Mendelian randomization provides a theoretically powerful approach to account for unmeasured confounding variables in the evaluation of relationships of biomarkers with disease outcomes. First proposed by Katan as a way to distinguish whether low cholesterol concentrations were a cause of cancer or a consequence of carcinogenesis (1), the approach seeks to capitalize on the random assignment of genotypes at meiosis in a methodologic strategy that mimics that of a randomized trial. A main strength of the approach is that whereas a biomarker typically varies throughout a person’s life in response to diverse and unmeasured lifestyle and age-related biologic processes, the genotypes are fixed at birth and their associations with the biomarker are apparently lifelong. An additional strength is the accuracy of genotyping, unlike many other risk factors that change with time and are measured with substantial error. However, several types of confounding can influence the results of a Mendelian randomization study, causal inference in observational research requires caution, and the interpretation of null Mendelian randomization studies is challenging, especially in the common situation of a weak association between the gene and the biomarker.

Causal Inference from Mendelian Randomization

Fig. 1 illustrates the approach of Mendelian randomization in the setting where interest focuses on the relationship of concentrations of a biomarker B with the subsequent occurrence of a disease D, and information is available on a polymorphism at a genetic locus associated with B. One often seeks to quantify the association of B with D that is independent of known relationships of other risk factors, C, that are correlated with B. If all risk factors for D are measured accurately and their relationship with D is correctly specified, then a standard analytic approach, such as a stratified analysis or a regression model (e.g., a logistic regression model), can obtain the correct adjusted estimate of the relationship of B with D. Commonly, however, some risk factors, U, are unmeasured or measured with error so that a standard analysis yields a biased estimate of the relationship of B with D because of residual confounding. In this situation, for causal inference on the impact of B on D, a genetic variant, G, which strongly influences the biomarker, can serve as a valuable instrumental variable. The validity of this approach requires that the genetic variant influences the biomarker of interest, is independent of other risk factors for D, and does not influence D through a pathway independent of the biomarker of interest. These are strong assumptions, with untestable aspects, yet, as for many modeling strategies, the approach can yield useful insights.

The methodology of Mendelian randomization uses the relationship of G with D, which presumably is unaffected by known confounding variables, C, and unknown confounders, U, to evaluate the relationship of B with D. The instrumental variable approach, long used in econometrics and other social sciences, and more recently applied in epidemiology (2), posits that integrating the estimates of the relationships of G with B and G with D can yield a more reliable estimate of the relationship of B with D. The main challenges to the method (3, 4) are as follows: the assumption that a gene influences disease solely through B is strong and unverifiable, as a single gene can influence disease risk through multiple pathways other than B (the phenomenon of pleiotropy); other alleles, G’, may be correlated with G (linkage disequilibrium) and influence D through other pathways, thereby inducing confounding and other characteristics of individuals at birth, C’, that independently predict the development of D can be correlated with G (population stratification) and influence D through other pathways, thereby inducing confounding and other characteristics of individuals at birth, C’, that independently predict the development of D can be correlated with G (population stratification) and influence the expression of G (epigenetics), leading to violations of the necessary conditions for a valid instrumental variable. Additionally, both other alleles and patient characteristics can modify the effect of G on B, the effect of G on D, or both.

Example: High-Sensitivity C-Reactive Protein and Coronary Heart Disease

A recent, large, genomewide association study conducted by Elliott et al. (5) found polymorphisms in

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several genetic loci with highly statistically significant associations with high-sensitivity C-reactive protein (hsCRP)\(^2\) concentrations, yet having little apparent association with the risk of coronary heart disease. The large size of the study yielded narrow confidence intervals about estimated odds ratios for the associations between single nucleotide polymorphisms and risk of coronary heart disease that excluded even moderate associations. Under the assumption of a simple model of causality without pleiotropy, population stratification, or genetic heterogeneity, the test of an association between the genotype and coronary heart disease provides a reasonable test of whether there is a causal association between hsCRP and coronary heart disease (3). Although simple models have the strength of parsimony, with multicausal diseases such as cardiovascular disease consideration of interactions among potential causes may be important.

A more complicated aspect of Mendelian randomization relates to the estimation of the average causal effect of a phenotype such as hsCRP, based on integration of the relationships of polymorphisms with the intermediate phenotype and with the disease of interest. If logistic regression is used to estimate this latter relationship, such as in the study of Elliott et al. (5), then an approach based on measurement error correction such as regression calibration (6) can be considered. In applications such as those of Elliott et al. (5), however, where none of the polymorphisms evaluated accounted for even 2% of the variance in hsCRP, the applicability of that approach is questionable (6). Often Mendelian randomization studies provide no adjusted estimate of the relationship between the phenotype of interest and the disease outcome that accounts for the multiple sources of uncertainty, including the often weak correlations between the genetic variants and the phenotype.

Negative Mendelian Randomization Studies in Light of the JUPITER Trial

Mendelian randomization studies provide limited information on the potential value of a biomarker to identify high-risk individuals and direct treatment. This is particularly the case when the studied polymorphisms account for little of the variability in the biomarker. With respect to risk classification, hsCRP is an independent risk factor for cardiovascular disease that has modest correlations with other risk factors (7). Moreover, addition of hsCRP to risk prediction models is known to improve the ability to identify high-risk individuals (8). Independent markers of disease processes can play an important role in identification of high-risk individuals, regardless of whether these markers are themselves causes of the disease.

The JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) tested the efficacy of statin therapy in individuals with lipid concentrations below those indicative of treatment in current guidelines, but who were at increased risk by virtue of increased hsCRP and who might particularly benefit by virtue of the anti-inflammatory effects of statins. The conception of the trial made no assumption of a causal relationship between hsCRP and cardiovascular disease; rather, it was based on substantial evidence that inflammation plays a causal role in the disease process, and that hsCRP is uniquely able to identify individuals both at increased risk due to inflammation and who might be particularly responsive to statin therapy. Thus, in Fig. 1, with hsCRP as the biomarker of interest, inflammation, I, was an important construct thought to affect cardiovascular risk, for which hsCRP served as a marker. Results of the JUPITER trial indicate that statin therapy guided by hsCRP has net clinical benefits that compare favorably to other accepted primary prevention strategies, including antihypertensive treatments and statins guided by other indications (9). Additionally, within the JUPITER trial as in several prior statin trials, subjects assigned to statin treatment who achieved low

\(^2\) Nonstandard abbreviations: hsCRP, high-sensitivity C-reactive protein; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin.
concentrations of hsCRP had better clinical outcomes (10). Thus, treatment-related changes in hsCRP, while not necessarily causally related to outcomes, are good indicators of treatment efficacy.

In some important subgroups, notably women and individuals 70 years or older, and for some endpoints, notably stroke, the JUPITER trial provides the strongest randomized evidence of a benefit of statin therapy in the setting of primary prevention. It is unclear what role a negative Mendelian randomization study of hsCRP should play in the translation of the JUPITER results to practice guidelines. The available evidence from Mendelian randomization studies provides little support for the idea that the benefits seen in JUPITER would also apply to treatment of individuals without increased hsCRP. Such an inference would be based on evidence that hsCRP not only was not causally related to cardiovascular disease, but also was not an independent marker of causal processes such as inflammation. The most straightforward application of trial results to clinical practice focuses on those individuals who meet the trial eligibility criteria. Overall, strong markers of causal processes can usefully guide therapy regardless of whether the marker is itself causally related to the disease outcome.

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