients with weight loss or in those with a strong family history of the disease [109].

Prevention of diabetes: on the horizon?

Given the sobering epidemiological data on the rise in types 1 and 2 diabetes, it is essential to query, Are the current trends in increases in the most common forms of diabetes, types 1 and 2 diabetes, a foregone conclusion? Is all hope lost? The answer is a firm “no.” In the case of type 1 diabetes, clinical trials aimed at new-onset and at-risk type 1 diabetes (the latter antibody-positive subjects), using various forms of immunotherapy and other strategies, are well under way. A key challenge and benchmark in this regard will be the identification and validation of prognostic and predictive biomarkers for the eventual diagnosis of type 1 diabetes. Such immune interventions are now viewed as best when used in “combination” strategies [110].

What about type 2 diabetes? As discussed above, obesity and reduced physical activity clearly are major risk factors. In the Diabetes Prevention Program (DPP) trial, subjects at high risk for type 2 diabetes (elevated fasting and post-load plasma glucose concentrations) were randomized to placebo, metformin, or lifestyle modification (weight loss and physical activity). Over a 2.8-year follow-up, the incidence of diabetes development was 11%, 7.8%, and 4.8% in the placebo, metformin, and lifestyle groups, respectively. Lifestyle intervention reduced the incidence of type 2 diabetes by 58% and metformin reduced the incidence of type 2 diabetes by 31% compared to that observed in placebo treatment. Interestingly, the lifestyle modification strategy arm was significantly more beneficial than the metformin arm [111].

These data strongly suggest that there is likely no “point of no return” in types 1 and 2 diabetes. Intense efforts aimed at reducing the development of type 1 and type 2 diabetes hold great promise to mitigate the devastation of these diseases.

Additional discussion about prevention of diabetes mellitus can be found in chapters 2 and 4.

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