CHAPTER 10.

The interplay of genes, lifestyle, and obesity

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This chapter reviews the evidence supporting a joint effect of genes and lifestyle factors in obesity, focusing mainly on evidence from epidemiological studies and clinical trials research.

Obesity is the scourge of most contemporary societies; about 40% of adults worldwide are overweight and 13% are obese (http://www.who.int/mediacentre/factsheets/fs311/en/). Much of the burden that obesity conveys arises from the life-threatening diseases it causes, although there are also direct consequences, because quality of life is often diminished in people with morbid obesity as a result of social stigma and other societal challenges.

Although intensive lifestyle modification leads to short-term weight loss in most people, weight regain typically begins within a year of intensive intervention, and only a small minority of the target populations are able to maintain reduced weight in the long term [1]. Success in pharmacotherapeutics for weight loss has also been meagre, and in some instances disastrous. A handful of anti-obesity medications have been approved by the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA). One of the most successful of these is the lipase inhibitor orlistat. However, because orlistat diminishes intestinal fat absorption, a frequent side-effect of the drug is fatty stool, which many patients cannot tolerate. Other weight-loss drugs, such as rimonabant, are approved for use in the European Union but are not widely prescribed because of safety concerns and limited effectiveness. Many other weight-loss drugs have been brought to market in the past few decades, only to be withdrawn because of serious side-effects, including death [2]. There are other drugs that do achieve safe weight loss, primarily those approved and marketed for treatment of diabetes: (i) metformin, which reduces hepatic gluconeogenesis (the production of glucose in the liver); (ii) sodium-glucose linked transporter 2 (SGLT2) inhibitors, such as empagliflozin, which reduce re-uptake of glucose in the kidneys and are diuretic; and (iii) glucagon-like peptide-1 (GLP-1) agonists, such as exenatide, which diminish appetite by delaying gastric emptying. However, because all of these drugs can cause side-effects and they are not all reimbursable by health insurance providers for treatment of obesity, they are rarely used primarily for weight reduction.

The third weapon in the anti-obesity arsenal is bariatric surgery. Unlike drugs and lifestyle intervention, which perturb the disease process, surgery can permanently alter the disease trajectory. Therefore, long-term weight loss through surgery is generally sustained to a much greater degree than weight loss from

lifestyle intervention or drug therapy. However, like drug therapies, bariatric surgery is expensive – although it is cost-effective for diabetes treatment compared with drug therapy [3] – and is not risk-free; serious adverse events [4] include about 4 in 1000 patients dying within 60 days of surgery [5]. Thus, although surgery is appropriate for a small minority of morbidly obese patients, it is no panacea for the obesity epidemic.

Of the three core prevention and treatment options for obesity, behavioural interventions that favourably affect chronic energy balance are by far the most compelling, not least because diet and exercise are generally safe, are relatively inexpensive, and convey numerous additional benefits to health and well-being that drugs and surgery do not. However, the considerable therapeutic idiosyncrasies of lifestyle therapy cause wide variability in its effectiveness at a population level. Some of this variability is due to the extent to which the participant adheres to the intervention, and some is due to differences in the participant's biology, which modulates the effects of lifestyle interventions on rates of weight loss and weight regain.

The common outcome variable in obesity research and clinical practice is weight change, because it can be assessed easily and inexpensively. Changes in the amount and deposition of adipose tissue and ectopic fat are probably more clinically important phenotypes, but they are more difficult to quantify. Beyond this, weight change should be more than merely aesthetic; thus, the many clinical sequelae of weight change should also be tracked. Nevertheless, whether the outcome of lifestyle intervention trials is weight or a related metabolic outcome, the response to the intervention is generally highly heterogeneous.

Therefore, quantifying and understanding the ways in which genetic factors modulate a person's response to weight-loss therapies might help to predict the response to different types of intervention, by guiding therapeutic choices in ways that are more precise and effective than conventional approaches, thereby avoiding unnecessary side-effects and reducing costs.

Why might gene–lifestyle interactions be relevant in obesity?

Germline DNA variants are especially appealing biomarkers for targeting obesity interventions, because they are randomly assigned at meiosis and are stable throughout a person's life, rendering their associations with phenotypes fairly robust to confounding and reverse causation. DNA variants are also the starting point of a process called the central dogma of molecular biology [6], downstream of which a complex molecular cascade ensues that translates the effects of extrinsic environmental exposures (of which diet and exercise are major components in obesity) to the clinical phenotypes that characterize health and disease. That molecular cascade is made up of gene transcripts and proteins, as well as epigenomic features (in the form of methylation marks, open chromatin, histone modifications, etc.), small circulating molecules (metabolites), and an array of peptide hormones and other biochemical components.

Studies in twins provided some of the earliest compelling evidence that obesity is under a high degree of genetic control. A study of children and adolescents showed that 82–90% of the phenotypic variance was explained by additive genetic factors [7]. The study used objective assessments of body composition (dual-energy X-ray absorptiometry [DEXA] and hydrostatic weighing), enabling the careful distillation of body corpulence into its constituent morphological features. Studies in adults have reported somewhat lower heritability estimates for obesity.

One of the most eloquent adult twin studies assessed the heritability of body mass index (BMI) in several hundred male and female Swedish twins; about half of them had been reared together, and the remainder had been reared apart, having been adopted into different families soon after birth [8]. The study showed that the concordance of BMI in monozygotic (identical) twins was about 70% regardless of whether the twins had been reared apart or together, whereas the concordance in dizygotic (fraternal) twins was substantially less, suggesting a strong genetic component to obesity. Importantly, however, as discussed later in this chapter, the genetic aberrations that cause obesity do so through a range of diverse mechanisms, including those that affect appetite, satiation, and energy expenditure.

However, knowing that obesity is highly heritable does not necessarily mean that it is the consequence of gene–lifestyle interactions. To determine this, one could test whether the obesogenic effects of lifestyle exposures (in epidemiological studies) or response to weight-perturbing interventions (in clinical trials) are heritable. In studies of twins in the USA exposed to long-term overfeeding [9] or exercise [10] interventions, the concordance in adaptive response to the interventions was significantly higher within twin pairs compared with the concordance between unrelated participants for a range of body composition measures, including waist circumference, body fat percentage, and fat-cell diameter (for exercise response).

Collectively, there is compelling evidence supporting the view that body corpulence in the free-living state and change in body corpulence with diet or exercise are governed to a considerable extent

by genetic factors. These are so-called *quantitative genetics* studies. Unlike the *molecular genetics* studies of the modern era, quantitative genetics provides a broad-strokes genome-wide overview of genetic influence on a phenotype but offers no insights into the specific molecular aberrations (e.g. single nucleotide polymorphisms [SNPs], insertions and deletions [indels], and copy number variations [CNVs]) that cause obesity or modify the effects of exposures and interventions on weight change.

Examples of gene–lifestyle interactions

Population-level molecular genetics studies of obesity, whether focused on associations or interactions, were once hopelessly unreliable. The evidence reported in most such studies before 2007 lacked any reasonable degree of replication. Sample sizes generally ranged from a few dozen to a few hundred participants, and most of the studies that focused on interactions lacked robust measures of lifestyle exposures. A recent systematic review [11] identified 212 studies published between 1995 and mid-2012 that tested gene–lifestyle interactions in obesity; the review found that only those studies that focused on gene–physical activity interactions at the *FTO* (rs9960939) locus and gene–diet interactions at the *PPARG* (Pro12Ala) locus had been independently replicated. As is explained later in this chapter, replication studies of gene–lifestyle interactions face many challenges that extend beyond those faced by association studies. Therefore, the absence of replication does not necessarily mean that the initial finding was false-positive. However, replication studies are a sentinel feature of science, and without replication results for interaction effects it would be difficult to justify major investments in expensive follow-up studies (such as clinical trials) to test whether a gene–lifestyle interaction has the potential for clinical translation.

The *FTO* example cited above was the first of several encouraging illustrations of gene–lifestyle interactions in obesity. The role of *FTO* variation in obesity was first described in three papers published in close proximity in 2007. Two of the studies made their discoveries using genome-wide association studies (GWAS) [12, 13], whereas the third [14] serendipitously identified the genetic association signal using a set of 48 intergenic SNPs intended for quality control, of which one was strongly associated with morbid obesity. Nevertheless, the three studies reached consistent conclusions and provided the first convincing evidence of an association of common genetic variation and obesity. The strongest signal for BMI emanated from the rs9960939 variant, which per copy conveys an odds ratio of 1.35 for obesity and amounted to a difference in body weight for a person 1.7 m tall of about 3 kg between the high-risk and low-risk homozygous genotype groups [12].

Soon after the publication of these papers, studies began to emerge reporting evidence of gene–lifestyle interactions at the *FTO* locus [12–17]. The first study to do so came from a Danish cohort study called Inter99 [15]. The authors used a cross-sectional subcohort of about 5500 Inter99 participants to show that the genetic effect of the rs9960939 *FTO* variant on BMI was about 2 kg/m^2 in people reporting little or no physical activity but was closer to 1 kg/m^2 in those reporting high levels of physical activity. Soon after this work was published, a second observational study and a clinical trial reported complementary results. The observational study was of a population isolate of Amish individuals living in Pennsylvania [17]. The authors undertook a comprehensive analysis of *FTO* variation and explored interactions with objectively assessed physical activity (via accelerometry). The trial tested for genotype–treatment interactions on changes in obesity-related traits in the Diabetes Prevention Program (DPP), a randomized controlled trial (RCT) of intensive lifestyle modification, metformin, and placebo control interventions [16]. Although there was no evidence of an interaction between the rs9960939 *FTO* variant and lifestyle intervention on weight change, there was nominal statistical evidence of an interaction on change in subcutaneous adipose mass (assessed using computed tomography). The interaction effect was consistent with the epidemiological data reported in the Danish and Amish studies.

Many studies were published in the following year, each addressing the *FTO* interaction hypothesis, but with mixed results. Given this state of equipoise, an analysis was undertaken involving about 220 000 adults and 20 000 children and adolescents, to seek replication of the original study's findings. To do this, a standardized analysis plan was executed in each of the 54 cohorts from which the 240 000 participants emanated. The meta-analysis of these data yielded a statistically significant interaction effect, one that was consistent in direction with the original reports, although of a much smaller magnitude (about one sixth of the magnitude of the original interaction effect) [19].

Replication studies: relevance and challenges

After the discovery of *FTO* in 2007, many subsequent GWAS analyses were performed, each larger than the last and each contributing to the burgeoning array of obesity-associated genetic variants [18]. With the emergence of these data came

studies modelling the combined effects of these loci (as genetic risk scores) and their interactions with lifestyle. Of the many that have now been published, three epidemiological studies stand out.

The first study examined the interaction of 12 obesity loci and physical activity in 20 000 adults in the United Kingdom [19a]. The study was a textbook analysis of gene–lifestyle interaction effects and yielded a highly statistically significant interaction effect, which showed that physical activity appeared to diminish the effect of the genetic loci on BMI.

These exciting results were published in one of the leading general medical journals, and the study was clearly well conducted, but without replication data the possibility that these findings might be population-specific or false-positive could not be ruled out. Therefore, a study attempted to replicate these findings in a combined sample of about 40 000 Swedish adults, but initially failed. For reasons outlined in detail by Ahmad et al. [11], a series of factors were identified that inhibited replication of gene–lifestyle interaction effects (listed in the "Key factors" box at the end of this chapter). It was subsequently determined that these factors are features that are likely to affect other replication studies of gene–lifestyle interactions, including the large study of interaction between *FTO* variation and physical activity discussed above [19]. Hence, although replication is the bulwark against false discovery, it is important to ensure that replication studies that fail to support the initial discoveries do so for the right reasons. In the replication study [11], it was shown that when inhibiting factors are considered, the sample size required to achieve sufficient power to test the hypothesis amounts to a cohort collection of more than about 100 000 adults, about 5 times as large as the original study. By testing this hypothesis in 111 000 adults, the authors were able to reproduce the original finding, albeit with an interaction effect of substantially smaller magnitude [11].

A third major study, performed in three epidemiological cohorts in the USA, focused on the interaction of a genetic risk score comprising 32 obesity-associated loci and consumption of sugar-sweetened beverages [20]. These analyses showed that the genetic predisposition to obesity tended to be stronger in people who consumed higher volumes of sugar-sweetened beverages. In a field that is plagued by a dearth of replication data, this study stands out as one of very few to report novel findings on gene–lifestyle interactions alongside robust replication data from independent cohorts.

Although replication is important, it provides no assurance of cause and effect in observational studies. There are many alternative explanations for why two variables might be associated with another that do not include causality, because the factors that might confound these relationships in one cohort could easily do so in others. Gene–lifestyle interaction studies are more prone to confounding and bias than studies that test the marginal associations of lifestyle or genetic exposures in disease. This is because interaction studies are prone to all of the major sources of bias and confounding that plague conventional association studies, as well as types of confounding and bias that are specific to interaction effects. For example, the way in which data are distributed can undermine the credibility of statistical interactions [21].

In the examples discussed above of gene–physical activity interactions in obesity (assessed using BMI, which is calculated as the weight in kilograms divided by the square of the height in metres), a further potential source of confounding exists. Although BMI is probably the most common estimate of adiposity in research studies and clinical practice, it is a proxy for the underlying degree of adiposity. In general, people with higher BMI scores are also fatter, but this is by no means always true. Consider, for example, muscular athletes such as major league basketball players, many of whom have BMI scores that classify them as "overweight" [22]. In population-based cohorts, one should expect there to be a subpopulation of people who are heavy and lean, in part because they are more physically active. One would expect few physically inactive people to be heavy and lean, and even fewer physically active people to be fat. Thus, if one were to model the association of obesogenic gene variants with BMI in the subpopulation of physically inactive people, one would anticipate a strong relationship, but if one were to model the same association in the physically active subpopulation, one should expect this relationship to be weaker, because BMI is a weaker proxy for total adiposity in physically active people compared with physically inactive people. Thus, because the interaction tests outlined in the studies discussed above compare the magnitude of the association between genotypes and BMI by strata of physical activity, statistically significant interaction tests could be driven entirely by confounding. Thus, when outcomes are assessed using imperfect proxies and the validity of that proxy varies across the distribution of the lifestyle exposure, this type of confounding, which is specific to interaction analyses, should be carefully considered.

Clinical trials

Epidemiology is a powerful tool for generating hypotheses about gene–lifestyle interactions, but it is prone to bias, confounding, and reverse

causality. RCTs of lifestyle interventions are more tightly controlled and monitored than epidemiological studies; they are prospective in design (most published epidemiological studies of gene–lifestyle interactions have been performed in cross-sectional data sets), thereby permitting the assessment of temporal relationships, and are less prone to confounding, because treatment (lifestyle vs control) is randomly assigned and hence should not be correlated with other factors that underlie an association between exposure and outcomes. However, because it is usually not possible to blind a participant to treatment allocation in a lifestyle trial (i.e. trials focused on changing diet and exercise behaviours), and because there is no placebo that can be given for exercise and most dietary factors, lifestyle trials are less robust to confounding than, say, placebo-controlled drug trials.

It is important to keep this in mind, because in lifestyle intervention studies behavioural compensation is known to occur, and this might lead participants assigned to treatment or control interventions to modify behaviours outside the hours of the intervention and thereby affect the trial's outcomes. Therefore, although much is made of the variability in treatment response in lifestyle intervention trials, perhaps most notably in the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study [23], it is reasonable to assume that some of the variability in response is due to behavioural compensation. The HERITAGE Family Study was an intervention-only exercise training (aerobic and resistance training) study administered over 20 weeks in about 1000 participants. The results from the study seem to suggest that there are "responders" and "non-responders" to exercise interventions, causing clinical phenotypes to improve dramatically in some participants (super-responders), whereas in other participants these same phenotypes do not improve (non-responders), or even worsen.

It is often the case that researchers interpret "phenotypic response" data from the HERITAGE Family Study and elsewhere as compelling evidence of biologically (genetically) encoded exercise-response potential [23]. However, genetic predisposition is only one of many plausible explanations for these results. For example, exercise intervention studies have shown that when people are encouraged to undertake structured exercise, non-exercise activity thermogenesis (the component of total physical activity that is not structured) decreases on average [24], a concept sometimes termed *behavioural compensation*. Importantly, because the time spent undergoing the lifestyle intervention in a trial (often about 150 minutes per week) is a very small proportion (< 2%) of the overall waking hours, a participant's behaviour during the hours when they are not participating in the intervention sessions will affect the extent to which their health phenotypes change during the trial, irrespective of the intervention's intensity or how faithfully the participant has adhered to it. Moreover, variability in measurement precision and accuracy (error), which are inherent features of all clinical trials and observational studies, causes a phenomenon called *regression dilution*, which contributes to the apparent variability in phenotypic response to interventions. Thus, measurement error is usually most abundant at the extremes of a trait's distribution, and this should be considered when using data from lifestyle intervention trials to understand human biology.

Nevertheless, some of the inter-individual variability in response to lifestyle interventions is likely to be under biological/genetic control, an assumption that is supported by heritability analyses undertaken in the HERITAGE Family Study and elsewhere, showing that the variability in trait response is larger between families than within families [25]. Studies of gene–treatment interactions performed in RCTs have the potential to identify specific genetic variants that underlie treatment response. Two of the largest and most comprehensive RCTs of lifestyle interventions were performed in the USA. The first, the DPP, was performed in about 3000 prediabetic overweight adults [26], whereas the second, the Action for Health in Diabetes (Look AHEAD) trial, focused on about 5000 people with clinically manifest type 2 diabetes [27]. The clinical interventions in both trials focused on inducing weight loss of about 7% of body weight through structured and personalized diet and exercise regimes, as well as comparison arms that provided standard of care; the DPP also included two drug arms (metformin and troglitazone). Extensive genetic analyses, including those relating to the *FTO* locus (discussed above for the DPP) have been performed in both trials, with several analyses focusing on weight change.

The first analysis of this nature in the DPP focused on the *PPARG* Pro12Ala locus [28]. These analyses in the DPP tested a hypothesis set forth by earlier epidemiological studies relating to the interaction of dietary fats. *PPARG* is a nuclear receptor that regulates many genes and pathways involved in energy metabolism, adipogenesis, and other metabolic processes. Long-chain unsaturated fatty acids bind with high affinity to *PPARG*, as do thiazolidinediones, a class of drugs used to improve peripheral insulin sensitivity. Therefore, the authors tested whether the Pro12Ala variant modified the weight-loss effects of (i) lifestyle intervention per se, (ii) dietary fatty acid consumption, and (iii) the

thiazolidinedione drug troglitazone. No statistical interaction was observed with lifestyle, but with both dietary fats and troglitazone, the hypothesized interaction effects were observed.

Many subsequent studies were conducted in the DPP; some involved detailed explorations of candidate genes, such as *MC4R* [29], *ADIPOQ* [30], *TCF7L2* [31], and *PPARGC1A* [32], and others focused on polygenic risk scores [33]. The most recent of these studies [34] assessed the effects of 92 variants that were recently reported for their associations with BMI by the Genetic Investigation of Anthropometric Traits (GIANT) consortium [18]. Joint analyses were conducted in the DPP and Look AHEAD trials to determine whether these variants, singly or in combination, modified the effects of lifestyle interventions focused on weight loss or prevention of weight regain. Overall, little evidence was found of interactions between lifestyle and these genetic variants, suggesting that GWAS-derived genetic loci for obesity have no clinically meaningful impact on response to lifestyle interventions. However, one variant (at *MTIF3*) yielded a statistically significant interaction effect on weight loss that was consistent in direction and magnitude in the DPP and Look AHEAD trials. The interaction manifested through a slightly elevated risk of weight gain in carriers of the G allele (the allele associated with higher BMI in the GIANT consortium meta-analysis [18]) who were assigned to the control intervention, which contrasted with the genetic effect in those assigned to the lifestyle interventions (where the G allele was associated with greater weight loss). The very similar results in the two trials represent some of the most robust evidence of a gene–lifestyle interaction in weight change published to date. Adding further credence to these findings is a large (N = 67 000), independent analysis of gene–diet interactions in BMI in a cross-sectional cohort collection [35]. This analysis of 32 of the 92 loci studied in the DPP and Look AHEAD trials found that the strongest evidence of interaction between a gene variant and diet was at the *MTIF3* locus. Obvious differences in study designs and outcomes make determining the comparability of the interaction effects across these studies challenging.

MTIF3 is involved in forming the initiation complex of the mitochondrial 55S ribosome [36, 37], which in turn synthesizes 13 of the inner mitochondrial membrane proteins. The regulation of *MTIF3* plays a key role in mitochondrial energy metabolism and reactive oxygen species production as part of the electron transport chain [37]. As was reported previously [34], although rs1885988 is an intronic variant, its close proximity (411 bp) to a triallelic missense SNP with a DNase peak indicates that the rs1885988 variant is a marker for a chromatin site involved in transcription factor binding regulation.

Functional implications

Notwithstanding the limitations of focusing on GWAS-derived (marginal-effect) loci for interaction analyses, the approach has the advantage that huge efforts have been invested in determining the functional basis of the genes to which the index maps. Importantly, locus mapping is still a fairly imprecise affair, and in many instances the region to which a variant with the strongest association signal in a GWAS maps spans several genes. Thus, leaping from an association signal to functional prognosis is fraught with caveats.

Nevertheless, in silico functional annotation performed by the GIANT consortium mapped BMI-associated loci to putative functional variants and transcription profiles across multiple human tissues [38], which indicated an overrepresentation of several of these loci across neural pathways involved in satiation and appetite. Those analyses were extended in the most recent GIANT publication on BMI-associated variants to include about 60 further variants [18]. Using the DEPICT software [39], the authors provided further evidence of enrichment across central nervous system pathways (i.e. synaptic function, long-term potentiation, and neurotransmitter signalling) but also found that some of the newly discovered loci mapped to pathways implicated in movement behaviour (physical activity and coordination) in mouse models. These intriguing functional implications add further support to the potential role of GWAS-derived loci in gene–lifestyle interactions. Although most common variants have roughly comparable effect sizes, *FTO* stands out given that the association and effect-modifying roles (of lifestyle exposures) in obesity are now well defined. However, despite huge efforts, the mechanisms through which *FTO* acts remain unclear. What is clear is that these mechanisms are complex, involving long-range interactions with other loci (e.g. *IRX3* [40]), and may be triggered by epigenomic factors (e.g. TRIM28 [41]).

Conclusions

There is an abundance of published evidence, predominantly from cross-sectional epidemiological studies, that supports the notion that lifestyle and genetic factors interact to cause obesity. However, few studies have been adequately replicated, and functional validation and specifically designed intervention studies are rarely undertaken; both of these are necessary to determine whether observations of gene–lifestyle interaction in obesity are causal and of clinical relevance.

Key points

- The patterns and distributions of obesity within and between ethnically diverse populations living in similar and contrasting environments suggest that some ethnic groups are more susceptible to obesity than others. Generally, when exposed to environments typical of industrialized countries, aboriginal peoples appear to be highly susceptible, whereas populations of European ancestry appear to be far less prone to obesity.
- More than 150 common loci have been robustly associated with measures of body composition.
- Evidence from several behavioural intervention studies suggests that response to caloric manipulation brought about by fasting, overfeeding, or exercise is heritable.
- There is now convincing epidemiological evidence of interactions between common variants at *FTO* and lifestyle on obesity. Almost all of these data are from cross-sectional studies, and temporal relationships are not clear. There are large studies supporting gene–lifestyle interactions at several other common loci, but the burden of evidence is far less for these loci than for *FTO*.
- The evidence from clinical trials supporting gene–lifestyle interactions at *FTO* or other loci is relatively weak compared with the epidemiological evidence.
- The magnitude of the interaction effects reported for *FTO* (or other common variants) is insufficient to warrant the use of those data for clinical translation.

Key factors

The following key factors affect the detection and replication of gene–lifestyle interaction effects.

- **Exposure variance.** When all else holds equal, statistical power is usually inversely related to the variance (usually expressed as standard deviation) of the exposure variable.
- **Outcome variance.** When all else holds equal, statistical power is usually positively related to the variance (usually expressed as standard deviation) of the outcome variable.
- **Categorization of variables.** For exposures (or outcomes) that are normally distributed and bear linear relationships with outcomes (or exposures), data stratification tends to reduce power [42]. Moreover, a variable that is stratified at the median point of its distribution will tend to yield higher statistical power than one that is stratified at other points in its distribution.
- **Measurement error.** Error in the assessment of exposure or outcome variables has a profound impact on statistical power, such that sample size requirements to detect interactions may differ by several orders of magnitude, depending on the quality of exposure and outcome measures [43].
- **Differential confounding.** Interaction effects detected in observational studies are prone to confounding. However, confounding variables often differ between populations. Thus, if an interaction effect that is detected in one population is driven by confounding, and the confounding variables are absent in a replication cohort, then the replication cohort is likely to fail to reproduce the results of the initial study. However, successful replication does not necessarily exclude the possibility that interaction effects are confounded, because confounding factors may be simultaneously present in the discovery and replication cohorts.
- **Publication bias.** Publishing negative findings, whether from studies of interaction or not, is generally more challenging than publishing results that appear statistically significant. Thus, the absence of negative-outcome replication studies in the literature may not mean that replication studies have not been performed.
- **Winner's curse.** The interaction effects featured in high-impact journals are often among the most striking. However, striking effects are sometimes overestimates of the true latent effect; thus, the results of subsequent studies are likely to be weaker, which in turn limits the statistical power of those later studies. This concept is often referred to as the "winner's curse".
- **Population-specific effects.** Although the logical conclusion when an adequately powered replication study fails is that the original discovery may be false-positive, one cannot exclude the possibility that the original finding was true-positive and population-specific. Further studies that explore three-way interactions (gene × lifestyle × population-specific parameters) would be needed to model these effects.

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