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Distinct Genetic Backgrounds in Quantitative Traits Preceding Type 2 Diabetes: One Reason for Missing Heritability

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors PRB and DKS wrote, edited and approved the manuscript.

Review Article

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ABSTRACT

Genome-wide association studies (GWAS) have identified variants associated with type 2 diabetes (T2D), but less than five percent of the genetic variance associated with T2D is accounted for in GWAS studies performed using T2D as endpoint. These findings suggest relevance of assessing preceding quantitative traits (QTs) or sub-phenotypes and their respective markers for contributing to the appearance of glycemic thresholds defining T2D. The QTs first appear in gestation and childhood when birth weight and childhood weight gain have an influence on T2D. Changes in fetal growth are associated with metabolic programming for T2D in adulthood. Subsequently gains in body fat leading to obesity, ectopic lipid deposition in liver and muscle, dyslipidemia and hypertension express in the β-cells, hypothalamus, adipocytes, myocytes, liver and kidney, and are associated with worsening insulin resistance and β-cell failure preceding overt T2D. Obesity, an increasingly common trait is associated with gene variants that regulate energy balance but have no association with T2D with few exceptions. For instance, *FTO* gene variants are associated with early onset of obesity and have also been associated with T2D and hypertension suggesting pleiotropism. Non-alcoholic fatty liver disease (NAFLD) has an independent genetic background and is considered as a new addition to the metabolic syndrome. Association of NAFLD with dyslipidemia,

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cardiovascular disease and hepatic insulin resistance contributes to progression to T2D. The classic dyslipidemia encountered in insulin resistant states consisting of increased triglyceride, low high-density lipoprotein cholesterol (HDL-C) and molding of triglyceride-containing lipoproteins to form atherogenic LDL particles and dysfunctional HDL particles, has strong environmental and genetic association. In addition evidence is accumulating to show that the abnormal lipoproteins have a direct effect on β-cells leading to failure and possible acceleration of diabetes onset. Consequently known variants affecting either the dyslipidemia or β-cell function have a compound effect on T2D. Like other preceding traits the genetic background for commonly encountered hypertension is independent of diabetes although it remains a predictive trait. However, increasing evidence supports a role for the renin-angiotensin system in causing oxidative stress and insulin resistance. Finally when glucose intolerance has progressed to overt T2D, reversibility is usually no longer possible; therefore recognition of preceding pre-diabetic glycemic thresholds and both their genetic and environmental associations may facilitate and serve as signals for prevention. Thus a better understanding of the sequential expression of the respective QTs and genetic variation affecting their metabolic effects leading to T2D could initiate more effective prevention strategies.

Keywords: Genetics; diabetes; pre-diabetes; quantitative traits; fetal growth; obesity; non-alcoholic fatty liver; hypertension.

1. INTRODUCTION

There is accumulating evidence that insulin resistance and associated traits, traditionally known as the 'metabolic syndrome', precede β-cell failure by several years [1,2]. These observations indicate that there is a window of time during which prediction markers could be used for timely intervention and prevention of T2D. The window may extend further back than adult life, since preceding traits have been identified in the fetus, child and adolescent; consequently they affect early pathogenesis of T2D (Fig. 1). Although the age of onset for risk factors has been decreasing, the likelihood of appearance of a risk factor increases with age. This has been shown in epidemiologic and genetic studies, which have mostly been conducted on adult populations over age 18 years, and usually age has been assessed as a confounder in data analysis. Nevertheless, onset of common predisposing traits such as obesity may represent more significant lifetime effects in childhood and adolescence than if they presented later. Moreover, since components of the metabolic syndrome are often present before the onset of T2D [3,4], it is important to recognize the commencement of the respective quantitative traits (QTs) during gestation, and their persistence through childhood to adulthood [5], leading to T2D [6]. However, it is interesting to observe from the genetic studies that the respective sub-phenotype or trait-associated genetic variants do not usually overlap with T2D variants suggesting relatively independent genetic backgrounds. These findings suggest that shared interaction with lifestyle- and obesity-related environmental factors is significant or that the traits themselves activate additional metabolic pathways leading to T2D.

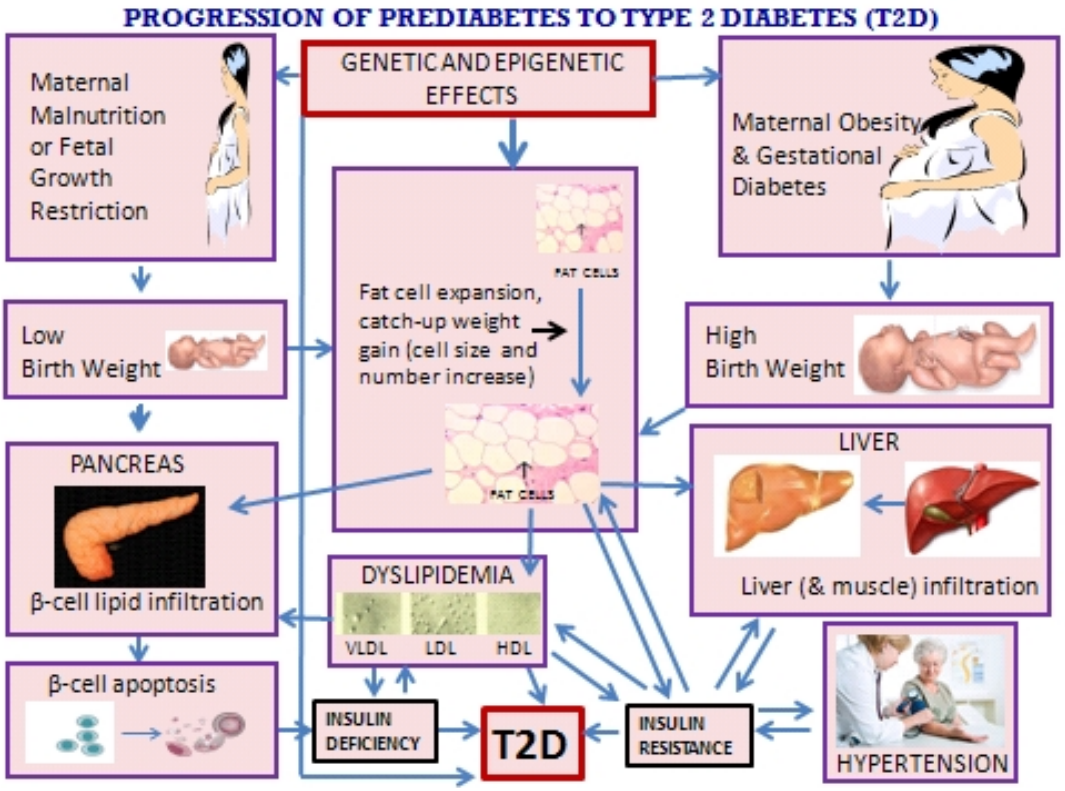

Fig. 1. A sequence of pre-diabetic phenotypes interacting with genetic and epigenetic effects begins in the fetus with effects of maternal gestational nutrition on genetic endowment, affecting growth of the baby. Maternal malnutrition or fetal growth restriction causes small babies, and maternal over-nutrition or hyperglycemia with gestational diabetes results in large babies. During childhood exposure to nutritional excess results in obesity associated with rapid catch-up fat deposition and weight gain in small babies. The onset of obesity, dyslipidemia and non-alcoholic fatty liver disease (NAFLD) occurs in children and adults and affect the β-cell resulting in insulin deficiency. Muscle and liver fat storage and hypertension are associated with insulin resistance but the biochemical relationships are complex and bidirectional. Both insulin resistance and β-cell failure lead to T2D and may be compounded by genetic variants

An analogy for the proposed model in which the genomic variation contributes to T2D is that of a train with a sequence of coaches loaded with cargo in a city of origin for travel and delivery of goods to a destination city (Fig. 2); but the goods are also delivered to towns *en route*, for example tractors for a rural agricultural town, and boats for a coastal town. When the train arrives at the destination the cargo from a significant number of the coaches has been depleted. An economist is hired by the railroad company to assess the effect of the initial load at the origin on the economy of the destination city over a ten year period. However, he finds only a small association and fails to account for empty coaches on arrival. Further investigation reveals that unloaded goods in five regions; for example tractors for farmers and boats for fishermen, resulted in increased production in the respective regions of the country. The resulting local produce resulted in substantially increased purchase and

consumption in the destination city. When the environmental contributions from five rural and coastal regions surrounding the stations for the two *en route* deliveries were accounted for, the initial loading of the train was calculated to be highly effective despite the changes in cargo and some empty coaches on arrival. This analogy based on indirect regional effects on the final outcome argues to support that gene variant association based on a final T2D phenotype such as overt diabetes may miss significant metabolic pathways and associated environmental interactions that have determined the outcome – or the onset of T2D.

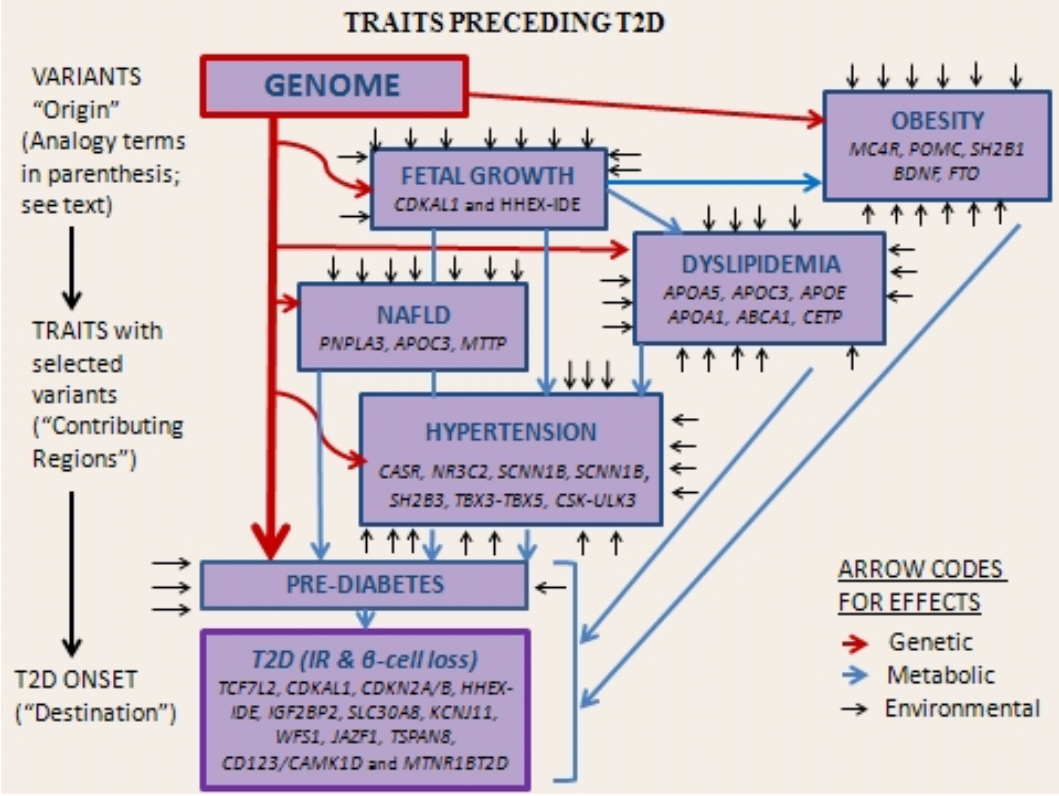

Fig. 2. Quantitative traits (QTs) with different genetic backgrounds precede and predict T2D but seldom share gene variants (see text). The red line represents connection of the genome to T2D and traits. Significant key genes are listed in each box. Metabolic effects (blue lines) attributable to each trait are increased throughout life, enhanced by the environment (small black arrows) and predict T2D onset. Pre-diabetes precedes T2D, is influenced by environment and is potentially reversible. Metabolic effects on β-cell function may have a compound effect on β-cell failure since most of the T2D-associated variants affect β-cell metabolism. The analogy of a train (see text) beginning at the station of origin, affecting each region *en route*, and arriving at the destination (T2D), is indicated in parentheses to the left of the diagram (large black arrows)

Since GWAS have only explained a small proportion of the genetic variance (up to 5%) - by most estimates, it is reasonable to propose that separate QTs and their respective biochemical effects preceding T2D onset may accelerate insulin resistance and β-cell failure and account for an essential proportion of the variance that is missed in studies restricted to

T2D as the only phenotype. This hypothesis is supported by findings that prediction models based on clinical risk factors such as age, sex, race, parental history of T2D, BMI, mean arterial pressure, fasting glucose, triglyceride and high-density lipoprotein cholesterol (HDL-C, predicted T2D as well as a genotype score in the CARDIA study [7] and the same was true for adolescents in the Bogalusa study when demographics, family history, physical examination and routine biomarkers were used to predict T2D [8]. These findings suggest that the clinical characteristics and biomarkers representing T2D-preceding traits have a strong effect on T2D and therefore could add to or alter genetic factors associated with T2D.

The case for significant effects of preceding T2D traits is supported by evidence that metabolic syndrome criteria in the Framingham Heart Study increased the 7- to 11-year risk for T2D more than it increased risk for cardiovascular disease. Furthermore, the odds ratio for T2D was proportionate to the number of criteria [9]. Also effects of the metabolic syndrome can be independent of obesity. Non-obese individuals with the metabolic syndrome had a 7-year cumulative incidence of T2D which was greater than obese individuals without the metabolic syndrome, representing a metabolically healthy obese sub-phenotype [10]. In this review we present current evidence for genetic determination of the main pre-diabetic traits such as fetal growth, obesity, non-alcoholic fatty liver disease (NAFLD), dyslipidemia, and hypertension during childhood and adolescence and how each of them may activate metabolic pathways that lead to pre-diabetes and T2D. The evidence is presented in support of the hypothesis that five traits with their respective QTs preceding the hyperglycemic traits predict T2D despite distinct genetic backgrounds. Table 1 summarizes major known genes associated with each QT preceding T2D.

2. FETAL GROWTH: PROGRAMMING IN PREGNANCY

Over the past two decades, there has been accumulating evidence to show a strong relationship of low birth-weight (usually defined as <2.5 kg) to metabolic syndrome traits in adulthood including obesity, hypertension and progression to T2D [11]. Based on initial studies that showed a relationship of birth-weight to impaired glucose tolerance at age 64 years, Barker et al. [12] proposed the 'thrifty phenotype' hypothesis. It states that T2D and metabolic syndrome traits result from the effects of poor nutrition in early life, which produces permanent changes in glucose-insulin metabolism. Their pioneering work showed a link between birth-weight and both diabetes and cardiovascular disease in adulthood. The initial observations were well replicated, supporting the argument that fetal growth restriction may result in permanent and progressive changes leading to T2D. It has also been shown that subsequent increase in body mass index (BMI) of the children who were born small predicts disease risk in adults. This suggests that risk is increased when infant nutrition exceeds gestational nutrition but not when the nutritional supply is matched [13,14]; for example weight gain in the first three months may determine insulin resistance as early as adolescence [15] or late childhood [16]. Furthermore, fetal growth restriction by genetic or nutritional causes could set the stage for the small baby to gain fat as the preferential tissue resulting in a "catch-up fat" phenotype [17].

Table 1. Established genetic determinants of the traits preceding type 2 diabetes

Trait	Gene	Chromo-some	Entrez Gene ID	Role
Fetal Growth				
	INS	11 p15.5	3630	Signaling hormone increases permeability to monosaccharides, amino acids and fatty acids
	INSR	19 p13.3	3643	Signaling hormone receptor tyrosine kinase
	IPF1/PDX1	13q12.1	3651	Activates insulin, glucokinase, and glucose transporter T2D gene transcription
	KCNJ1	11q24.3	3758	Associated with Bartter syndrome characterized by salt wasting, hypercalciuria, and low blood pressure
	ABCC8	11p15.1	6833	Regulator of ATP-sensitive K(+) channels and insulin release
	HNF1B	17q12	6928	Involved in diabetes syndrome and noninsulin-dependent diabetes mellitus
	ADCY5	3q21.1	111	Gene variants influence fasting glycemic traits and insulin resistance
	HHEX-IDE	10q23.33	3087/3416	Transcription factor involved in hematopoietic differentiation, pancreatic development and insulin secretion
	GCK	7p14	2645	Modulates insulin secretion, glycolysis, energy pathways
	TCF7L2	10 q25.2	6934	Transcription regulator influences insulin secretion
	HNF1A	12q24.31	6927	Regulates tissue-specific expression of genes especially in pancreatic islets and liver
Obesity				
	FTO	16q12.2	79068	Severe obesity/insulin resistance
	MC4R	18q21.32	4160	Member of G-protein coupled receptor family, signaling hormone involved in energy homoeostasis
	PPARG	3p25.2	5468	Transcription factor involved in adipogenesis and type 2 diabetes risk
	ADIPOQ	3q27.3	9370	Adipose tissue specific protein involved in insulin sensitizing and anti-atherosclerotic properties
	LEPTIN	7q31.3	3952	Signaling hormone affects central nervous system to inhibit food intake and energy expenditure
	POMC	2p23.3	5443	Mutations in this gene linked with early onset obesity
	SH2B1	16p11.2	25970	Obesity locus associated with myocardial infarction in T2D patients
	BDNF	11p14.1	627	Development, survival and differentiation of selected neuronal populations

NAFLD*				
	ADIPOR2	12p13.31	79602	Hormone secreted by adipocytes and acts as an anti-diabetic factor
	MTTP	4q23	4547	Catalyzes the transport of triglyceride, cholesterol ester, and phospholipids between phospholipid surfaces
	APOCIII	11q23.3	345	Inhibits lipoprotein lipase; delays catabolism of triglyceride-rich particles
	PNPLA3	22q13.31	80339	Triacylglycerol lipase that mediates triacylglycerol hydrolysis in adipocytes
Dyslipidemia				
	APOE-CI-CII-CIV	19q13.32	2282	Cluster of triglyceride-rich lipoprotein receptor ligands for LDL receptor–related proteins
	APOAV-AIV-CIII-AI	11q23.3	117536	Cluster of apolipoproteins plays an important role in regulating the plasma triglyceride levels
	PCSK9	1p32.3	255738	Decreases plasma- and LDL- cholesterol and provides protection from coronary artery disease
	CETP	16q13	1071	Exchanges cholesterol esters for triglycerides from HDL and triglyceride-rich lipoproteins
	LCAT	16q22.1	3931	Required for remodeling HDL particles into their spherical forms
	ABCA1	2p23.3	2646	Mutations in this gene cause Tangier disease and familial HDL deficiency
Hypertension				
	WNK1	12p13.33	65125	A key regulator of blood pressure by controlling the transport of sodium and chloride ions
	KCNJ1	11q24.3	3758	Associated with Bartter syndrome characterized by salt wasting, hypercalciuria, and low blood pressure
	NPR3	5p13.3	4883	Regulate blood volume and pressure, pulmonary hypertension, and cardiac function
	GUCY1A3	4q32.1	2982	Regulate blood volume and Na+ balance
	GNAS	20q13.32	4686	Involved as modulators or transducers in various transmembrane signaling systems
	NPPA-NPPB	1q36.22	9757	Associated with intracellular guanylyl cyclase activity and involved in homeostasis of body fluid volume
	CYP17A1	10q24.32	1586	Gene variants associated with hypertension
	ARRDC3	5q14.3	57561	Gene variants associated with diastolic blood pressure
	C21orf91	21q21.1	54149	Gene variants associated with hypertension

Hyperglycemic Traits (Pre-T2D) Fasting Glucose	*GCKR*	2p23.3	2646	Enzyme regulator, controls activity of glucokinase in liver and brain
	G6PC2	2q24.3	57818	Transport channel, key role in glucose homeostasis
	MTNR1B	11q21-q22	4544	Melatonin receptor regulates physiological and neuroendrocrine functions
	DGKB-TMEM195	7p21.2	1607/392636	Plays a key role in cellular processes
	GCK[†]	7p14	2645	Modulates insulin secretion, glycolysis, energy pathways
	ADCY5	3q21.1	111	Gene variants influence fasting glycemic traits and insulin resistance
	MADD	11p11.2	8567	Gene variants influence fasting glycemic traits and insulin resistance
	CRY2	11p11.2	1408	Gene variants influence fasting glycemic traits and insulin resistance
	FADS1	11q12.2	3992	Gene variants influence fasting glycemic traits and insulin resistance
	GLIS3	9p24.2	169792	Gene variants affect fasting glucose and T2D
	SLC2A2	3q26.1	6514	Plays role in human β-cell function and impacts glycemic traits
	PROX1	1q41	5629	Gene variants affect fasting glucose and insulin
2 hour Glucose	*GIPR*	19q13.3	2696	Stimulate insulin release in the presence of elevated glucose
	VPS13C	15q22.2	54832	Gene variants influence glycemic traits and insulin resistance
β-cell function (T2D)	*TCF7L2*	10 q25.2	6934	Transcription regulator influences insulin secretion
	SLC30A8	8q24.11	169026	Facilitates transportation of zinc from cytoplasm into insulin containing vesicles
	IGF2BP2	3q27.2	10644	Regulatory enzyme influences insulin secretion
	CDC123	10p13	8872	Involved in transcription regulation, insulin secretion
	HHEX-IDE	10q23.33	3087	Transcription factor involved in hematopoietic differentiation, pancreatic development, insulin secretion
	CDKN2A/B	9p21.3	1029	Enzyme, anti-oncogene involved in pancreatic carcinomas, type 2 diabetes
	KCNJ11	11 p15.1	3767	Ion channel transporter
	KCNQ1	11p15.5	3784	Encodes a voltage gated K channel required for repolarization phase of the cardia action, associated with T2D

**NAFLD- Nonalcoholic fatty liver disease*

It is also evident that gestational weight gain of the mother is an independent predictor of obesity during infancy, even occurring when the maternal pre-pregnancy weight is normal [18]. Thus maternal weight gain during pregnancy predisposes the child to become obese continuing to adulthood [19]. In addition exposure to high maternal glucose during gestation can result in large babies giving rise to observations that risk for T2D is determined by large birth-weight; meaning that the relationship of both low and high birth weight to subsequent T2D can be characterized as bimodal [20]. Also, maternal fat intake during gestation influences glucose tolerance of the offspring. Therefore it appears likely that excess maternal nutrient supply, particularly as fat, may have long-term effects [21]. A review of eleven animal models investigating glycemic control in offspring of mothers exposed to a high fat diet during gestation has identified risk for T2D and obesity in the offspring. The effect was stronger in males, and glucose intolerance was independent of maternal obesity, birth-weight or post-weaning macronutrient intake [22]. Studies have shown that fetal systems are also modulated by metabolic factors such as the hypoxic effect of changes in blood supply, oxidative stress, DNA methylation, histone acetylation, transcription factors and hormones such as cortisol, insulin and leptin. These factors could serve as a basis for prevention, treatment and for further studies to determine interaction of the metabolic factors with genotypes [23]. Epigenetic effects in the form of biochemical modification of DNA, such as methylation, may not only occur in the fetus but continue in later life [24,25] and influence traits such as NAFLD [26].

Evidence for a genetic background for fetal growth is increasing significantly supporting possible interaction with gestational factors. Observation that mutations in the glucokinase gene (*GCK*) resulted in reduced birth-weight gave rise to the hypothesis that rare variants that modify insulin secretion or action could not only cause monogenic diabetes, but also low birth-weight [27,28]. This has been supported by findings that reduced birth-weights occur due to other known monogenic mutations causing early onset diabetes, such as *INS, INSR, IPF1, KCNJ11, ABCC8* and *HNF1B* [29-32]. GWAS on birth-weight has revealed association of fetal loci near *ADCY5*, *CDKAL1* and *HHEX-IDE* genes. The same risk allele at the *ADCY5,* associated with low birth-weight, also predisposes to T2D [33]. Incidentally, the effects on fetal growth restriction can potentially be offset by maternal alleles at *GCK* and *TCF7L2* that result in reduced maternal insulin and consequent growth stimulation by fetal hyperinsulinemia secondary to trans-placental passage of maternal glucose [33]. The hyperglycemic effect on the fetus is known to interact with fetal *HNF1A,* a known maturity onset diabetes of young (MODY) gene, resulting in earlier onset of the diabetes [34]. GWAS conducted by the “Meta-Analyses of Glucose- and Insulin-related traits Consortium” (MAGIC) has identified seven maternal loci associated with birth-weight accounting for a similar proportion of variance to maternal smoking. Two of the loci, *ADCY5* and *CDKAL1* were replicated from previous studies [35] and predicted T2D supporting association of common variants with fetal growth and subsequent metabolic events predisposing to T2D [36]. However, due to possible population differences, the low birth-weight was not explained by genetic variation in the *ADCY5* in Asian Indians, although these variants were associated with elevated glucose and reduced insulin response in early adulthood [37].

3. OBESITY

Obesity continues to be of great concern since the QTs associated with obesity have been robust predictors of T2D and remain highly associated with metabolic events leading to T2D. Although there is some leveling off in obesity trends in the United States [38], global trends in overweight and obesity are increasing [39] with serious implications for health. Obesity is often unrecognized and screening and treatments are generally inaccessible and often

ineffective [40] accounting for secular changes in BMI in adults [41] and children [42]. This leads to obesity-associated disease beginning in childhood [43] that progresses over time [44] with increased loss of β-cell function and insulin resistance [45]. Associated metabolic changes include ectopic fat deposition [46], fat-induced insulin resistance [47], β-cell dysfunction [48], and endoplasmic reticulum stress leading to β-cell apoptosis [49,50]. Waist circumference or BMI are strongly associated with insulin resistance [51] and are both highly correlated however BMI has been used as an obesity trait in most genetic studies because of its availability and widespread acceptance.

To explain the high heritability of obesity, it has been proposed that T2D results from an array of "thrifty" genes that are latent in the normal state and efficiently store nutrients for times of need [52]; but with constant nutritional excess over extended periods, dyslipidemia and ectopic fat deposition in the liver and muscle lead to insulin resistance and diabetes [47,53]. Constant and excessive nutritional excess common in modern cultures has contributed to the world-wide escalation in obesity and subsequent T2D incidence by interacting with the genetic backgrounds of the QTs for fetal growth, fatty liver disease, dyslipidemia and hypertension, and their metabolic effects that lead to T2D as described in this review. However, common genetic variation in very few obesity or T2D genes have been identified to promote excess nutrient storage, suggesting that trait-specific metabolic processes are primed to promote excessive nutrient storage, and subsequently lead to dysfunctional carbohydrate homeostasis and fat metabolism. For example accumulation of excessive diacylglycerol in the liver is associated with accumulation of liver and muscle fat leading to defective insulin action [54], particularly in genetically susceptible populations such as Asian Indian men [55].

Hypothalamic control of appetite has been associated with uncommon forms of monogenic obesity, which have provided insight on mechanisms for the development of obesity in the general population [56]. Studies on monogenic obesity cases and their families have led to definition of metabolic pathways in animal models; in particular the leptin-melanocortin pathway involved in satiation [57]. *MC4R* encoding the melanocortin-4 receptor, is the commonest of the clinically occurring single gene defects associated with severe obesity [58]. Furthermore it, is realistic to hypothesize that polymorphisms within these known genes are involved in polygenic inheritance of obesity in the general population, since 60% of the BMI variance within a population is accounted for by genetic variance [59]. A large BMI GWAS conducted in 123,865 individuals using 2.8 million single nucleotide polymorphisms (SNPs) and follow-up in significant numbers revealed 14 known obesity susceptibility loci and identified 18 new loci [60]. Some of the variants near loci, such as *MC4R, POMC, SH2B1* and *BDNF* are known hypothalamic regulators of energy balance and a locus near *GIPR*, an incretin receptor present on β-cells, is regulated by intestinal incretins [60]. This observation supports a predisposition to T2D with pleiotropic effects in the hypothalamus and β-cell.

An intronic variant (rs9939609) in the *FTO* (fat mass and obesity-associated) gene was found to be associated with T2D, but the association was abolished when adjusting for BMI suggesting that the *FTO* variant influences T2D by its effect on obesity, a powerful diabetes-determining factor [61]. The association with obesity has been confirmed in longitudinal studies in childhood [62,63] including a Dutch study showing association with higher BMI, fat mass index, and leptin concentrations during puberty but declining at ages 13-14 years, a finding thought to be consistent with hormonal effects at pubertal onset [64]. The association of severe obesity with *FTO* has been studied using a haplotype approach. Using linkage disequilibrium (LD) block structure of a region surrounding the candidate *FTO* rs9939609

SNP, a haplotype composed of a three SNP combination was shown to be associated with severe obesity. The calculation of a risk score based on the haplotype yielded an attributable risk of 34% for severe obesity suggesting that the approach has clinical use for examining risk in predisposed families [65].

FTO mRNA is abundant in mouse hypothalamic nuclei and codes for 2-oxoglutarate-dependent nucleic-acid demethylase, supporting a regulatory role in energy balance, appetite and sympathetic outflow to the circulatory system [66]. The effects on obesity occur early since they affect the rate of weight gain in African and European American youth (63), consistent with the finding that high fat intake and low physical activity modify the association between genetic variation in the *FTO* genotype and obesity [67]. Since rs9939609 in *FTO* was initially associated with T2D, and phenotypic interactions appear to be diabetogenic, the question has been further explored in a meta-analysis of South Asian populations. Obesity QTs such as BMI and waist were associated with *FTO* as was seen in Europeans but the association with T2D is only partly accounted for by BMI [68] suggesting that *FTO* has pleiotropic effects. This observation has been supported by a recent large-scale meta-analyses study conducted on 96,551 individuals from East and South Asia confirming the association of rs9939609 with T2D independent of obesity [69]. Also the *FTO* gene has been associated with hypertension and obesity in adolescents within a French Canadian founder population [70].

4. NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

There is a growing evidence to support that QTs representing NAFLD are also significant pre-T2D phenotypes. NAFLD is also regarded as a new component of the metabolic syndrome [71] with an independent genetic background. Predisposition to T2D is supported by observations that liver fat is increased in T2D compared to equally obese non-diabetic patients [72]. Also patients with NAFLD have insulin resistant adipose tissue and tend to have higher rates of glucose intolerance which is associated with increased risk for T2D [73,74]. The association of NAFLD with insulin resistance begins in childhood and adolescence [75] and with increased visceral fat and low adiponectin in adolescence [76], supporting association with adiponectin's effects via adiponectin receptor 2 (*ADIPOR2*) in three independent Finnish cohorts [77]. The association between insulin resistance and NAFLD has been confirmed in a meta-analysis of 21 prospective population-based studies of fatty liver disease diagnosed by liver ultrasonography [78]. Also there is evidence for NAFLD progressing to T2D [79]. Excess storage of fat in the liver is associated with activation of inflammation and production of cytokines, particularly IL-6 [80] , which may lead to further insulin resistance activated via signaling pathways such as toll-like receptors [81] and possibly by the receptor activator of nuclear factor-kB [82]. Simple steatosis progresses to inflammation with risk for cirrhosis and liver cancer (71) and is independently associated with increased risk of coronary artery disease (CAD) [83].

Large sized VLDL has been observed in NAFLD in an adolescent population independent of adiposity and insulin resistance, and the NMR (nuclear magnetic resonance) lipid profile was characterized by small dense LDL and reduced number of large HDL particles [84], revealing the association of NAFLD with a lipid profile predisposing to atherosclerosis in adults [85] and with increased intima-media thickness (IMT) in adolescents [86]. These data suggest pleiotropic effects, or alternatively, the effects arise from a biochemical cascade leading to excessive hepatic fat storage, inflammation and lipoprotein abnormalities.

Maturation of the VLDL particle in the golgi, at the stage when triglyceride is transferred to apoB by microsomal triglyceride transfer protein encoded by *MTTP*, determines liver fat storage and if defective may lead to NAFLD [87]. Carriers of the *MTTP*-493 G/T allele also have a more atherogenic lipid profile [88], which may have a deleterious effect on β-cell function [89]. Furthermore, the *MTTP-I128T* variant is associated with central obesity, elevated liver enzymes in fatty liver disease with and without association with alcoholism [90]. In addition, a manganese superoxide dismutase (*MnSOD*) variant was associated, possibly working by reducing mitochondrial fatty acid oxidation. Genetic determinants of VLDL formation and disposal may result in both atherosclerosis and fatty liver disease. Asian Indian carriers of two *APOC*3 variant alleles (C-482T, T-455C, or both) had a 30% increase in apoC-III levels and a 60% increase in triglyceride, as compared with the wild-type homozygotes. The prevalence of NAFLD was 38% among variant-allele carriers compared to 0% among wild-type homozygotes, and association with insulin resistance was significant [91]. Furthermore, the apoC-III overexpression model is predisposed to diet-induced hepatic steatosis and hepatic insulin resistance [92]. These observations are explained by the role of apoC-III in increasing triglyceride-rich lipoproteins by two mechanisms. It enhances hepatic VLDL assembly and inhibits VLDL lipolysis [93]. Missense mutation in *APOC3* gene within the C-terminal lipid binding domain results in impaired assembly and secretion of VLDL providing evidence that apoC-III plays a role in the formation of lipoproteins [94], whereas apoC-III non-competitively inhibits lipolysis by direct interaction with lipoprotein lipase [95]. It is also of interest that the apoE2 isoform may protect from development of NAFLD [96] supporting opposing effects of apoC-III and apoE, as has been observed in studies on atherosclerosis [97].

A GWAS of 2111 participants of the Dallas Heart Study revealed a robust association of liver fat defined by magnetic spectroscopy with the I148M allele of the *PNPLA3* gene [98] and the association was replicated in children and adolescents [99], when it may act jointly with *GCKR* [100]. A meta-analysis of 16 studies showed association of *PNPLA3* with disease severity with strong effect on more aggressive disease susceptibility indicated by higher inflammation indices and progression to fibrosis [101]. The gene *PNPLA3* codes for patatin-like phospholipase domain-containing protein 3, or adiponutrin, which plays a role in hepatic triglyceride hydrolysis. It catalyzes conversion of lysophosphatidic acid into phosphatitidic acid, an important regulatory reaction in lipid synthesis. Adiponutrin is up-regulated by sucrose feeding in the mouse model and the *PNPLA3* I148M variant results in increased cellular lipid accumulation providing a plausible mechanism for its impressive association with NAFLD [102]. In addition to *PNPLA3,* diet-induced obesity increases adiponutrin expression [103] which is associated with increased alanine transaminase, a marker of fatty liver disease, in Europeans, Hispanics and Asian Indians [104,105]. The homozygous carriers of the *PNPLA3 I148M* variant showed increased fasting glucose levels [106], and the *PNPLA3* S453I allele was associated with lower hepatic fat content and was more frequent in African Americans who had the lowest hepatic fat content, suggesting a protective effect from NAFLD [98].

5. DYSLIPIDEMIA

The classic dyslipidemia associated with the metabolic syndrome not only precedes and predicts T2D but abnormal LDL and HDL have biochemical associations with T2D pathogenesis. Insulin-resistant states such as obesity promote increased triglyceride, low HDL-cholesterol and molding of triglyceride-containing lipoproteins to form atherogenic LDL particles and dysfunctional HDL particles particularly when there is increased abdominal fat [107]. As in the case of other prediabetic traits, independent genetic determinants of

dyslipidemia (discussed below) interact with nutritional excesses and obesity-generated insulin resistance.

In vitro studies have shown that addition of LDL to human and rat islets decreases glucose-stimulated insulin secretion and is attributed to cholesterol uptake by islet LDL receptors and intracellular cholesterol-mediated toxicity [108]. Intracellular accumulation of cholesterol is dependent on HDL-mediated cholesterol efflux via the ATP-binding cassette transporter A1 (ABCA1), which is rate-limiting supporting a critical protective role for HDL [109]. Further studies have revealed that high cholesterol content in the β-cell membrane down-regulates insulin secretion by influencing membrane depolarization, the signal for calcium influx and calcium-mediated insulin secretion [110]. Since the classic dyslipidemia associated with the metabolic syndrome precedes T2D onset by several years [6], the effect of low HDL is operative over an extensive time period depending on the duration of low HDL.

In addition elevated triglyceride is associated with increased fatty acid levels, which enter the β-cell, undergo glucose-dependent esterification resulting in lipotoxicity [111]. Since not all obese individuals have elevated triglycerides, and non-obese cases can present with elevated levels [112,113]. Genetic predisposition can account for abnormal levels and for gene-environment interactions with obesity and dietary intake. Four commonly encountered classic hypertriglyceridemia phenotypes (IIb, III, IV and V) originally described at the National Institutes of Health have been characterized as having an elevated level of triglyceride. Type III hyperlipidemia is an exception since it has a distinct monogenic association with *APOE* polymorphism with homozygous effects of apoE2 isoform when the individual becomes obese. The IIb, IV and V phenotypes were associated with common variants which had previously been identified in GWAS performed on subjects with mild triglyceride elevations [114]. Thus clinically relevant dyslipidemia with high triglyceride can often be associated with common triglyceride-associated variants. Homozygous expression of rare variants such as *APOAV* and *APOE* , can result in severely increased triglyceride [115]; severe cases were found to be carriers of *APOAV* variants, S19W or -1131 T>C.

The -455 T>C variant in the *APOC3* gene promoter region is associated with increased triglyceride levels. The -455C and -482T alleles, located in the insulin response element (IRE), fail to respond to insulin-mediated down-regulation via Foxo1 so that transcription remains active and plasma apoC-III is increased [116]. This mechanism explains the association of apoC-III levels in non-HDL lipoproteins with insulin resistance in children and adolescents [117]. Since apoC-III transcription is activated in insulin resistance, increases in plasma apoC-III and triglyceride [118] occur in insulin-resistant obesity. Since transfer proteins and lipolytic enzymes mediate triglyceride and apoC-III transfer, there are increases in the atherogenicity of both LDL [119] and HDL [120]. The finding that apoC-III content of HDL predicts T2D [121] could be attributed to change in HDL function. Furthermore the higher diabetes prediction in females [121] follows the appearance of higher levels of plasma apo-CIII relative to apoA-I in teenage girls possibly accounting for a higher prevalence of T2D in young females than males [122]. In a multi-ethnic population sample, the serum triglyceride levels were 20% higher among individuals carrying -455C, particularly in females who were also shown to have low HDL-C [123]. Findings in GWAS and meta-analysis studies have reported a strong association of common variants near the *APOAV-AIV-CIII-A1* gene cluster with serum triglycerides [124]. Furthermore, these variants affect apoC-III and apoA-V, thus influencing triglyceride secretion as VLDL and its subsequent disposal by lipoprotein lipase.

It has long been known that cultural, environmental and hormonal factors determine HDL-C. However, a genetic component accounts for up to 76% of its variation [125], suggesting that genetic variants may affect HDL regulation and expression of HDL-associated traits with environmental interaction [126]. Regulatory genes involved in HDL metabolism mediated by apoA-I, LCAT, endothelial lipase and ABCA1 have been associated with severe HDL deficiencies [127], but only 20% of cases have mutations, and the population frequencies of the major gene abnormalities are small. However, association of these rare variants with atherosclerosis has been ambivalent [128], supporting a case for functional assays to represent the HDL phenotype such as measures of cholesterol efflux [129].

Quantitative trait loci were found in 6p, 9q and 15q regions containing known candidate genes [130], using apoA-I and HDL-C levels as QTs. Since the studies were done in a predominantly American Indian population, the findings could lead to association of SNPs with insulin resistant phenotypes including T2D [131]. The 15q region has been recognized to have a significant interaction with diabetes, BMI, smoking, alcohol intake and gender [132]. After serial adjustments, the LOD score increased from 1.75 to 4.52, supporting multiple endogenous and environmental influences including obesity. The region contains the gene for hepatic lipase suggesting that it has HDL-determining polymorphisms. A region on 9q contains the *ABCA1* gene coding for the cholesterol transporter regulating efflux from cells to HDL. The gene was found to contain the *ABCA1*-C230 variant which was associated with low HDL-C in exclusively American Indian populations who have increased risk for T2D [133]. This is important since carriers of loss of function mutations in *ABCA1* display pancreatic β-cell dysfunction supporting a role for *ABCA1* in removing cholesterol from β-cells [134].

Susceptibility to changes in HDL composition and function occur in obesity in part due to triglyceride elevation. Triglyceride-enrichment of HDL is mediated by cholesterol ester transfer protein (CETP) and is followed by degradation of HDL by hepatic triglyceride lipase, dissociation of apoA-I and subsequent renal catabolism [135]. It follows that in hypertriglyceridemic conditions, CETP activity has an HDL-reducing role. Conversely, CETP deficiency secondary to a gene defect results in extreme elevations in HDL-C [136], while maintaining function. Consequently CETP inhibition is the basis for use of pharmaceutical agents designed to raise HDL-C with encouraging recent trial results despite preceding setbacks [137]. Genetic variation in the *CETP* gene has been studied for association with variation in HDL-C in different populations [138,139]. A meta-analysis reported *CETP* genotypes to be associated with moderate inhibition of CETP activity and inverse association with cardiovascular disease but the findings are inconsistent [140]. Other studies have reported greater risk associated with low CETP activity secondary to severe genetic deficiency [141]. A recent prospective study from the community-based Framingham Heart Study also reported greater cardiovascular risk with low CETP activity [142]. More recently it has been shown that polymorphisms in the *CETP* promoter region determine activity. GWAS in Caucasians has revealed association of the variant -2568 C/A (rs3764261) with HDL-C and the finding has been replicated in different ethnic groups [143,144].

SNPs in the *CETP* promoter region (-2568 C/A, -1700 C/T), -998 A/G) and the well-known non-coding SNP (397 A/G) identified as a restriction fragment (Taq1b) in the first intron, were studied in the unique Sikh population of Northern India who are known to have a high prevalence of T2D and cardiovascular disease despite much lower obesity rates [145]. The -2568 C/A allele showed a strong association with increased HDL-C and decreased blood pressure. Although none of the SNPs were individually associated with CETP activity, low activity was associated with greater risk for CAD and there was significant interaction

between the *CETP* SNPs studied as haplotypes and CETP activity for affecting HDL-C [146]. These results suggest that more complete genotyping could serve to define individual risk and response to therapies designed to raise HDL-C by inhibiting CETP.

Since fatty acids, LDL and HDL interact with the β-cell, it is possible that the levels and functions interact with SNPs, which determine β-cell function and survival. If so, those populations that have very high T2D incidence may be collectively predisposed by influx of cholesterol, fatty acids and variants affecting β-cell metabolism. For example in the Khatri Sikhs, four of six SNPs for the *TCF7L2* gene and two variants within the *KCNQ1* gene were associated with T2D [147,148]. Three of the four *TCF7L2* SNPs were associated with LDL-C levels [147]. In separate studies the *CDK5* gene contained an allele associated with decreased HDL-C [149]. In addition a linkage scan of blood lipid phenotypes in diabetic families from the same population has identified significant linkage signals for HDL-C at 10q21.2 and for LDL-C at 10p11.23 and 9q21.13 [150].

6. HYPERTENSION

The high heritability of blood pressure, ranging from 30-40% [151] and abundance of evidence showing association of high blood pressure with insulin resistance [152] strongly supports its consideration as a pre-diabetes phenotype but with an independent genetic background. This association is attributed to enhanced sympathetic nervous activity, oxidative stress and enhanced renin-angiotensin-aldosterone system [152,153]. Angiotensin II has a direct effect on increasing insulin resistance independent of alterations in blood flow and interstitial insulin concentration [154], but angiotensin II-mediated oxidative stress may play a role [155]. The TG (mREN2)27 rat, a monogenic model of both hypertension and insulin resistance is characterized by locally elevated tissue angiotensin II levels and impairment of the IRS-1-dependent insulin signaling pathway [156]. The insulin resistance is reversible by selective inhibitors of angiotensin II at AT1 receptors [157]. Similar selective antagonism using irbesartan, a clinically used AT1 receptor blocker (ARB) has been shown to improve insulin action in the obese Zucker rat- associated with up-regulation of GLUT4 the main glucose transporter in skeletal muscle [158].

Given substantial experimental evidence for the renin-angiotensin system's involvement in hypertension, there has been interest in investigating association of common variants such as *ACE* (angiotensin-converting enzyme) and *AGT* (angiotensinogen) with hypertension but results have not been conclusive [159] and they have not been associated with T2D [160]. However variants in *ACE* and *CYP11B2* genes have been associated with insulin resistance in hypertensive families in Taiwan [161] Gene variants in *ACE*, *AGT*, and *AT1R* predicted T2D in a Tunisian population [162]. Data from the National Health and Nutrition Examination Survey (NHANES) showed the prevalence of hypertension to be 40% in African Americans compared to 27% in European Americans [163,164] leading to the hypothesis that part of the excess burden in African Americans is due to genetic susceptibility [165]. GWAS and candidate genes examined in the Candidate Gene Association Resource Consortium consisting of 8591 African Americans identified novel associations for diastolic blood pressure on chromosome 5 near *GPR98* and *ARRDC3* and for systolic blood pressure on chromosome 21 in *C21orf 91.* However, none of these variants were associated with T2D [165].

Interestingly, monogenic forms of hypertension have provided evidence for a regulatory role of key metabolic pathways and have been the basis for candidate gene population studies but none have involved carbohydrate metabolism or insulin action. Using such an approach,

24-hour ambulatory blood pressure has been associated with five polymorphisms in the *KCNJ1* gene, which has the potential to cause Bartter Syndrome Type 2 when the abnormal allele is inherited [166]. Also ambulatory blood pressure is associated with common variations in the *WNK1* gene known to cause pseudo-hypoaldosteronism Type 2 or Gordon Syndrome. Association of *WNK1* with blood pressure in childhood underscores its possible association with evolving hypertension at young ages [167]. Additional association with variants in *CASR, NR3C2, SCNN1B and SCNN1B*, all of which are known to have had mutations causing rare Mendelian defects in blood pressure regulation, provide support for the hypothesis that relevant polymorphisms influence conventional pathways involved in blood pressure regulation [166]. However, GWAS has shown that only some of the associations are in or close proximity to nearby genes involved in known hypertension-related metabolic pathways, suggesting new pathways involved in hypertension. A GWAS performed by the International Consortium for Blood Pressure on 200,000 individuals of European descent and identified sixteen loci of which only six contained genes that are known or suspected to regulate blood pressure, which include *NPR3, GUCY1A3-GUCY1B3, ADM, GNAS-EDN3, NPPA-NPPB, CYP17A1* and their known metabolic roles have been comprehensively reviewed [168]. *CYP17A1* achieved the most robust GWAS significance and is the site for a known Mendelian-inherited mutation causing hypertension by increasing mineralocorticoids in the adrenal steroid pathway and causing a rare form of congenital adrenal hyperplasia.

Clearly evidence from genetic association studies point to separate genetic backgrounds for hypertension and T2D. However, secondary or primary activation of the renin-angiotensin system is associated with insulin resistance and may predispose to insulin resistance leading to T2D.

7. HYPERGLYCEMIC TRAITS

7.1 Pre T2D

The risk factors preceding T2D vary in sequence and in the number of components reflecting both individual and population differences in inheritance and environments. However, detectable changes in glucose-insulin metabolism precede T2D and have been studied as QTs in genetic studies and as targets for reversal or prevention of T2D onset. Therefore there has been interest not only in searching for genetic association but also in finding the glucose levels which accurately reflect T2D and risk for T2D. Consequently, The American Diabetes Association Expert Committee established the impaired fasting glucose range as 100-125 mg/dl and impaired glucose tolerance levels after ingestion of a glucose load as 140-199 mg/dl [169]. These cut points were selected to facilitate early diagnosis of risk and have subsequently been of great significance since the defined pre-diabetic state is reversible by lifestyle [170], suggesting that differences in lifestyle could affect outcomes in association studies.

HbA1c, the glycosylated fraction of hemoglobin, has also been useful in defining the pre-diabetic state in population studies [171,172]. In an African American population with a normal range from 3.3 to 6.4%, subjects in the upper tertile were found to have abnormalities in insulin action but not β-cell secretion [173]. These findings support the use of HbA1c levels in the upper ranges of normal as being pre-diabetic. Furthermore it has been associated with other pre-diabetic traits constituting the metabolic syndrome [174].

Approximately 60% of people who develop diabetes have either IGT (impaired glucose tolerance) or IFG (impaired fasting glucose) about five years before T2D onset, with 40% having normal glucose tolerance [175]. Some studies suggest that IGT is more strongly associated with hypertension and dyslipidemia with worse cardiovascular outcomes [175]. Also, it is known that progression of IGT to T2D is potentially reversible with lifestyle [170]. Recognition of IFG as a distinct phenotype is important since it is associated with increased gluconeogenesis rates. However, insulin-mediated glucose disappearance becomes impaired in cases with IFG and IGT, who have also increased cardiovascular risk [176,177]. Association of IFG with NAFLD is reported in Chinese males [178,179]. IFG has been linked to calpain 10 [180] and to CD36 [181] in patients with essential hypertension. The rs553668 of the *ADRA2A* gene predicts worsening of fasting glucose values in a pre-diabetic cohort [182]. Variants associated with fasting glucose levels in the normoglycemic population such as *GCK, GCKR, G6PC2, MTNR1B* and *DGKB-TMEM195* [183], do not always influence risk for T2D (in contrast to *TCF7L2* and *SLC30A8)*, but their effect appears confined to fasting glucose homeostasis [184,185]. The data support recognition of early hyperglycemic phenotypes derived from regulatory polymorphisms on the genes affecting interacting pathways leading to T2D. Meta-analysis of 21 GWAS identified 9 new loci influencing fasting blood glucose (*ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1* and *C2CD4B*) but of these only *ADCY5* and *PROX1* was associated with T2D. These data suggested that although there is overlap, the genetic background for fasting glucose is different from that of T2D [186]. Similarly, the 2-hour glucose levels, after a standard oral glucose load can be defined as a separate trait to T2D with overlap in the associated variants. Meta-analysis identified new loci, *GIPR* and *UPS13C*, uniquely influencing 2-hour glucose [187] supporting the hypothesis that there are separate glucose-related QTs representing specific modes of carbohydrate metabolism [188].

7.2 T2D

Increased prevalence of T2D in adolescents over the past two decades has coincided with an increase in adults with descending age of onset for both obesity and T2D [189]. Resistance to insulin action occurs in the liver, fat cell, and muscle, and respective pathways may contribute to T2D [47]. Furthermore, longitudinal studies indicate association of insulin resistance and obesity in youth with gender and ethnic-specific tracking of BMI, and lipids to middle-age adulthood [190]. Not only is the metabolic syndrome a predictor of T2D [6,191], but also the syndrome traits or QTs are associated with insulin resistance [192]. Also, the prevalence of the metabolic syndrome increases with progression from normal glucose tolerance to impaired glucose tolerance to onset of T2D when it often exceeds 60% depending on the definition and study population [193,194]. Based on population studies and animal models, it has been proposed that T2D has a progressive pathogenesis beginning with insulin resistance and progressing to β-cell failure [195] and that it may involve several genes, sometimes with significant interaction [196]. For example, using knock-out models for both *IRS-1* and the insulin receptor, it was shown that neither model alone had much effect on diabetes onset but the combined effect resulted in more than 50% developing diabetes at young ages [196].

Glucose cut points for the diagnosis of diabetes have been based on arbitrary glucose thresholds. Based on evidence for a bimodal distribution, the National Diabetes Data Group in the United States initially used the glucose levels that best distinguished overlapping populations [197]. Two decades later it was observed that the levels appeared too high since cases below the cut points developed retinopathy, consequently the American Diabetes Association Expert Committee decreased the thresholds based on cross-sectional

association between glucose levels and the development of retinopathy [198]. Accordingly, a fasting glucose greater than or equal to 126 mg/dl and 2-hour post-glucose load level ≥ 200 mg/dl have become cut points for diabetes. In addition, HbA1c is increasingly being introduced as supportive diagnostic evidence for T2D [199] in addition to its use in defining pre-diabetes (see above). Since it does not require fasting conditions and has become generally available as a standardized assay with less inter-individual biologic variability than glucose, it has potential for diagnostic use in clinics and for screening. However, conditions that influence hemoglobin production or disposal influence the levels and when used alone can lead to missed diagnoses even when using a relatively high cut point of 6.5% [200].

Since the diagnosis of T2D has been based on glucose thresholds defining overt diabetes, it is possible that variants determining β-cell function interact with secondary metabolic derangements accounting for GWAS results since subjects are assessed at a point when the β-cells are likely to be undergoing failure or cell death. This is supported by observations that the majority of gene variants associated with T2D such as *TCF7L2, CDKAL1, CDKN2A/B, HHEX-IDE, IGF2BP2, SLC30A8, KCNJ11, WFS1, JAZF1, TSPAN8, CD123/CAMK1D* and *MTNR1B*, are implicated in β-cell functions such as glucose-stimulated insulin secretion, incretin effects on β-cell stimulation, and proinsulin to insulin conversion [201,202]. It also appears likely that many of the secondary events described for each of the preceding phenotypes have significant effects on both insulin resistance and β-cell failure.

8. CONCLUSIONS

In the ongoing quest to identify measurable phenotypes or QTs to fully account for T2D susceptibility, recognition of traits and their respective QTs preceding T2D may account for interaction both at the gene level by gene-gene interaction and epigenetic modifications, and at the pathway level as shown by disorders that are susceptible to environmental effects. T2D-predisposing traits such as fetal growth restriction, obesity, NAFLD, dyslipidemia, hypertension and early hyperglycemia constituting pre-diabetes, interact with environmental factors and possibly confound gene association when T2D is the sole phenotype. Few trait-associated genetic variants were identified to be linked with T2D in GWAS supporting a role for primary and secondary metabolic events interacting with pathways involving insulin resistance and insulin secretion. Early metabolic programming during gestation not only has a genetic background but also is susceptible to metabolic and nutritional changes in the fetal environment. Obesity, also known to have significant genetic background, is sensitive to early environmental influences beginning during gestation and continuing in childhood to adulthood; however obesity itself like the other phenotypes generates insulin resistance and worsens dyslipidemia. NAFLD, associated with significantly increased hepatic fat synthesis and storage, is associated with hepatic insulin resistance, a T2D predictor, suggesting that liver-expressed variants may indirectly affect glucose intolerance and T2D. The classic lipid derangement observed in insulin resistance consisting of elevated triglyceride (often associated with increased free fatty acid levels), small LDL particles in increased numbers, and low HDL-C has significant association with insulin resistance and progression of β-cell failure, and predicts T2D onset. Hypertension and T2D have separate genetic backgrounds. However, primary and secondary activation of the renin-angiotensin system may predispose to insulin resistance leading to T2D. This suggests that cross-sectional and prospective clinical investigations beginning at early developmental phases could allow assessment of more precise inter-relationships of phenotypes to each other, to T2D and to their respective genetic backgrounds. To achieve this goal it will be necessary to understand overlapping relationships of polymorphisms with the respective QTs for each phenotype and identify the causal variants in the identified target genes by the use of functional models, gene

expression studies during the lifespan, pleiotropism, gene-gene interactions, epigenetics, and pathway interrelationships. In addition, clinical studies on natural history and pharmacogenomics studies showing impact of genetic variation on treatment responses to pharmacological agents are continuing to provide additional insights.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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