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Invited Commentary

Invited Commentary: Gene × Lifestyle Interactions and Complex Disease Traits—Inferring Cause and Effect From Observational Data, Sine Qua Non

Paul W. Franks* and Jennifer A. Nettleton

* Correspondence to Prof. Paul W. Franks, Department of Clinical Sciences, Building 91, Clinical Research Center, Malmö General Hospital, Lund University, 20502 Malmö, Sweden (e-mail: paul.franks@med.lu.se).

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Observational epidemiology has made outstanding contributions to the discovery and elucidation of relations between lifestyle factors and common complex diseases such as type 2 diabetes. Recent major advances in the understanding of the human genetics of this disease have inspired studies that seek to determine whether the risk conveyed by bona fide risk loci might be modified by lifestyle factors such as diet composition and physical activity levels. A major challenge is to determine which of the reported findings are likely to represent causal interactions and which might be explained by other factors. The authors of this commentary use the Bradford-Hill criteria, a set of tried-and-tested guidelines for causal inference, to evaluate the findings of a recent study on interaction between variation at the cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) locus and total energy intake with respect to prevalent metabolic syndrome and hemoglobin A_{1c} levels in a cohort of 313 Japanese men. The current authors conclude that the study, while useful for hypothesis generation, does not provide overwhelming evidence of causal interactions. They overview ways in which future studies of gene × lifestyle interactions might overcome the limitations that motivated this conclusion.

CDKAL1 protein, human; energy intake; hemoglobin A1c protein, human; Japan; metabolic syndrome X

Abbreviations: CDKAL1, cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1; FFQ, food frequency questionnaire; GWAS, genome-wide association study(ies); HbA1c, hemoglobin A_{1c}; TEI, total energy intake.

In contrast to clinical trials, epidemiology may be well suited to the discovery and elucidation of gene × lifestyle interaction effects that are small in magnitude and manifest over long durations. The limitations inherent in epidemiology, however, necessitate careful consideration of whether observed relations are likely to reflect cause and effect; this process is fundamental when considering whether epidemiologic data on gene × lifestyle interactions are of value for disease prevention or personalized medicine. In this commentary, we apply Hill's established criteria for causal inference (1) to a study of gene × nutrient interactions published in this issue of the *Journal* (2) and discuss alternative explanations for the observed interaction effects.

Susceptibility to disease given specific lifestyle exposures varies greatly from one person to the next, as do responses to lifestyle interventions in clinical trials (3–5). Family-based clinical trials show that there is considerably less phenotypic variability between family members than between members of different families (6), suggesting that inherited factors, such as genes, modify the phenotypic response to interventions. This supposition is supported by clinical trials in which specific gene variants appear to modify the effects of interventions (7–10). Copious numbers of (mainly cross-sectional) epidemiologic reports also describe the influence of interactions between candidate loci and lifestyle exposures on disease traits (11).

Despite abundant literature on gene × lifestyle interactions, many epidemiologists remain skeptical that these reports are authentic, largely because few are adequately replicated (12, 13). The same was true for almost all genetic association studies until recently. The turning point was the advent of the genome-wide association study (GWAS), a high-throughput massively parallel genotyping technology that motivated a transition away from the widely used

hypothesis-driven biologic candidate gene approach and toward one which is hypothesis-free and reliant on enormous sample sizes, extensive replication efforts, and extremely conservative *P* values to demonstrate associations. For type 2 diabetes, more than a decade of genetic association studies yielded barely a handful of reliably associated loci, yet in little more than 3 years since the first type 2 diabetes GWAS was published, more than 40 have emerged (14–16). A similar story exists for almost all other complex traits.

So far, 2 main approaches have been adopted to transfer the GWAS concept to studies of gene × lifestyle interactions. The first is a simple extension of existing GWAS, where highly ranked loci from GWAS of main genetic effects are tested for interaction with lifestyle factors in much the same way as biologic candidate gene variants have been tested in the past. The second approach, which is more innovative but also more time-consuming, involves undertaking de novo genome-wide analyses, where interaction terms (or some variation thereof) are modeled on disease outcomes and the highest-ranked interaction effects are carried forward for replication in independent samples. There are numerous consortia-based efforts under way in which this second approach has been deployed, but so far no group of investigators has published its findings. A third approach, which has not yet been realized, is one where, from the outset, the study is designed solely to test specific hypotheses of interaction, such as genotype-based recall randomized clinical trials, where persons with starkly contrasting genetic risk profiles are randomized to treatments in order to determine whether one group responds differently from the other, thereby demonstrating genetic modification of treatment effects.

Using the first of these approaches, in a study published in this issue of the *Journal*, Miyaki et al. (2) describe analyses of gene × lifestyle interactions (cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) gene × total energy intake (TEI)) in a cross-sectional cohort of 313 ''healthy'' Japanese men. The gene variant examined (rs9465871) was initially identified through a type 2 diabetes GWAS of whites (17, 18) and was later confirmed as a diabetes-predisposing locus in Japanese (19). Miyaki et al. examined these interactions in relation to hemoglobin A_{1c} (HbA1c) levels and metabolic syndrome (2). They also reported on associations between the rs9465871 variant and HbA1c levels, fasting glucose concentrations, and metabolic syndrome and between TEI and HbA1c levels. These tests yielded a series of nominally statistically significant results. Miyaki et al.'s main finding was that the relation between the rs9465871 variant and HbA1c was significant only in persons with the highest caloric intakes (2). They also reported associations between the rs9465871 variant and HbA1c, fasting glucose levels, and metabolic syndrome risk, where the C allele was associated with elevations in these traits.

Examination of the findings from this study, and others like it, in the context of causal inference criteria may help determine which published examples should be carried forward to experimental studies specifically designed to examine the effects of gene × lifestyle interactions.

CAUSAL INFERENCE AND ALTERNATIVE EXPLANATIONS FOR AN INTERACTION STUDY'S FINDINGS

In the Appendix, we provide a description of Hill's criteria for causal inference (1) and briefly apply them to Miyaki et al.'s findings (2). We conclude that overall there is limited evidence of causal interactions in Miyaki et al.'s study. Indeed, studying gene × lifestyle interaction effects is a major challenge, and if one were to apply these criteria to the majority of existing studies, including one of our own (20), one might reach a similar conclusion. Thus, the alternative explanations for observed interaction effects outlined below apply to much of the published literature, and not all points are specific to Miyaki et al.'s study. It is also important to bear in mind that, as Hill himself pointed out, none of the 9 criteria ''can bring indisputable evidence for or against the cause-and-effect hypothesis... What they can do, with greater or less strength, is to help us to make up our minds on the fundamental question—is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?'' (1, p. 299).

Multiple hypothesis-testing

An α level below 0.05 is conventionally used to justify rejecting the null hypothesis. Under a normal probability distribution, a hypothesis test yielding a *P* value of 0.01 suggests that 1 in 100 tests where we rejected the null hypothesis of no association is in fact false-positive (type 1 error rate = 1/100). If 2 completely independent hypotheses are tested, the type 1 error rate at $P = 0.01$ is now 2 in 100, and so on. A Bonferroni correction attempts to overcome the disparity between the nominal (uncorrected) *P* value and the underlying type 1 error rate by multiplying the observed *P* value for each test by the number of tests performed; hence, a nominal *P* value of 0.01 obtained from 10 independent tests would not be considered statistically significant after Bonferroni correction (corrected $P = 0.10$). Parenthetically, the reason a probability threshold of $P < 1.0 \times 10^{-8}$ is used in GWAS is to account for the million or more tests that might be performed; type 1 error rates in GWAS approximate those from studies in which single tests were performed, yielding *P* values of approximately 0.01. There are limitations to the Bonferroni correction (21), and other less conservative and more idiosyncratic approaches have been advanced (21). The prevailing issue, however, is that the greater the number of tests performed, the greater the risk of false discovery when using nominal *P* values to guide the decision as to whether to accept or reject the null hypothesis.

Miyaki et al. corrected the interaction *P* values presented in their paper (2) for 2 tests, meaning that where *P* values were less than 0.025, they chose to reject the null hypothesis of no interaction. As they acknowledge (2), they performed at least 16 additional hypothesis tests for the main effects of the *CDKAL1* variant. Thus, type 1 error rates in Miyaki et al.'s study may have been higher than was apparent at face value, although it could be argued that these additional 16 tests were secondary and therefore independent of the

interaction tests. Only with thorough replication efforts could one make a reasonable conclusion on this point.

Measurement error and information bias

Miyaki et al. used a food frequency questionnaire (FFQ) to estimate caloric intake. However, this method is well known to be prone to error and bias (22). FFQs are touted for their ability to appropriately rank people from high intake to low intake (23), but FFQs are not designed to measure absolute TEI. Biomarker studies indicate that correlations between FFQ-derived TEI and TEI estimated by the gold-standard doubly labeled water method are generally fairly weak (24). Owing to this and other limitations, FFQs are rarely used as the primary exposure or outcome (23). TEI estimated by FFQ is, however, frequently used to adjust other nutrient or food estimates in epidemiologic studies and clinical trials.

Miyaki et al. categorized TEI into 3 broad groups (low, medium, and high) but apparently did not exclude persons with extremely high or low reported TEIs. Such exclusions are commonly performed in nutritional epidemiology, largely because measurement error tends to segregate at the extremes of the trait distribution. Thus, it is likely that the approach used by Miyaki et al. to characterize TEI augmented exposure misclassification. Assuming that the misclassification was nondifferential, one would expect this to have resulted in diminished statistical power. If, however, the error was related to other factors—factors correlated with the outcome but not lying on the causal pathway (e.g., types and amounts of foods selected, hunger cues, societal factors, hormone levels, basal metabolic rate, physical activity level, and/or genotype)—the reported interactions may be biased.

Small-study bias and type 1 error

Small-study bias relates to issues concerning the reporting of data from small studies that might lead to spurious conclusions about the strength, magnitude, or direction of associations. Underrepresentation in the literature of negative results from small studies is one form of small-study bias. Other forms pertain to the manner in which data are analyzed and how methods are reported. Large, well-funded studies tend to strictly regulate data analysis and reporting. Oftentimes, large studies have steering committees which oversee the creation and execution of analysis plans, minimizing reporting biases. Studies lacking such infrastructure may be more prone to analytical errors and misreporting. One major problem that is perhaps more frequent in small studies which lack statistical power is the fact that investigators conduct extensive data exploration in order to identify associations which are significant at the nominal level but are not significant when appropriately corrected for multiple testing. Where these findings are reported as emerging from primary hypothesis tests, without a thorough description of the circumstances in which they were obtained, the risk of false discovery may be high. Interaction analyses are often performed on an exploratory basis when the primary hypothesis test has not yielded publishable results. Therefore, small studies in which interactions are reported may be particularly prone to these forms of bias; the abundance of published interaction studies lacking adequate replication suggests that this problem is common. An important consideration when reviewing data on gene × environment interactions is whether the *P* value for the interaction effect is roughly consistent with what one would predict given the study's level of statistical power. If the reported effect sizes are implausibly large or if the study seriously lacks power to detect the reported effects at the specified α level, one should remain cautious about the validity of the study's findings. Therefore, replication studies are generally considered necessary to confirm or refute initial reports of interaction.

SUMMARY

For more than a decade, scientists have sought to quantify how interactions between gene variants and lifestyle factors affect variations in type 2 diabetes risk or its antecedent quantitative traits. Despite many publications purporting to have detected such interactions, very few of these studies have been adequately replicated, highlighting the considerable challenges facing those seeking to achieve this end and the need for a whole-scale reevaluation of the way such studies are conducted and reported (25). While studies such as Miyaki et al.'s (2) do not in and of themselves provide concrete evidence of gene × lifestyle interactions, they do contribute to the foundations which will support larger, more comprehensive future studies. Those endeavors, particularly when comprised of multiple smaller studies, will face other challenges (26), but the result will almost certainly be the detection of statistically reliable interactions; determining whether these are of clinical relevance is another matter.

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Author affiliations: Department of Clinical Sciences, Clinical Research Center, Malmö General Hospital, Lund University, Malmö, Sweden (Paul W. Franks); Genetic Epidemiology and Clinical Research Group, Department of Public Health and Clinical Medicine, Section for Medicine, Umeå University Hospital, Umeå, Sweden (Paul W. Franks); and Division of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas Health Sciences Center, Houston, Texas (Jennifer A. Nettleton).

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APPENDIX

Hill's Criteria for Causal Inference

Effect size: The size of the effect estimate is positively related to the probability that the effect is causal.

In the study by Miyaki et al. (2), the difference in mean hemoglobin A_{1c} (HbA1c) levels between the lowest and highest tertiles of total energy intake (TEI) was 0.8%. The difference in mean HbA1c between the CT + TT and CC genotypes in the lowest tertile of TEI was −0.2%, and the difference in the highest tertile was 0.7%. As these data indicate, the magnitude of the observed interaction effect was large. Indeed, had it not been so large, it is highly improbable that with such a small sample the interaction would have reached a nominal level of statistical significance. It is worth bearing in mind that large interaction effects in small studies are a virtual prerequisite for a paper to be competitive for publication; small studies reporting negative results are not prioritized by most journals, owing to the high risk of type 2 error.

Consistency: Effects observed across different study settings, times, and subgroups are more likely to be causal than those observed in isolation.

Several epidemiologic and intervention studies have examined the relations of interactions between cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) variants and lifestyle factors with diabetes-related traits. The results of 2 intervention studies (27, 28) provide no support for Miyaki et al.'s findings. Similarly, a cohort study of more than 16,000 Swedish adults (29) found no evidence that the rs7754840 variant (in near-perfect linkage disequilibrium with rs9465871 in other Asian populations) modifies the relations of physical activity and body mass index (both of which are correlated with TEI) with type 2 diabetes incidence or fasting or 2-hour glucose concentrations.

Specificity: Mechanisms of action that are specific to the observed effect strengthen the probability that the effect is causal.

The etiology, clinical presentation, and treatment requirements of type 2 diabetes and the metabolic syndrome are often highly heterogeneous and in some senses lack specificity. When considering the mechanisms of action that might underlie the interactions reported by Miyaki et al. (2), one must first ask how TEI and *CDKAL1* transcription or translation, singularly or in combination, might influence variations in glycemia and the manifestation of the metabolic syndrome. Miyaki et al. offer no clear explanation in their paper, and while excess caloric intake can cause obesity, the latter being a major risk factor for cardiovascular and metabolic disease, little is known of how, or even if, the *CDKAL1* rs9465871 variant *causes* these diseases.

Temporality: The disease trait emerges at an appropriate time and/or rate following exposure to the risk factor. Where the effect has emerged before risk factor exposure, the effect cannot be causal, and reverse causality should be considered as a possible explanation.

In a cross-sectional study such as that conducted by Miyaki et al. (2), temporality of exposures and outcomes cannot be easily determined. However, in most genetic studies of germ-line variants, investigators can be confident that the genotype is unlikely to be altered by the outcome (reverse causality). In the case of gene × environment interactions, this issue of temporality is more complex than in conventional genetic association studies. For example, because glucose levels can influence energy intake (30), it is possible that in the present study the effects of the *CDKAL1* variant were modified by glucose levels and not by TEI, and that the true outcome was TEI level and not variations in glycemia.

Biologic gradient: Levels of quantitative disease traits or disease incidence rates increase (or decrease) in a manner dependent on the extent of risk factor exposure (i.e., ''dose-response'' relations exist).

In the study conducted by Miyaki et al. (2), both genetic (*CDKAL1* genotype) and dietary (TEI) exposures were evaluated. No linear trend was observed across genotype groups with respect to the outcomes of interest. Instead, a recessive effect was observed, where both copies of the risk allele were required to yield a statistically significant association with HbA1c concentrations. With respect to TEI, a dose-response relation with HbA1c was evident only in persons carrying both copies of the C allele. Furthermore, the strength of the association between *CDKAL1* genotype (CC vs. CT + TT) and HbA1c was significant only in subjects whose TEI was in the upper third of the sample distribution. Thus, overall, Miyaki et al.'s study partly fulfills this criterion of causality.

Plausibility: Is a plausible mechanism known for the observed effect?

Laboratory studies show that the gene product of *CDKAL1* is involved in β-cell function, particularly under glucotoxic conditions (31). Thus, it is possible that polymorphisms in this gene region that compromise function and affect insulin secretion could be further perpetuated as glucose concentrations become chronically elevated. High TEI (relative to energy expenditure) can lead to elevated fasting glucose levels and therefore provides a potentially plausible mechanism for an enhanced effect of *CDKAL1* variation on HbA1c when TEI exceeds energy expenditure. However, one should consider what the TEI variable used in Miyaki et al.'s study (2) really represents. Food frequency questionnaire validation studies conducted in other cohorts (24) clearly show that TEI estimated from this method is only weakly correlated with TEI measured using gold-standard approaches. Food frequency questionnaire TEI estimates are also likely to be correlated with a variety of other dietary, as well as nondietary, factors (32). Disentangling these factors is a formidable challenge but is necessary in order to understand the putative mechanisms underlying gene × TEI interactions. Thus, while the study by Miyaki et al. can be viewed as hypothesis-generating, because of the weak methods used to assess TEI and a dearth of supportive evidence from elsewhere, it is difficult to advance a plausible mechanism that is specific to an effect of interaction between TEI and *CDKA1* genotypes on glycemia.

Coherence: The availability of supportive laboratory evidence increases the probability that the effects are causal, but the absence of such evidence does not mean that the effects are not causal.

To our knowledge, no laboratory studies have addressed parallel interaction hypotheses.

Experiment: The availability of experimental evidence is perhaps the strongest single criterion for causal inference, although in experiments where the treatments

cannot be fully blinded, as is the case in lifestyle intervention studies, observed effects may still be susceptible to confounding.

Two intervention studies have examined the influence of interaction between *CDKAL1* variants and lifestyle interventions on fasting glycemia, insulin sensitivity, or diabetes incidence (27, 28). The first of these studies was the Diabetes Prevention Program, in which approximately 3,200 people at high risk of type 2 diabetes were randomized to receive placebo, a program of intensive lifestyle modification focused on weight loss, or metformin treatment. After 3 years, the results of this study showed no evidence that the *CDKAL1* rs7754840 variant modified the effects of lifestyle intervention on diabetes incidence (27). Results from the HERITAGE Study, a nonrandomized family-based trial ($n = 481$), showed no evidence of association between the rs7754840 variant and change in fasting glucose, fasting insulin, or acute insulin response after 20 weeks of exercise training (28). A nonsignificant borderline effect was observed for change in insulin sensitivity, whereby the G allele (analogous to the T allele at rs9465871) was associated with greater improvements in insulin sensitivity following exercise training as compared with the low-risk A allele. This finding, that the G allele apparently increases responsiveness to changes in energy expenditure (exercise), is not supportive of Miyaki et al.'s findings (2), which suggest that the T allele diminishes the effect of energy intake on HbA1c.

Analogy: Where examples exist of similar effects in different scenarios, the effects may be more likely to be causal.

We have already outlined the lack of supporting evidence from other studies which have examined the influence of interactions between *CDK1A* variants and energy intake or energy expenditure on metabolic traits. However, many other analogous examples of effects of gene × nutrient interactions on related traits have been reported (summarized by Franks et al. (11)). Probably the strongest example of gene × nutrient interaction is that of the peroxisome proliferator-activated receptor gamma (*PPARG*) Pro12Ala variant, dietary fat intake, and obesity (33). A second promising example is variation in the fat mass and obesity-associated (*FTO*) gene and nutrient intake. For example, in a recent Scandinavian study, Sonestedt et al. (34) reported strong effects of interactions between the rs9939609 *FTO* gene variant and fat or carbohydrate intake on obesity predisposition—findings which are support by clinical trial data from the United States (35) in which the high-risk *FTO* genotype group appeared more responsive to the effects of lifestyle modification on reductions in abdominal adipose mass during the first year of the trial.