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Gene-Environment and Gene-Treatment Interactions in Type 2 Diabetes

Progress, pitfalls, and prospects

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Type 2 diabetes has rapidly emerged as a global health crisis. Because population-level genetic changes take many generations to occur, this epidemic is almost certainly primarily a consequence of recent environmental changes; nonetheless, diabetes does appear to occur preferentially in genetically predisposed populations, which suggests that the effects of pre-existing susceptibility genes have been triggered by recent shifts in nongenetic factors.

Predisposition is influenced by the level of certain environmental exposures, personal factors, access to good-quality primary care, and by genotype. Interactions between genetic and nongenetic risk factors are hypothesized to raise diabetes risk in a synergistic manner; reciprocally, health-enhancing changes in behavior, body composition, or medication may reduce the risk of disease conveyed by genetic factors. Defining the nature of these interactions and identifying ways through which reliable observations of gene-environment interactions (GEIs) can be translated into the public health setting might help 1) optimize targeting of health interventions to persons most likely to respond well to them, 2) improve cost- and health-effectiveness of existing preventive and treatment paradigms; 3) reduce unnecessary adverse consequences of interventions; 4) increase patient

adherence to health practitioners' recommendations; and 5) identify novel interventions that are beneficial only in a defined genetic subgroup of the population. In this Perspective, we describe the rationale and evidence relating to the existence of gene-environment and gene-treatment interactions in type 2 diabetes. We discuss the tried, tested, and often-failed approaches to investigating gene-lifestyle interactions in type 2 diabetes; we discuss some recent developments in gene-treatment interactions (pharmacogenetics); and we look forward to the strategies that are likely to dominate these fields of research in the future. We conclude with a discussion of the requirements for translating findings from these future studies into a form where they can be used to help predict, prevent, or treat diabetes. Here we describe the rationale and evidence concerning GEIs and gene-treatment interactions in type 2 diabetes, provide an interpretation of current findings and strategies, and offer a view for their future translation.

What is GEI?—The definition of GEI varies somewhat depending on the field of diabetes research. In this review, we adopt epidemiological definitions of interaction, also known as *effect modification* or *effect modulation*. For binary outcomes,

an interaction would be present if the combined risk attributable to genetic and environmental exposures is significantly greater or less than expected if their effects were additive. For quantitative traits, an interaction would be present if the magnitude of the genetic effect estimate differs across the range of an environmental exposure or treatment. Although the word *environment* when used in the context of GEI can relate to any nongenetic factor to which a person is exposed, extending from the macro (e.g., urban planning) to the micro (e.g., circulating proteins) environment, in the fields of complex disease research, the word *environment* has most often referred to lifestyle behaviors (e.g., diet or physical activity), although this view is evolving (Fig. 1). The word *interaction* is sometimes used to describe the joint effects of a genetic exposure and a second factor that is positioned on the causal pathway between the genetic exposure and a disease phenotype; in epidemiology, this process is termed *mediation*, which differs in meaning from *interaction*. The term *epistasis* refers to the interaction between two or more genetic loci.

Why do we think GEIs cause type 2 diabetes?—The evidence supporting the existence of gene-lifestyle interactions in type 2 diabetes comes primarily from 1) the pattern and distribution of diabetes across environmental settings and ethnic groups, 2) family-based intervention studies, in which response to interventions varies less between biologically related individuals than between unrelated individuals; and 3) animal studies in which genetic and environmental factors are experimentally manipulated to cause changes in the expression of metabolic phenotypes. A brief overview of pertinent literature from human studies is given below.

There is considerable global variation in the prevalence and incidence of type 2 diabetes (1). In societies of European origin, the prevalence of type 2 diabetes is generally 10% or less, with the disease confined primarily to overweight and

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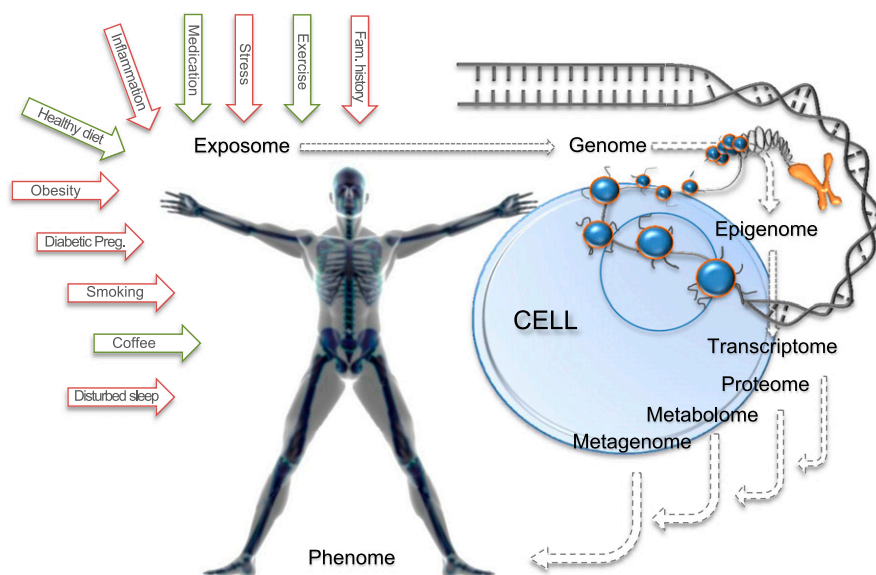


Figure 1—The future of research on stratified diabetes medicine: a systems epidemiology approach to the discovery of interactions between the exposome (all nongenetic elements to which we are exposed) and the quantifiable elements of the human physiome.

obese older adults. However, in some nonwhite populations, including Native Americans (especially Pima Indians), Alaskan Natives, Micro-Indonesia islanders (especially Nauruans), and some Middle Eastern (especially Saudis and Emiratis) and Canadian First Nation populations (2,3) (www.phac-aspc.gc.ca/cd-mc/publications/diabetes-diabete/facts-figures-faits-chiffres-2011/index-eng.php), the prevalence of type 2 diabetes is substantially higher than in the rest of the world.

The Pima Indians of Arizona have the highest recorded prevalence of type 2 diabetes, with more than half of the adult population affected by the disease (3), and diagnoses are often made in adolescence and occasionally in childhood (4). The damming of the Salt and Gila rivers around 1911 brought an abrupt end to the Pima's traditional subsistence farming lifestyle and with it a sharp reduction in occupational physical activity and the consumption of fresh produce. A second group of Pima Indians live in the Sierra Madre Mountains of northern Mexico. Although Arizona and Mexican Pima are phylogenetically similar (5), their lifestyles stand in stark contrast, with the Mexican Pima still living a traditional way of life left behind by Arizona Pima almost a century ago. Probably because of this, the prevalence of type 2 diabetes in Mexican Pima is roughly five-times lower than that seen in their Arizonan cousins, with diabetes prevalence in the

Mexican Pima comparable to that of other non-Pima populations of northern Mexico (5). This observation emphasizes how environmental changes can awaken an underlying, possibly genetic, susceptibility to obesity and type 2 diabetes.

A popular yet contentious explanation for why indigenous groups (whose evolution has involved long periods of migration, hunter-gatherer lifestyles, and frequent famine) are so susceptible to the adverse consequences of industrialized environments is termed the “thrifty genotype hypothesis,” first proposed by Neel in the 1960s (6). Whereas the original description of the thrifty genotype hypothesis focused on the over-production of insulin after meals and a corresponding period of hypoglycemia that induces appetite, the idea that efficient storage and utilization of energy in adipose tissue is a selected trait has also been widely discussed and attributed to thrifty genes (7). The hypothesis hinges on the notion that frequent exposure to famine and other physiologically stressful events, such as migration and cold temperatures, over thousands of years of evolution may have enriched certain populations with gene variants that promote metabolic thriftiness, which in turn conveyed a survival advantage during famine or other periods of energetic stress. In the modern world, however, where excessive automation and almost effortless access to energy-dense foods are rife, calorie accumulation and storage may become

metabolically deleterious. Of note, however, there is little evidence of positive selection genetic signatures around established type 2 diabetes loci (8), suggesting these diabetes loci at least are not thrifty genes.

Caveats of the literature on gene-lifestyle interactions

A recent simulation study on the role gene-gene and GEIs are likely to play in risk prediction and targeted medicine reached a rather sobering conclusion (9). The authors estimated that the average improvement in predictive accuracy, as defined by the area under the receiver operating characteristic curve, for type 2 diabetes was ~5% when between 4 and 20 interactions were added to a prediction model. To conduct their simulations, Aschard et al. (9) made a series of assumptions about the magnitude and frequency of interaction effects, based on published epidemiological studies that had focused on common diseases, common exposures, and common variants; however, it is possible that as geneticists begin to study lower frequency variants, fairly large magnitude interaction effects may be discovered, albeit affecting relatively few individuals, which would likely increase the value of data on interactions for disease prediction.

Epidemiological studies have been the predominant source of literature on gene-lifestyle interactions in cardiovascular and metabolic disease. Dozens of case-control and cohort studies have been published since the late 1990s purporting to have identified gene-lifestyle interactions in type 2 diabetes or related quantitative metabolic traits. Until recently, however, most of these studies were small and often relied on imprecise estimates of environmental exposures and outcomes. These are prone to error and bias, and exposures may not be assessed at the time when they conveyed their effects; for example, the causative exposures may have occurred very early in life, perhaps even in utero. Moreover, the complexities of modeling interaction effects have forced geneticists to focus primarily on very simple models of interaction, whereas clinically relevant interaction effects likely involve multiple genetic and nongenetic biomarkers. In addition, barely a handful of studies have examined incident type 2 diabetes as an outcome, with most focusing on cross-sectional measures of glucose and others relying

on analyses that include prevalent cases of diabetes; this may introduce labeling bias, where the recall of well-known diabetes-associated behaviors is less likely to be accurate in individuals recently diagnosed with disease than in those who have not been diagnosed with disease.

In a systematic review published in 2006 (10), we found that almost all studies published at that time included fewer than 1,000 participants and none included more than 3,000 participants. Although all studies lacked rigorous replication, we identified four classes of genes harboring loci that showed most consistent evidence of gene-lifestyle interactions in diabetogenic traits: the $\beta 2$ adrenergic receptor (*ADRB2*), uncoupling proteins (*UCP*) 1-3, lipid-related loci (*LIPC*, *LPL*, *FABP2*, *APOC3*, and *APOE*), and the peroxisome proliferator-activated receptor- γ (*PPARG*). Of these, *PPARG* (at its common missense polymorphism Pro12Ala) was perhaps the most promising candidate, with a number of studies reporting interactions with dietary fatty acids or exercise in relation to insulin concentrations (11–13), adiposity (12,14,15), and type 2 diabetes (16). Studies examining interactions between dietary fats and the Pro12Ala genotype have continued to accrue, and attempts have been made to formally summarize this literature through meta-analysis. These attempts have been unsuccessful, however, owing to the challenges of pooling unstandardized data and the inadequate descriptions of the methods and results in many published studies (17).

Other key caveats to small gene-lifestyle interaction studies include their likelihood to be underpowered and that they are prone to reporting biases (18). The problems with measurement imprecision in studies of gene-lifestyle interaction are eloquently outlined and discussed by Wong et al. (19), where the authors provided estimates of sample-size requirements to detect gene-lifestyle interactions in the presence of varying degrees of environmental exposure assessment and phenotyping measurement error. The authors show that for the detection of fairly large magnitude gene-lifestyle interactions ($\beta_{GE} = 2$), a study of $\sim 2,000$ individuals in which precise measures of environmental exposure and outcome had been made would be adequately powered (95% power, critical $\alpha P = 1 \times 10^{-4}$); however, studies that imprecisely estimated environmental exposures and phenotypes, as is often the case in epidemiological

studies, would require a sample collection ~ 50 -fold larger to afford comparable power.

Although the expected range of effects that are realistic for gene-lifestyle interactions in type 2 diabetes remains unclear, a doubling of the genetic risk estimate in the group exposed to adverse lifestyle factors compared with those who are unexposed ($\beta_{GE} = 2$) is at the upper end of the interaction effect estimate ranges reported for common variants and common exposures (10). It is reasonable to conclude, therefore, that most of the interaction studies published to date report “lucky” true-positive results or false-positive results that may be underpinned by analytical and reporting biases. The replication of few examples of gene-lifestyle interactions in type 2 diabetes suggests that the literature is composed largely of the latter. Despite this, recent developments in the ways genetic association studies are performed, such as adoption of hypothesis-free approaches, the availability of comprehensive genotype arrays in large sample collections, global collaborations, and more rigorous analysis and reporting of data, have led to the emergence of many reproducible genetic association signals for type 2 diabetes and related glycemic traits, which has spurred a number of large-scale studies of gene-lifestyle interactions.

Using genome-wide association studies to inform the selection of loci for studies of gene-lifestyle interactions

The identification of more than 50 genetic loci that are reproducibly associated with type 2 diabetes (20) (Fig. 2) and 53 additional loci for glucose and insulin concentrations (21) has fueled multiple studies in which these loci have been tested for interactions with lifestyle risk factors for type 2 diabetes. One of the first publications of this kind focused on the interaction between the *FTO* rs9939609 variant and physical activity. Two cross-sectional cohort studies (22,23) and one clinical trial analysis (24), published at approximately the same time, provided nominal evidence that physical activity modifies the effects of the *FTO* variant on BMI or adipose tissue accumulation. Replication studies achieved mixed results; thus, we sought a definitive answer by conducting a prospective meta-analysis including 45 adult ($n = 218,166$) and 9 pediatric ($n = 19,268$) cohorts (25). Although the study

yielded a statistically significant interaction summary statistic ($P_{interaction} = 0.005$), which was directionally consistent with the original studies' findings (22), the effect estimate was heterogeneous ($I^2 = 36\%$), suggesting the presence of one or more latent effect-modifiers (i.e., unidentified factors that change the magnitude of *FTO*'s effect on BMI). Further data exploration determined that the source of this heterogeneity was the geographic origin of the cohorts, with the interaction effect being driven almost entirely by the North American cohorts. Although this geographic difference remains unexplained, the observation strongly suggests that physical activity is not the causal effect-modifier; instead, factors that correlate with physical activity in North American but not in European cohorts, such as specific dietary factors, are likely to be the causal modifiers of *FTO*'s obesogenic effects. It is also important to bear in mind that almost all studies reporting significant *FTO*-lifestyle interactions are cross-sectional observational studies, from which causal effects and causal direction are almost impossible to ascertain. Thus, even if a causal relationship underlies the results reported above, it is possible that the direction of effect is reversed (i.e., a direct effect of *FTO* variation on lifestyle behaviors, which is stronger in fatter compared with leaner people). These alternative explanations are important to consider when discussing, as many do, the potential translational implications of studies of gene-lifestyle interactions.

The first large-scale study examining the interaction of established type 2 diabetes loci and physical activity included 16,000 men and women from southern Sweden, of whom 2,200 went on to develop diabetes during the ensuing 25 years of follow-up (27). Of the 17 established diabetes loci examined, the study identified a single locus, the noncoding polymorphism rs4430796 at the diabetes gene *HNF1B*, that interacted with baseline physical activity levels as estimated by questionnaire (Bonferroni corrected $P_{interaction} = 0.015$). In homozygotes for the nonrisk allele (A), baseline physical activity apparently protected against the development of type 2 diabetes, as one would predict from previous studies of physical activity and diabetes; however, in carriers of the rs4430796 risk allele, the protective effect of physical activity appeared to be diminished in a dose-dependent fashion.

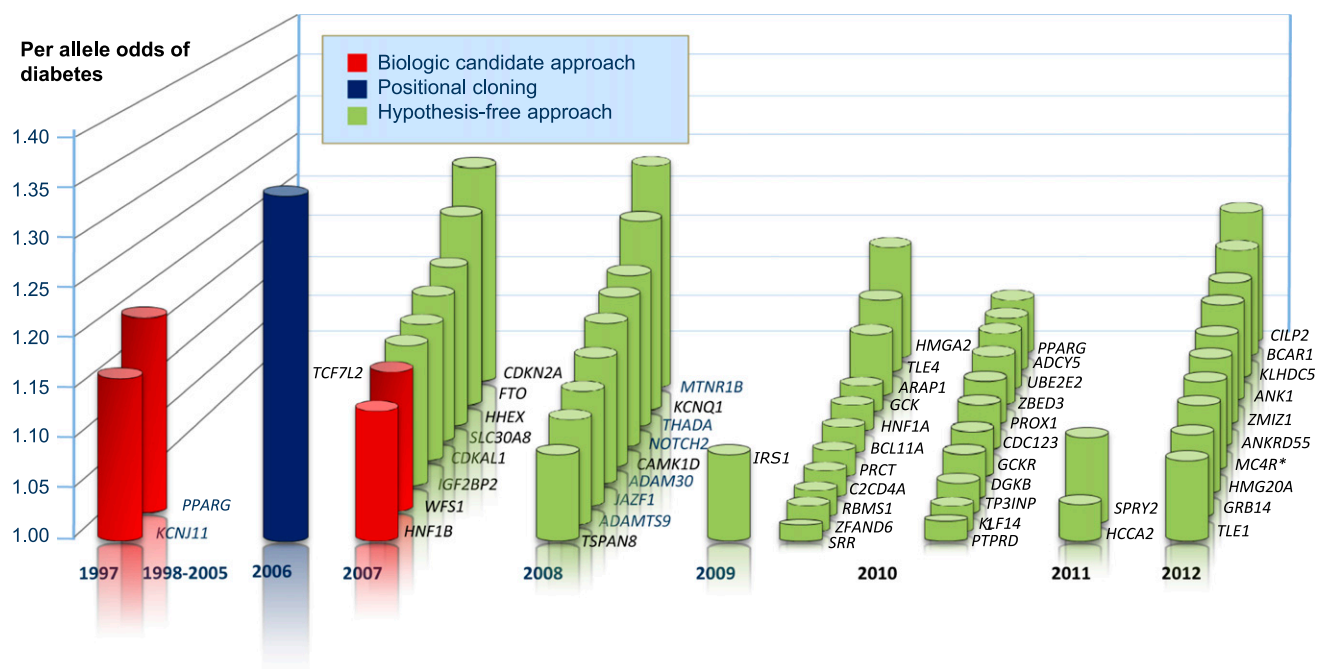


Figure 2—The evolving landscape of established type 2 diabetes-associated genetic loci. Effect estimates and list of loci are adapted from reference 21. Variants in *CCND2* and *GIPR* (not shown in Fig.) have sex-heterogeneous effects, with the former having a larger effect in males and the latter in females (21). *This locus was initially associated with type 2 diabetes by Renström et al. (67).

In a separate cohort from southern Sweden of ~25,000, two gene-diet interactions have been reported in relation to type 2 diabetes. Sonestedt et al. (28) reported that dietary fat and dietary carbohydrate intake obtained by questionnaire modified the effect of the rs10423928 variant at *GIPR* on incident diabetes, such that the odds of diabetes associated with the established diabetes risk allele (A) were highest in individuals who consumed low levels of dietary fat or high levels of dietary carbohydrate. In the second study, Hindy et al. (29) reported that the diabetogenic effect of the *TCF7L2* variant rs7903146 was augmented in individuals consuming high levels of dietary fiber; although the test of interaction was nominally statistically significant ($P_{\text{interaction}} = 0.049$), it was no longer significant after correction for multiple testing. The findings of Hindy et al. contrast with those reported earlier in the Nurses Health Study, where Cornelis et al. (30) reported that for a correlated variant (rs12255372; $r^2 = 0.73$ and $D' = 0.93$ with the rs7903146 *TCF7L2* variant in white Europeans: www.hapmap.org), the effect of the diabetogenic genotype was greatest in women who reported consuming diets with a high glycemic load or index (uncorrected $P_{\text{interaction}} = 0.03$). A further study of 46,000 individuals (31) reported no evidence of interaction

between the rs4506565 variant ($r^2 = 0.92$ and $D' = 1.00$ with the rs7903146 *TCF7L2* variant in Europeans: www.hapmap.org) and dietary whole-grain intake in relation to insulin or glucose concentrations (nominal $P_{\text{interaction}} = 0.88$).

Qi et al. (32) studied the interaction of a Western dietary pattern assessed by questionnaire and a genetic risk score (GRS) consisting of 10 genetic loci that had previously been reproducibly associated with type 2 diabetes (20). Cross-sectional analyses were conducted in a cohort of 1,196 prevalent and incident case participants with diabetes and 1,337 matched control participants from the Health Professionals Follow-up Study (32). The Western diet score was more strongly associated with diabetes risk in the health professionals with a higher GRS and less so in those with a lower GRS ($P_{\text{interaction}} = 0.02$). Interaction analyses focusing on specific components of the Western diet score indicated that consumption of red and processed meat underlies the interactions described above and that heme iron intake, in particular, may be the central component of the diet score driving the interaction with the GRS. However, an analysis of ~50,000 nondiabetic individuals by the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium failed to find any evidence that established glucose- or

insulin-associated loci modify the effects of Western dietary pattern on fasting insulin or glucose levels (33).

Individual groups and large consortia have embarked on genome-wide association study (GWAS) analyses stratified by potential effect modifiers such as sex and BMI. These studies are often much larger than single-cohort analyses but are restricted to cross-sectional data, which may hinder the interpretation of results for the reasons discussed above. This is so, particularly when focusing on BMI, because the onset of diabetes can correspond with weight loss as a consequence of lifestyle changes in the immediate aftermath of a diagnosis, treatment, or of the disease process itself. Nevertheless, in an analysis of 2,112 lean type 2 diabetes case subjects, 4,123 obese type 2 diabetes case subjects, and 54,412 unstratified non-diabetic control subjects, Perry et al. (34) identified a *LAMA1* variant that conveyed a significantly higher odds of diabetes in lean compared with obese diabetic case subjects. Similar BMI stratum-specific genetic effects were observed for 29 of the 36 type 2 diabetes loci (binomial $P = 0.0002$) that had been identified previously in unstratified GWAS meta-analyses performed by the DIABetes Genetics Replication and Meta-analysis (DIAGRAM) consortium (20). These results indicate that when diabetes develops in a person

who is lean, genetic risk factors are more likely to be present than in someone who is obese and develops the disease or that weight loss enhances the genetic risk of diabetes.

Genetic analyses performed in clinical trials involving intensive lifestyle modification provide an important adjunct to the epidemiological literature on gene-lifestyle interactions in type 2 diabetes. On one hand, a major advantage of randomized controlled trials is that interaction effects observed in trials are likely to reflect causal processes, whereas those observed in epidemiological investigations are more prone to confounding and reverse causality. Other advantages of well-designed clinical trials include relatively precise estimates of the environmental exposures (treatments) and of the phenotypes, careful ascertainment of participants, randomization of exposures, close predetermined follow-up, and hypothesis-driven design. On the other hand, trials are often smaller than epidemiologic cohorts, control poorly for changes in behavior outside the intervention sessions, and typically consist of individuals at high risk of diabetes; hence, results may not be generalizable to other population subgroups.

Only two randomized controlled trials, the Diabetes Prevention Program (DPP) from the U.S. (35) and the Finnish Diabetes Prevention Study (DPS) (36), have reported results for gene-lifestyle interactions in relation to diabetes incidence. Both studies focused on people at high risk of developing type 2 diabetes and implemented almost identical lifestyle intervention protocols. The DPP randomized 1,079 participants to an intensive lifestyle intervention, 1,082 to a placebo control arm, and 1,073 to metformin treatment. In the Finnish DPS, 522 participants were randomized to a lifestyle or control intervention. The DPP is well powered (~80%) to detect genetic effects, with a hazard ratio of 1.2, but has appreciably lower power to detect gene-treatment interactions (37,38); statistical power to detect interactions in the Finnish DPS is less than in the DPP owing to its smaller sample size.

Notwithstanding the sample size constraints of the DPP, a number of interesting findings relating to gene-treatment interactions have emerged from the trial. For example, lifestyle intervention offsets the risk conveyed by the diabetogenic alleles at the *TCF7L2* rs7903146 (39) and *ENPP1* K121Q (40) loci, or by a genetic

risk score consisting of 34 type 2 diabetes-associated variants (41). Elsewhere, the DPP investigators reported that the *CDK2NA/B* rs10811661 variant diminishes the effects of lifestyle intervention on diabetes risk and on estimated insulin secretion ($P_{\text{interaction}} \leq 0.05$) (37); interestingly a subsequent cohort study of 8,600 nondiabetic Swedish adults reported directionally consistent interactions between the same genotype and physical activity levels on the odds of impaired glucose regulation and on continuous 2-h glucose concentrations ($P_{\text{interaction}} \leq 0.015$) (27).

Hypothesis-free discovery of gene-lifestyle interaction effects

The decision to carry forward findings from conventional GWAS experiments to detect gene-lifestyle interactions is a simple, pragmatic, and relatively cost-efficient strategy. However, of the many loci associated with cardiometabolic traits, few have been reproducibly shown to interact with environmental factors; *FTO* (physical activity interactions in obesity) (25), chromosome 9q21 variants (prudent diet interactions in cardiovascular disease and myocardial infarction) (42), and an obesity GRS (sugar-sweetened beverages interaction in obesity) (43) are rare examples of gene-lifestyle interactions in cardiometabolic traits that have been robustly replicated.

The paucity of replicated examples of GWAS-derived loci that interact with lifestyle factors may be due to the low prioritization of follow-up studies by investigators and journal editors or that not all GWAS-derived loci have been examined for interactions in well-designed studies. Alternatively, it is possible that the statistical approaches used in conventional GWAS experiments bias against the detection of variants that interact with environmental factors that are reasonably prevalent within the populations in which the GWAS are performed. Indeed, the GWAS ranking system is typically based on the *P* value derived from the main effect regression model for each single nucleotide polymorphism (SNP). Of note, common, disease-associated variants have relatively small effect sizes (typically odds ratios <1.4 per risk allele); thus, for a genetic association signal to exceed the conservative genome-wide probability threshold used in most GWAS ($P = 5 \times 10^{-8}$), the estimates of the genetic effect are relatively consistent in magnitude

within and between the populations included in GWAS meta-analyses, as reflected in the narrow CIs for the odds ratios of the top-ranked loci and low heterogeneity estimates. Broadly speaking, one would expect that the larger the magnitude of a GEI or gene-gene effect, the greater the variance associated with the main effects for the genetic and/or environmental components (44). Hence, with some exceptions (25,27), it is likely that the gene variants that are most relevant for GEI are those that rank poorly in most GWAS meta-analyses.

Genome-wide interaction studies have potential to identify gene variants that influence diabetes risk that might not be detected using hypothesis-driven approaches. However, the statistical power limitations of such studies when applying conventional tests of interaction, combined with the challenges of identifying large cohort collections with appropriately characterized environmental, genetic, and phenotypic data, pose challenges that conventional genetic association studies do not face. Several methods have been developed to mitigate these challenges; among the most promising is the joint meta-analysis approach, which is derived from the model with two degrees of freedom popularized by Kraft et al. (45) and developed further by Manning et al. (46). Manning et al. (47) went on to apply the joint meta-analysis approach in a genome-wide study of 52 cohorts in which they tested for SNP main effects and interactions (with BMI) on fasting glucose and insulin levels. The analysis yielded novel experiment-wide association signals for main effects, but none was discovered for interactions.

Recognizing that heterogeneous effect estimates are a signature of loci involved in interactions, Paré et al. (48) and Visscher and Posthuma (49) developed methods that model genetic associations with genotypic variance estimates rather than with phenotypic means, as is the case in conventional GWAS experiments. Both approaches involve two key steps: in step 1, a phenotypic variance estimate is obtained for each of the genotypes at a SNP locus. A statistical comparison of these variance estimates is then made and a *P* value obtained. These *P* values are ranked from lowest to highest, and those that exceed an experiment-wide threshold are carried forward to step 2, where conventional, pairwise tests of GEI are performed for an array of environmental exposures, with the intent of identifying one or more

that underlie the SNP's heterogeneous effect estimate revealed in step 1. In a recent application of method described by Visscher and Posthuma, Yang et al. (26) performed a meta-analysis for height and BMI in 170,000 samples and identified a single locus for BMI that met the genome-wide significance threshold; intriguingly, this locus was *FTO*, the most plausible candidate for GEI in obesity currently known. There was no genome-wide significant discovery for height, which is perhaps unsurprising given that this trait is under much tighter genetic control than weight and varies much less than weight across the adult life span.

Pharmacogenetics—Pharmacogenetics is a specialized example of GEI. Here, the environmental exposure is drug treatment; by studying interactions between gene variants and treatment, investigators seek to identify variants that are associated with adequate or inadequate response to diabetes therapies. The pharmacogenetics of diabetes therapies have been extensively reviewed elsewhere (50–52). However, three examples illustrate successful approaches and the potential clinical utility of pharmacogenetics in diabetes:

Firstly, studies of *HNF1A* mutations that cause a form of maturity-onset diabetes of the young showed that carriers of these mutations, who are often misdiagnosed as having type 1 or type 2 diabetes, respond better to sulfonylureas than metformin, thus facilitating their transition off insulin or metformin (53,54).

Secondly, common genetic variation in the gene that encodes a transporter responsible for disposing of metformin (*MATE1*, encoded by *SLC47A1*) has been associated with metformin response in a retrospective patient cohort (55), a preliminary finding corroborated in the DPP clinical trial (56).

Finally, in a discovery GWAS of 1,000 metformin-treated patients from the Genetics of Diabetes Audit and Research Tayside (GoDARTS) study, a locus including the *ATM* gene was associated with metformin response. This discovery was initially replicated in independent GoDARTS and UK Prospective Diabetes Study cohorts (57) as well as subsequent cohorts that were similarly ascertained (58); however, it was not reproduced in the DPP clinical trial, which differs from the earlier studies by its experimental, prospective design and its enrollment of nondiabetic participants in whom

metformin was used for diabetes prevention (56). If *ATM* is eventually established as a causal regulator of metformin response, this will provide a novel unexpected role for this established oncogene in diabetes treatment. The challenge for pharmacogenetics is to establish clinical utility, which in adult diabetes is currently limited to the *HNF1A* paradigm. An elegant example of how pharmacogenetics influences therapy in neonatal diabetes has been reviewed by Greeley et al. (59).

Future directions—We have emphasized GEI in this Perspective because this is where most of the published research has been focused to date. A complementary set of disease predictors is being generated with the emergence of comprehensive *metabolomic* approaches, in which circulating small molecules present in human fluids are assayed in a high-throughput manner through liquid chromatography and mass spectroscopy. These molecules represent metabolic readouts of cellular states at a systems level and reflect the output of gene products and also their interactions with the environment. Using these platforms, independent groups have established a metabolomic signature of branched chain and aromatic amino acids as associated with obesity and insulin resistance (60) as well as future diabetes (61). How genes regulate circulating levels of these molecules, what they tell us about gene function, how much they reflect environmental factors, and to what extent they provide orthogonal information for diabetes prediction and treatment response is the subject of intense investigation. The participant-level integrated assessment of variation in the genome, metabolome, and other aspects of the physiome (e.g., microbiome, transcriptome, and proteome) in large cohorts has not previously been possible, but with recent advances in technology and analytical methods, and cost reductions, this is now feasible and is evolving into a new field called *systems epidemiology* (Fig. 1). This topic is eloquently reviewed elsewhere (62,63).

Although recent genetic discoveries in metabolic traits have typically illustrated novel pathways, pointed toward fundamental biology, confirmed prior epidemiological observations, highlighted the role of β -cell dysfunction in type 2 diabetes, and provided possible targets for pharmacotherapy, their role

in genetic prediction is less clear. This is partly so because even in aggregate, they only explain a relatively small fraction of the disease's heritability (41,64–66). The latter is likely due to insufficient sample sizes to detect small effects, a nearly exclusive focus on populations of European descent, an imperfect capture of infrequent genetic variants, an incomplete ascertainment of alternate (non-SNP) forms of genetic variation, and the limited exploration of additional genetic models, including those involving GEI. As the community embraces complementary approaches that include systematic fine-mapping, custom-made replication, denser genotyping arrays, platforms that focus on functional variation, next-generation sequencing techniques, expansion to non-European populations, and integration of other global biological measurements with genetic data, the coming years will continue to elucidate the genetic architecture of metabolic phenotypes and its interaction with the environment. A more refined characterization of the molecular basis of type 2 diabetes can then be translated into more detailed disease nosology, appropriate targeting of more effective and better tolerated therapeutic or preventive strategies, more rational and efficiently designed clinical trials, and stratification of risk groups so that costly public health interventions can be deployed intelligently.

We speculate that the future of diabetes medicine may involve genetic and molecular biomarker screening in patients to inform the prescription of lifestyle or drug therapy for diabetes prevention or management. However, the translation of this vision into clinical practice will require structured research programs that combine observational epidemiology to generate relevant hypotheses and experimental studies that test these hypotheses and demonstrate cause and effect. When reliable and causal interactions are discovered, it will be necessary to conduct studies proving that the inclusion of this information into conventional risk prediction algorithms improves predictive accuracy and/or reclassification, or that stratified medicine informed by biomarker data improves treatment outcomes; in addition, it will also be necessary to show that these strategies are cost-effective compared with conventional approaches.

The concept of stratified medicine (otherwise known as personalized or

precision medicine) is an area of considerable research interest both within the pharmaceutical industry and academia. The European Union-Innovative Medicines Initiative (IMI)—funded Diabetes REsearch on patient sTratification (DIRECT) study (www.direct-diabetes.org) is an academic-industry program that addresses most of these questions, spanning a discovery phase of comprehensive phenotyping, and large-scale omic analysis through to a validation and clinical trial phase to establish utility of a biomarker-stratified approach to diabetes medicine. The study focuses on two phenotypes where stratification approaches can be applied (glycemic deterioration of prediabetes and diabetes) and therapeutic response (to sulfonylureas, metformin, GLP-1R agonists, and obesity surgery). For each phenotype, the plan is to integrate physiological parameters with lifestyle measures, genetic (GWAS and sequencing), transcriptomic, metabolomic, proteomic, and metagenomic data to enable a comprehensive analysis for discovery of stratification and surrogate biomarkers. Biomarker stratified clinical trials will then be done to establish the utility of biomarker led therapeutics over traditional nonbiomarker led studies.

Similar technologies can be applied in the framework of comparative medicine. The National Institutes of Health-sponsored Glycemia Reduction Approaches for Diabetes: A Comparative Effectiveness Study (GRADE) trial will for the first time perform head-to-head comparisons of representative agents from four major drug classes for type 2 diabetes treatment—the sulfonylurea glimepiride, the DPP-4 inhibitor sitagliptin, the GLP-1 agonist liraglutide, and the basal insulin glargine—as adjuncts to metformin in achieving glycemic control. This large randomized clinical trial, planning to enroll 6,000 participants in 40+ centers throughout the United States, will be launched in the spring of 2013 and will collect phenotypes, covariates, end points, and biomaterials on all participants to enable the deployment of omics techniques to examine prediction and response to pharmacological manipulation.

Conclusions—We are witnessing a time in biomedical research when systems can be queried globally to establish the metabolic state of the organism in a single experiment. Such technologies can also be deployed across populations, tissues, and environmental conditions. The integration

of all this information and its interpretation into a cogent vision presents enormous challenges, not least of which is the scientific imperative of reproducibility. As rigorous analytical standards are implemented and international collaborations enable the pursuit of these fundamental questions at an adequate scale, we stand on the verge of a true transformation of medicine as applied to the individual patient. While discovering and replicating evidence of GEIs is proceeding, the process of discussing and planning how such data can be translated into the clinical arena should already be underway. Current discoveries should also prompt us to consider the benefits and challenges (e.g., ethical, economic, logistic) that using genetic information in diabetes medicine is likely to present, which may enable the rapid translation of human genetics research into clinical practice.

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P.W.F. planned and wrote the initial draft of the manuscript, revised and edited its content, and approved its submission. E.P. and J.C.F. wrote key sections of the manuscript, revised and edited its content, and approved its submission.

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