Emerging Applications of Metabolomic and Genomic Profiling in Diabetic Clinical Medicine

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Clinical and epidemiological metabolomics provides a unique opportunity to look at genotype-phenotype relationships as well as the body's responses to environmental and lifestyle factors. Fundamentally, it provides information on the universal outcome of influencing factors on disease states and has great potential in the early diagnosis, therapy monitoring, and understanding of the pathogenesis of disease. Diseases, such as diabetes, with a complex set of interactions between genetic and environmental factors, produce changes in the body's biochemical profile, thereby providing potential markers for diagnosis and initiation of therapies. There is clearly a need to discover new ways to aid diagnosis and assessment of glycemic status to help reduce diabetes complications and improve the quality of life. Many factors, including peptides, proteins, metabolites, nucleic acids, and polymorphisms, have been proposed as putative biomarkers for diabetes. Metabolomics is an approach used to identify and assess metabolic characteristics, changes, and phenotypes in response to influencing factors, such as environment, diet, lifestyle, and pathophysiological states. The specificity and sensitivity using metabolomics to identify biomarkers of disease have become increasingly feasible because of advances in analytical and information technologies. Likewise, the emergence of high-throughput genotyping technologies and genome-wide association studies has prompted the search for genetic markers of diabetes predisposition or susceptibility. In this review, we consider the application of key metabolomic and genomic methodologies in diabetes and summarize the established, new, and emerging metabolomic and genomic biomarkers for the disease. We conclude by summarizing future insights into the search for improved biomarkers for diabetes research and human diagnostics.

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Diabetes is a rapidly increasing metabolic disorder precipitated by complex and poorly understood interactions between multiple environmental and genetic factors. The consequences of diabetes are far reaching, and disturbances in both the secretion and action of insulin impact on the global regulation of metabolism, affecting the composition of blood and other body fluids. Understanding of this process and identification of potential disease biomarkers have been greatly facilitated in recent years by the upsurge in new technologies for comprehensive metabolic profiling, which are often collectively termed metabolomics.

Metabolomic profiling in clinical medicine

Metabolomics is defined as the analytical description of biological samples accompanied by the characterization and quantification of small molecules. It can often be confused with the term metabonomics, which represents the global, dynamic metabolic response of living systems to biological stimuli or genetic manipulation. Both terms are closely affiliated with each other owing to the analytical and experimental technologies used in each field. The observation of the characteristics and changes in metabolism by metabolomics allow the resulting data to be merged with data from the other "-omic" technologies. Genomic, metabolomic, and proteomic state-of-the-art technologies are now used increasingly by researchers to identify clinical methodologies for the early diagnosis and monitoring of human degenerative diseases such as diabetes. Classical risk factors still have an important role to play in diabetes assessment; however, powerful methodologies are now available for exploitation of novel quantitative and qualitative disease-related biomarkers. Novel biomarkers are needed that are independent of known clinical risk factors.

Fundamentally, metabolomics aims to monitor changes in products of metabolism and provide valuable information on a range of influencing factors and gene-related outcomes. Exploitation of genomic technology in recent times has resulted in many technical advances, and genomic analysis has now emerged as a valuable tool in predicting the body's response to stimuli caused by disease or injury. Indeed, methodologies such as epigenetic profiling, sequencing technologies, microarrays, functional fingerprinting, and analysis of genomic alternations are all well-established methodologies in practice. Complementing these technologies with computational methods/bioinformatics that integrate large amounts of heterogeneous genetic and genomic information has helped provide meaningful results to aid our understanding of the complex changes of genes and macromolecules. There is now a clear need to discover novel and effective clinical biomarkers using technologies that encompass an array of different methodologies. Chromatography, two-dimensional electrophoresis, mass spectrometry, functional magnetic resonance, positron emission tomography, and protein/gene sequencing are some examples being used to unravel the body's complex biological systems. Sensitive and high-resolution techniques used in clinical metabolomics, such as nuclear magnetic resonance, gas chromatography–mass spectrometry, and liquid chromatography–mass spectrometry, are sensitive and robust and have the capacity to process large volumes of data from population studies (1,2). However, overinterpretation of data remains one of the key limitations to be overcome for successful exploitation of metabolomics and metabonomics.

In this brief review, we consider recent applications of metabolomic and related technologies in diabetes together with their use in relation to clinical diagnostics. Technical details of the methodologies involved and their use in basic diabetes research have

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Table 1—*Established, new, and emerging metabolomic biomarkers for type 2 diabetes*

Predictor	Abbreviation	Reference no.
Metabolic markers		
Insulin		7 and 8
Glucose		7 and 8
γ-Glutamyl transferase	GGT	4 and 8
Alanine aminotransferase	ALT	5 and 6
Ferritin	FTH1	7 and 8
Pancreatic polypeptide	PP	9
Fibronectin		10
Fetuin A		11
Sex hormone–binding globulin	SHBG	7 and 12
Free testosterone		12
Insulin-like growth factor I	IGF-I	13
Insulin receptor		8
Creatine kinase-MB	CKMB	8
MR-Pro atrial natriuretic peptide	MR_PRO_ANP	8
NT-Pro B-type natriuretic peptide	NT_PRO_BNP	8
B-type natriuretic peptide	BNP	8
Biomarkers of glycemia		
Glycated hemoglobin	HbA_{1c}	
Fructosamine		14
1,5-Anhydroglucitol	1,5AG	15
Glycated albumin		16
Glycated insulin		17
Glycosylated amylin		18
Glycated LDL		19
Markers of oxidative stress and nutrient status		
Glutathione	GSH	20
Advanced glycated end products receptor	RAGE	24
Ascorbic acid	Vitamin C	21
25-Hydroxyvitamin D	Vitamin D	22
Homocysteine		8
Branched-chain and aromatic amino acids	Leu, Ile, Val, Tyr, Phe	23
Lipid-related markers		
Leptin	LEP	6 and 25
Adiponectin	ADIPOQ	6, 8, and 25
Apolipoprotein B	ApoB	8
Apolipoprotein A	ApoA	8
Endothelial and inflammatory markers		
C-reactive protein	CRP	6–8 and 26
Interleukin-18	IL-18	8 and 27
Interleukin-1 receptor antagonist	IL-1ra	28
Interleukin-2 receptor antagonist	IL-2ra	7
Interleukin-6	IL-6	6–8
Plasminogen activator inhibitor-1	PAI-1	6 and 29
Cell adhesion molecule	CAM	30
Tissue plasminogen activator antigen	t-PA antigen, PLAT	31
Neopterin		8
Von Willebrand factor	vWF	31

been covered in several excellent articles and reviews (1,3).

Metabolomics applied to the clinical diagnosis and prognosis of diabetes

The American Diabetes Association officially recommends HbA_{1c} testing for the diagnosis and monitoring of diabetes, and the global comparison of HbA_{1c} values is now possible as a result of the International Federation of Clinical Chemistry and Laboratory Medicine establishing true international reference methods for HbA_{1c} (in millimoles per mole) and the successful preparation of pure HbA_{1c} calibration material. However, there is clearly a need to discover new markers, as illustrated in gestational diabetes mellitus, where there is a drive to reconsider diagnostic criteria recognizing the possibility of adverse pregnancy outcomes of milder levels of glucose intolerance than hitherto appreciated.

Metabolic markers of type 2 diabetes. Whereas glucose and insulin are the most well-established biomarkers, there are many new and emerging biomarkers of diabetes (Table 1). Positive associations have been reported between serum γ-glutamyl transferase and incident type 2 diabetes (4). Pathophysiological mechanisms underlying how serum γ-glutamyl transferase relates to type 2 diabetes risk have not been elucidated, but insulin resistance, oxidative stress, and chronic low-grade systemic inflammation may be involved. Alanine aminotransferase (ALT) is elevated in some patients with type 2 diabetes independently of confounding factors such as obesity (5,6), and evidence now indicates that markers associated with fatty liver may predict future development of type 2 diabetes. In most cases of nonalcoholic fatty liver disease, the hepatic component of metabolic syndrome, ALT is elevated, and studies have associated raised ALT with metabolic syndrome and type 2 diabetes (5).

A strong association has been found between raised ferritin levels (below the range indicative of clinical hemochromatosis) and development of incident diabetes (7,8). Ferritin was associated with diabetes independently of established risk factors (age, BMI, sex, family history, physical inactivity, and smoking), as well as dietary factors and alcohol intake. The mechanism is thought to involve insulin resistance, free radical damage, and accumulation of iron in hepatocytes.

Pancreatic polypeptide, believed to act as a regulator of pancreatic and gastrointestinal functions, has been proposed as a possible marker of β-cell failure in diabetes (9). Likewise, fibronectin levels change in insulin resistance, and Amrein et al. (10) proposed that levels could be used in the diagnosis of insulin resistance and monitoring of disease progression. Fetuin-A, a hepatic secretory protein that binds the insulin receptor and inhibits insulin action, has been shown to be associated with incident diabetes independent of other markers of insulin resistance (11). Sex hormone–binding globulin (SHBG) is known to be downregulated by insulin, and low levels have been reported to reflect insulin resistance and incident diabetes in women (7,12). Population studies have shown that low testosterone levels are

commonly associated with the prediction of type 2 diabetes and the metabolic syndrome. Although the inverse association of testosterone with diabetes is partially mediated by SHBG, low testosterone is linked to diabetes via a bidirectional relationship with visceral fat, muscle, and possibly bone (12). IGF-I, which is involved in somatic growth, cellular differentiation, and regulation of metabolism, is potentially another marker of diabetes, given its insulin-like effects and involvement in glucose homeostasis. Large-scale gene association and prospective observational studies are needed to fully elucidate the involvement of IGF-I (13).

In a recent study of 31 novel biomarkers, Salomaa et al. (8) demonstrated an association between clinically incident diabetes and insulin receptor, creatine kinase-MB, MR-Pro atrial natriuretic peptide, NT-Pro B-type natriuretic peptide, and B-type natriuretic peptide. The utility of these as potential biomarkers and the nature of their links to diabetes clearly deserve further study.

Biomarkers of glycemia in diabetes. HbA_{1c} is the most widely known glycosylated protein in diabetes, and its assay has been the gold standard for the evaluation of glycemic status for many years. Limitations of the HbA_{1c} measurement do exist, but it remains an important tool in the management of diabetes along with self-monitored blood glucose profile data. Fructosamine is another marker used in practice (14) and refers to the ketoamine rearrangement product formed by the interaction of glucose with the ε-amino group on lysine residues of albumin. The assay is thought to be less accurate than HbA_{1c} because of factors affecting the half-life of its many components and is thus considered of less clinical value. Other markers of glycemic control that have been considered but not widely used are 1,5-anhydroglucitol (15) and glycated albumin (16).

Much interest has surrounded the role of glycosylated regulatory proteins as biomarkers. Because insulin glycation is dependent on the degree and duration of hyperglycemia, monitoring of glycemic status in diabetic patients using glycated insulin could aid approaches to the diagnosis, management, and treatment of diabetes (17). Other peptides such as amylin and amylin-like peptides have been disclosed as potentially useful in the detection and/or evaluation of diabetes. Glycosylated amylin (18) has been proposed as a predictor of the onset of diabetes in patients who otherwise show normal glycemic control, and another group has filed a patent based on monoclonal antibodies against glycated LDL for monitoring glycemic control (19).

Markers of oxidative stress and nutrient status in type 2 diabetes. Since diabetes is associated with overproduction of different reactive oxygen species leading to long-term development of diabetes complications, a number of candidate biomarkers have emerged (Table 1). Reduced levels of antioxidants such as glutathione, vitamin C, and vitamin E (20–22) and changes in serum malondialdehyde and activities of superoxide dismutase and glutathione peroxidize have been found in diabetic patients as well as changes in other oxidative stress biomarkers such as catalase, glutathione reductase, lipid peroxidation, and nitrite concentration (20).

Ascorbic acid (vitamin C) (21) and 25-hydroxyvitamin D (vitamin D) (22) are both associated with diabetes risk, but because of the many confounding determinants, levels need further investigation. Homocysteine also has promising links to diabetes (8). These and other biomarkers in Table 1 have yielded promising results, but most have been tested one at a time, with lack of independent validations. Many of these apparently "independent" risk factors may in fact be related by virtue of their common origins or shared metabolic pathways (6). There may even be different patterns of biomarkers of diabetes associated with early or late-stage diabetes. Recently, amino acid profiles were proposed as important in assessing diabetes risk as elevated levels of five amino acids were shown to predict the development of diabetes at early stages (23). Combinations of the five branched-chain and aromatic amino acids—leucine, isoleucine, valine, tyrosine, and phenylalanine—rather than a single amino acid, served as a more accurate predictor of diabetes risk (23).

In diabetes, advanced glycation end products form as a consequence of long-term hyperglycemia, and a number of truncated forms of the advanced glycation end product receptor (RAGE) have been identified. The C-terminally truncated form, named endogenous secretory RAGE, has potential as a biomarker and in the estimation of the risk of atherosclerotic disorders and occurrence of metabolic syndrome (24).

Lipid-related markers of type 2 diabetes. Adipokines are involved in a broad range of physiological processes such as insulin sensitivity, lipid metabolism, vascular hemostasis, blood pressure regulation, angiogenesis, and appetite control. Leptin and adiponectin are associated with increased risk of type 2 diabetes even after adjustment for BMI, lifestyle factors, and cardiovascular disease (6,25). It is well recognized that adiponectin increases insulin sensitivity, regulates glucose and lipid metabolism, and enhances insulin action in the liver. Serum levels of adiponectin have been shown to decrease with increasing obesity, and interestingly, elevated adiponectin has been associated with a lower incidence of diabetes (6,25). Leptin is already a marker of percentage fat mass in healthy individuals and regulates body weight by effects on food intake and metabolism. The association between leptin and incident diabetes has been difficult to determine but may also reflect insulin resistance. Adiponectin is more strongly associated with type 2 diabetes risk than leptin (25). Apolipoprotein (Apo)B, and to a lesser extent ApoA, was a particularly strong predictor of diabetes even when controlling for BMI and waist-to-hip ratio (8).

Endothelial and inflammatory markers of type 2 diabetes. C-reactive protein (CRP) is a predictor of diabetes independent of other clinical indicators such as BMI, fasting triglyceride, and glucose (26), but circulating levels correlate with lipids, SHBG, and adiponectin. The value of CRP is promising, but further evaluation is needed. Elevated levels of the cytokine interleukin (IL)-18 are linked with an increased risk of type 2 diabetes, independent of a generalized proinflammatory state (27). Studies have also reported upregulation of anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra) in individuals with obesity and insulin resistance (28). These studies indicate that individuals with high risk of type 2 diabetes can be characterized by the presence of an early compensatory, anti-inflammatory response that precedes the development of the disease and inflammatory markers. Like IL-1ra and CRP, IL-2ra is involved in inflammatory pathways; however, only one study to date has identified IL-2ra as a diabetes marker (7), which may be due to oxidative stress in diabetes culminating in T lymphocyte activation.

IL-6 has been reported to be elevated in incident diabetes, independent of obesity and fasting glucose (6–8). Studies are needed to determine the relationship between IL-6 and diabetes and whether there is a causal link. Plasminogen activator inhibitor 1 levels reflect an acute phase response, and elevated levels are found with incident diabetes independent of obesity and insulin resistance (29). The association between high plasminogen

activator inhibitor 1 and incident diabetes may be due to associations with liver fat (6). Circulating levels of several other inflammatory endothelial-derived factors such as cell adhesion molecules (30), tissue-plasminogen activator antigen (31), neopterin (8), and von Willebrand factor (31) have been linked to diabetes risk. Recent studies such as the MONICA/KORA study have shown that when a risk prediction model of multiple inflammation markers is used, the prediction of incident type 2 diabetes and coronary events is significantly improved compared with cardiometabolic risk factors (25,27).

Increased clinical value of evaluating panels composed of different biomarkers

Advances in technology plus awareness of an increasing number of diabetes-related metabolomic analytes are likely to facilitate use of panels of combined biomarkers rather than reliance on single biomarkers for diabetes. If these are selected from different tissue origins/pathways, their ability to predict diabetes risk is likely to be increased, thereby facilitating earlier interventions. This view is supported by two comprehensive studies (7,8). Kolberg et al. (7) evaluated the potential of 58 candidate diabetes-related biomarkers plus six clinical factors for predicting 5-year risk of diabetes in 160 of 632 individuals from the Danish Inter99 cohort who went on to develop type 2 diabetes. A six-biomarker model (adiponectin, CRP, ferritin, IL-2ra, glucose, and insulin) showed improved performance over single markers such as HbA_{1c} and fasting glucose, being equivalent to a 2-h oral glucose tolerance test (7). Similarly, Salomaa et al. (8) evaluated the potential of 31 novel biomarkers as predictors for clinically incident diabetes in a combined total of 12,804 individuals from the FINRISK97 and Health 2000 cohorts of whom 596 later developed diabetes in 10-year follow-up. This study revealed that adiponectin, ApoB, CRP, and ferritin improved diabetes prediction even after taking BMI, glucose, and other classical risk factors into account (8). Sex-specific analysis further showed potential value of including IL-1ra and insulin as biomarkers. These data suggest that biomarker scores reflecting different pathological processes may have significant potential for improving future prediction of diabetes. Similarly, evaluation of amino acid profiles appears to be more effective in prediction than are single amino acids (24).

Genomic variations and DNA profiling of those at risk for type 2 diabetes

Despite many candidate gene studies and genome-wide linkage studies, very few susceptibility loci for type 2 diabetes have been identified until the recent emergence of genomic-wide association (GWA) data and large-scale replication studies (Table 2). Meta-analysis of GWA studies provides the unique opportunity to investigate the heterogeneity or consistency of genomic associations across diverse datasets and study populations. Recently, Voight et al. (32), using large-scale association analyses combining the data from eight GWA studies, identified 12 new susceptibility loci for type 2 diabetes.

Despite identification of many putative causative genetic variants, few have generated credible susceptibility variants for type 2 diabetes. Indeed, the most important finding using linkage studies is the discovery that the alteration of *TCF7L2* (*TCF-4*) gene expression or function (33) disrupts pancreatic islet function and results in enhanced risk of type 2 diabetes. Candidate gene studies have also reported many type 2 diabetes–associated loci and the coding variants in the nuclear receptor peroxisome proliferator–activated receptor-γ (34), the potassium channel *KCNJ11* (34), *WFS1* (35), and *HNF1B* (*TCF2*) (36) are among the few that have been replicated (Table 2). Recently, there have been great advances in the analysis of associated variants in GWA and replication studies due to high-throughput genotyping technologies, the International HapMap Project, and the Human Genome Project. Type 2 susceptibility loci such as *JAZF1*, *CDC123-CAMK1D*, *TSPAN8-LGR5*, *THADA*, *ADAMTS9*, *NOTCH2*, and *ADCY5* (37,38) are among some of the established loci (Table 2). *CDKN2A/B*, *CDKAL1*, *SLC30A8*, *IGF2BP2*, *HHEX/IDE*, and *FTO* are other established susceptibility loci for diabetes (Table 2) (34,39,40). GWA studies have also identified the potassium voltage-gated channel *KCNQ1* (32) as an associated gene variant for diabetes. A recent GWA study reporting a genetic variant with a strong association with insulin resistance, hyperinsulinemia, and type 2 diabetes, located adjacent to the insulin receptor substrate 1 (*IRS1*) gene, is the C allele of rs2943641 (41). Interestingly, the parental origin of the single nucleotide polymorphism is of importance because the allele that confers risk when paternally inherited is protected when maternally transmitted. GWA studies for glycemic traits have identified loci such as *MTNR1B* (42), *GCK* (glucokinase) (42), and *GCKR* (glucokinase receptor) (42); however, further investigation of genetic loci on glucose homeostasis and their impact on type 2 diabetes is needed. Indeed, a recent study by Soranzo et al. (42) using GWA studies identified ten genetic loci associated with HbA_{1c}. Genetic factors affecting expression, turnover, and abnormal glycation of hemoglobin may be associated with changes in levels of HbA_{1c}.

Significant effects of many susceptibility loci are still to be determined and replicated, and further large-scale association studies will be required. Recently, Schleinitz et al. (43) found some of the type 2 diabetes risk alleles or related subphenotypes to be weak, including those of JAZF1, CDC123/CAMK1D, NOTCH2, ADAMTS9, THADA, and TSPAN8-LGR5. The *TNF/LTA* locus has been a long-standing type 2 diabetes candidate gene, whereas a recent study found no evidence of an association between TNF/LTA region variation and type 2 diabetes (44). The association of polymorphisms in *TNFa* and type 2 diabetes has been extensively reported. Recently, the *TNFa* variant rs3093662, linked to higher serum levels of tumor necrosis factor-α, was shown to be associated with elevated insulin (45). Mutated transcription factors, hepatocyte nuclear factor (HNF)1A and HNF4A, have received substantial attention, and there is evidence for susceptibility of the variants to maturity-onset diabetes of the young (MODY) and type 2 diabetes. Recently, high-sensitivity CRP was shown to discriminate HNF1A-MODY from other subtypes of diabetes (46).

Interestingly, many of the established susceptibility loci are involved in insulin secretion signaling, supporting an important role for defects in β-cell function and β-cell mass in type 2 diabetes. The exciting potential of genetic testing for susceptibility of diabetes appears to be some way off, apart from rare forms of monogenic diabetes (44). Moreover, it is well known that nongenetic factors such as obesity and lifestyle factors play an important role in the disease. New phenotyping approaches to studying metabolite and protein abundance and data integration are needed to bring genomic and metabolomic goals together. In this context, the Human Metabolome Project in Canada (47), aimed at providing a linkage between the human metabolome and the human genome, has identified and quantified normal concentration ranges for a large number of metabolites in cerebrospinal fluid, serum, urine, and other

Table 2—*Type 2 diabetes susceptibility loci established through candidate-gene, genome-wide linkage, and GWA studies*

Gene/region	Gene name	Chromosomal location	Identification	Reference no.
TCF7L2	Transcription factor 7-like 2	10q25.3	Linkage study	33 and 39
PPARγ	Peroxisome proliferator–activated receptor γ	3q25	Candidate gene	34
KCNJ11	Potassium channel, inwardly rectifying subfamily J, member 11	11p15.5	Candidate gene	34
WFS1	Wolfram syndrome 1 (wolframin)	4p16.1	Candidate gene	35
HNF1B	HNF1 homeobox B	17q12	Candidate gene	36
JAZF1	Juxtaposed with another zinc finger gene 1	7p15	GWA	37
CDC123-CAMK1D	Cell division cycle protein 123 homolog/ calcium/calmodulin-dependent protein kinase 1D	10p13-p14	GWA	37
TSPAN8-LGR5	Tetraspanin 8 and leucine-rich-repeat-containing G-protein coupled	12q21	GWA	37
THADA	Thyroid adenoma-associated	2p21	GWA	37
ADAMS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9	3p14	GWA	37
NOTCH2	Notch homolog 2, *Drosophila*	1p12	GWA	37
ADCY5	Adenylate cyclase	3	GWA	38
CDKN2A/B	Cyclin-dependent kinase inhibitor 2A/B	9p21	GWA	40
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1	6p22.2	GWA	34 and 40
SLC30A8	Solute carrier family 30, member 8	8q24.11	GWA	34 and 40
IGF2BP2	Insulin-like growth factor 2 mRNA binding protein 2	3q28	GWA	34 and 40
HHEX/IDE	Hematopoietically expressed homeobox and insulin-degrading enzyme	10q23-q25	GWA	34 and 40
FTO	Fat mass and obesity associated	16q12.2	GWA	34
MTNR1B	Melatonin receptor 1B	11q21-q22	GWA	42
KCNQ1	Potassium channel, voltage-gated, KQT-like subfamily, member 1	12q21	GWA	32
IRS1	Insulin receptor substrate 1	2q36	GWA	41
GCK	Glucokinase	7p15.3-p15.1	GWA	42
GCKR	Glucokinase regulator	2p23	GWA	42
C6PC2	Glucose-6-phosphatase, catalytic 2	2q24.3	GWA	42
TNFa	Tumor necrosis factor-α	6p21.3	Candidate gene	45
HNF1A	Hepatocyte nuclear factor 1α	12q24.2	Candidate gene	46
HNF4A	Hepatocyte nuclear factor 4α	20q13.12	Candidate gene	46

tissues and biofluids. There are currently 7,900 entries in the Human Metabolome Database (http://www.hmdb.ca), which contains detailed information about small molecule metabolites and will be useful for applications in metabolomics, clinical chemistry, and biomarker discovery.

Susceptibility gene markers in type 1 diabetes

In type 1 diabetes, the study of susceptibility genes has been facilitated by the availability of large collections of families with affected sibling pairs as seen in the Type 1 Diabetes Genetics Consortium (48). Type 1 diabetes is a multifactorial disease where loci within the HLA account for most of the genetic susceptibility. The major susceptibility locus maps to the HLA class II genes at 6p21, accounting for up to 30–50% of genetic type 1 diabetes risk (48). The association of genes of the class II region is thought to reflect their role in the T-cell immune response. The major susceptibility class II loci are HLA-DRB1 and HLA-DQB1/DQA1 on chromosome 6p21 and, to a lesser extent, HLA-DPB1/DPA1 (48,49). Association studies are complicated by the high polymorphism of the HLA DPA1 and DPB1 loci. The HLA loci, DRB1, DQA1, DQB1, DPA1, DPB1, A, B, and C, have repeatedly been shown to be involved in type 1 diabetes susceptibility. However, conflicting results have been obtained for the HLA loci involved in susceptibility or protection as a result of coinherited loci, population-specific differences, typing approaches used, differences in study design, or low-powered studies (49).

The highest-risk DR/DQ haplotypes, DR3 and DR4, exhibit a spectrum of risk from increased to neutral to protective (50). Type 1 diabetes incidence is increasing worldwide each year, and it appears that as the disease increases the percentage of cases with the high-risk HLA DR3/4 genotype is decreasing, suggesting an increased environmental pressure or contribution of other non-HLA class II alleles to diabetes risk (51). Type 1 diabetes risk is also linked to the major histocompatibility complex independently of HLA-DR/DQ such as HLA class I alleles (52). Studies have supported a role for HLA class I alleles in type 1 diabetes susceptibility including B*3906 and B*5701 (53). The large datasets generated by studies such as the Type 1 Diabetes Genetics Consortium are crucial for the generation of sufficient class I data

for disease association studies (48). Studies are ongoing, investigating HLA class I and class III alleles.

More than 40 non-HLA susceptibility gene markers have been identified that contribute to type 1 diabetes risk. However, for many of these genetic predictors of risk the effect is small, even for the strongest loci (54). These non-MHC loci include the insulin gene (*INS*) on chromosome 11p15 (55), which confers ~10% of the genetic susceptibility to type 1 diabetes. The cytotoxic T cell–associated protein 4 (*CTLA4*) gene on chromosome 2q33 is associated with type 1 diabetes (56). Also, the protein tyrosine phosphatase, nonreceptor type 22 (lymphoid) (*PTPN22*) gene on chromosome 1p13 (57), involved in preventing spontaneous T-cell activation, is linked to type 1 diabetes risk (57). A number of other associations have been proposed such as IL-2 receptor, -α (*IL2RA*), and interferon-induced with helicase C domain 1 (*IFIH1*) genes (58) and KIAA0350 (59) and small ubiquitin-like modifier 4 (SUM04) (60). Many new candidate genes are emerging such as *IL10*, *IL19*, *IL20*, *GLIS3*, *CD69*, and *IL27* (58), but further genotyping and functional studies are needed to determine whether the genes are causal. With important breakthroughs in DNA sequencing technology and mapping of diabetes cases, the determination of extreme genetic risk of type 1 diabetes in the general population could eventually lead to intervention or prevention trials.

Future developments in the search for improved biomarkers for the diagnosis and treatment of diabetes

Translating research findings to useful and reliable clinical tests has been challenging; however, the discovery of ideal biomarkers for diabetes is improving along with the development of biomarker panels and new methodologies. In the future, diagnostic tests may be used to select individuals who are likely to benefit from treatment or those who demonstrate an objective indication of treatment efficacy. Emergence of diabetes-associated genetic variants represents a powerful tool for improving our understanding of the pathogenesis of diabetes. However, translation of these novel findings to genetic screening and personalized medicine is still at an early stage. Characterization of functional variants and an understanding of the mechanisms by which these loci confer susceptibility to disease are needed. With discovery of genes linked to fasting glucose, it may be possible to identify other loci associated with additional features of the type 2 diabetes phenotype such as impaired glucose tolerance, defective first-phase insulin release, and insulin resistance. Combination of genetic and biomarkers screens may provide further opportunities. The challenges in harnessing the potential of new biomarkers should be alleviated by new and exciting collaborations between pharmaceutical agencies, diagnostic companies, and academic institutions, with the harnessing of skills from the different clinical, biomedical, diagnostic, and pharmacological areas.

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