

outcome than a direct intervention because of the specific pathway affected by the variant, such as the effect of kringle IV type 2 size polymorphisms on lipoprotein (a), and the subsequent association with myocardial infarction(5).

While statistical guidance on assessment of the validity of IVs in Mendelian randomization is welcome (6), there is a danger of overreliance on empirical testing at the expense of biologic knowledge (7). The statements provided by Glymour et al., while providing useful guidance, should not be seen as absolute indicators of the invalidity of an IV and should supplement rather than replace sound scientific judgment (8).

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the bounds does not take into account sampling variability. To address this, Ramsahai and Lauritzen (6) proposed a relevant hypothesis test for the bounds. In related work, Richardson et al. (7) also proposed a Bayesian approach to estimating bounds for the ACE and other causal parameters.

In conclusion, when the exposure and outcome in a Mendelian randomization analysis are binary variables and the instrument is a categorical variable, gross violations of the instrumental variable assumptions, including the exclusion restriction, can sometimes be detected by checking certain inequality restrictions on the observed relative frequencies. Further empirical evidence for violations of the instrumental variable assumptions can sometimes be obtained using multiple instruments and overidentification tests (8). However, we caution researchers that it is generally not possible to establish the *validity* of the instrumental variable assumptions, particularly the exclusion restriction assumption, on the basis of data and statistical tests alone. In general, the exclusion restriction should always be justified from subject matter background knowledge—in this example, the biochemical and behavioral mechanisms underlying the *FTO* gene (9).

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THE AUTHORS REPLY

We appreciate the comments of Palmer et al. (1) and Burgess (2), as well as Palmer et al.'s provision of their useful Stata command, `bpbounds` (StataCorp LP, College Station, Texas). As Palmer et al. note (1), the assumptions required for a valid instrumental variable (IV) cannot be established from any data or statistical tests, but these assumptions can sometimes be falsified. One approach to falsification, applying the instrumental inequality tests, is applicable with categorical IVs and phenotypes (3, 4). As we demonstrated in Web Appendix 3 of our article (5), these tests are straightforward to implement in Excel (Microsoft Corporation, Seattle, Washington) when the phenotype is dichotomous and the instrument is either dichotomous or trichotomous. Trichotomous instruments are particularly common in Mendelian randomization studies. In our example, we used *FTO* allele count as a trichotomous instrument, classified as homozygous for the common allele, heterozygous, or homozygous for the rare allele.

A valid IV places inequality constraints on the observed data distribution. An instrumental inequality test assesses whether these constraints hold in the data. The ability (i.e., power) of the test to detect an invalid instrument increases with the number of constraints being tested; hence, it is optimal to test all of the inequality constraints. Pearl (6) first derived constraints implied by a valid IV; Bonet (4) subsequently recognized that when either the IV or the endogenous variable has more than 2 possible values, additional inequality constraints are implied by the IV assumptions. Bonet demonstrated how to derive and thus test all inequality constraints; Bonet's tests are implemented in Web Appendix 3 of our original article (5) and in the `bpbounds` command. In Web Figure 1 (available at <http://aje.oxfordjournals.org/>),