General Discussion

Main findings

* summaries of findings by chapter

Methodological considerations

-this seems to be limitations mainly

Clinical implications

Future directions

Practical implications

Conclusion

Tomer

Summary

Pros and cons of different utility functions

Criteria for comparison schedules

Recommendations for future improvements

General conclusion

General Idea of Outline

Summary of findings by chapter

1. MR Lit Review
2. investigators using prenatal MR often describe and attempt to mitigate forms of bias common in the general MR literature, including selection bias due to loss to followup, population stratification, and certain types of pleiotropy.
3. However, researchers rarely discuss issues specific to the prenatal MR context, including selection on pregnancy, pleiotropy via offspring genotype, of pleiotropy via pre- or postconceptional exposure status.
4. Although the majority of prenatal MR studies present point estimates, the additional assumptions necessary for point estimation are rarely discussed. This is especially concerning in the context of prenatal MR, where certain estimands are defined differently, and there is biologic evidence that the point estimating conditions cannot hold for certain exposures of interest.
5. Instrumental Inequalities
6. Our results provide a concrete example of how, in the context of multiple proposed instruments, the instrumental inequalities can be to detect violations of the instrumental conditions.
7. we found that, within our sample, the MR assumptions were violated for at least half of the 4 maternal SNPs proposed as instruments.
8. Our simulations suggest that the inequalities will be increasingly violated as the magnitude of violations grow, are more sensitive as larger numbers of variables are proposed as joint instruments, and that small sample sizes seem to increase the probability of finding true violations of the IV model.
9. Applying the instrumental inequalities across differing combinations of proposed joint instruments may enable researchers to detect patterns consistent with a single “bad apple” proposed instrument.
10. These results show how, consistent with previous calls, the inequalities should be more broadly applied in applications of IVs to falsify clearly invalid models.
11. They may be especially useful in the context of mendelian randomization, where investigators frequently propose large numbers of SNPs as joint instruments
12. Vitamin D EWAS
13. we did not find evidence of associations between maternal mid-pregnancy vitamin d sufficiency and offspring DNA methylation at any measured site in cord blood.
14. Bounds Paper
15. We found that, when single SNPs were proposed as instruments, bounds on the average causal effect barely improved on the assumption free bounds. However, when larger numbers of SNPs were proposed as joint variants, the bounds narrowed and sometimes identified directions of effect, and were sometimes able to identify the direction of effect (though bounds from different subsets of SNPs identified different directions of effect).
16. Our results were slightly limited by the fact that the results of the inequalities showed that at least 8 of the SNPs could not be valid instruments in MoBa, and at least 6 could not be valid instruments in ALSPAC.
17. Our results show that, when multiple SNPs are proposed as instruments, it is possible to narrow bounds on the average causal effect and, on occasion, to identify the direction of effect without additional homogeneity assumptions.
18. In addition, the variation we observed across bounds calculated using different sets of proposed instruments highlights the utility of the bounds for comparing and critically evaluating assumption sets used in MR.

* in our results, bounds computed using different sets of proposed joint instruments sometimes identified opposite directions of effect

1. Pooled Bounds
2. We show that, under the IV conditions and some additional assumptions, bounds computed within different study populations can be pooled using the set intersection methods described by Manski 2020.
3. Bounds can be computed assuming that the identifiability conditions hold within each study, or assuming that the identifiability conditions hold in at least some number of studies (though this second approach may only be plausible in very specific settings).
4. In addition to assumptions regarding the internal validity of each study, these pooled bounds require an assumption of consistency of potential outcomes across study populations, and an assumption of no effect modification by study population.
5. While these assumptions are strong, they are consistent with the assumptions required in person (rather than study) –centered meta-analyses
6. Pooled bounds could be used alongside more traditional point estimation meta-analyses to help researchers and readers clarify how strongly the conclusions of a meta-analysis depend on the assumptions used in the model.

Broader Implications

1. Greater attention needs to be paid to the validity of the MR conditions in prenatal MR studies, especially for offspring behavioral outcomes
2. prenatal MR is subject to more forms of bias than previous articles using this method have made clear (MR lit review)
3. the fact that there were violations of the instrumental inequalities for SNPs whose relationship to the exposure is relatively well understood in multiple cohorts, suggests that either pleiotropic effects of these snps are stronger than was previously understood, or that the prenatal MR model is being affected by more severe structural biases (selection on pregnancy, pre- or postnatal exposure status, assortative mating)
4. this second possibility is a concerning indictment of prenatal MR more broadly, as these researchers often describe these studies as being superior to other observational designs, and sometimes encourage their use in policy determinations
5. further research is needed to evaluate the impact of some biases in applied prenatal MR (selection on pregnancy, assortative mating)
6. researchers need to be made aware of the unique issues of prenatal MR, and should discuss these limitations in their work
7. If maternal vitamin D or maternal vitamin D related genes do affect offspring psychiatric outcomes, it does not appear to operate through offspring DNA methylation
8. The instrumental inequalities and IV bounding approaches should be more broadly applied in the IV studies, especially MR with multiple proposed instruments
9. these studies provide multiple concrete examples of cases where the instrumental inequalities were able to provide meaningful information (either clearly falsifying a model or identifying a direction of effect), without additional homogeneity assumptions or other potentially implausible assumptions (e.g., the inside assumption)
10. the bounds in particular also provide an opportunity, in both single studies and meta-analyses, to contextualize point estimates

🡪 could give readers a clearer sense of how much information is the data alone

1. on a more meta level, these results argue for the importance of considering existing methods based on minimal assumptions, and trying not to reinvent the wheel

🡪 recent years have seen an explosion in sensitivity analyses and robust methods for MR

🡪 however, these methods typically take some form of homogeneity for granted, although biologic knowledge suggests this homogeneity is implausible for many exposures of interest and proposed instruments

🡪 neither the bounds nor the instrumental inequalities require such homogeneity assumptions, and were originally described long before MR became broadly popular

🡪 while innovation is vital to science, these results suggest that useful methods incorporating minimal assumptions sometimes are dropped by the wayside

Future Directions/Limitations

1. Further research needed on selection on pregnancy and assortative mating
2. in multiple analyses of different exposure-outcome relationships, in different cohorts, we found strong evidence of violations of the MR conditions for prenatal exposures, including for SNPs with relatively well understood relationships to the exposure of interest
3. maybe look into whether there is existing evidence of pleiotropy through more common sensitivity analyses for these
4. almost no attention has been paid to selection on pregnancy and assortative mating, although these biases have the potential to powerfully impact the results of MR analyses, and are note easily detected or ameliorated through commonly used robust methods

🡪 selection on pregnancy violates the INSIDE assumption and would affect all SNPs (thus violating the requirements for weighted median regression)

🡪 assortative mating would likely similarly affect all SNPs

🡪 not sure if there is existing evidence on whether SNPs have shown pleiotropic associations in other studies – might look into this

1. both require somewhat unique samples, in that they require samples that include information on pregnancy exposures, parental genotypes, and offspring outcomes in study populations that were not selected on the basis of pregnancy
2. Need for development of methods that account for sampling variability/uncertainty (95% confidence intervals and hypothesis testing) for bounds and inequalities
3. Considerable debate on how to generate confidence intervals or conduct statistical inference for bounded parameters

🡪 maybe briefly summarize swanson 2018/tamer 2010 on this topic, or just reference

1. importance of development of confidence intervals for bounds

🡪 our samples are not infinite, and the ability to incorporate this source of uncertainty will be critical to using bounding approaches for decisionmaking

🡪 development of 95% confidence intervals will also maximize comparability to point estimating approaches

1. complexity of confidence intervals and testing for instrumental inequalities

🡪 random confounding and random violations of the instrumental inequalities

🡪 in their existing state, still work as a sharp test within a sample

🡪 however, development of confidence intervals could allow us to better differentiate between random violations and structural violations.

🡪 This has some key advantages : first, identification of structural violations in one cohort provides evidence against the same study in similar populations (minimizing sunk costs)

🡪 Second: when pooling studies or conducting meta-analyses, determining whether a violation is structural or random would allow us to better understand whether an entire analysis should be eliminated (structural violation) or whether a single cohort should be eliminated from the analysis (random violation)

🡪 this pre-assumes that the violation is not a form of selection bias: this will need to be evaluated further

🡪 third: structural violations of the instrumental inequalities can also provide information about the pleiotropic relationships of a particular SNP to multiple outcomes

- Cai 2008: under the assumption that there are no common causes of the proposed instrument and outcome, the instrumental inequalities can themselves be used as bounds on the controlled direct effect of the proposed instrument on the outcome

- Zaidi 2020: can also be used as bounds on the average causal effect within particular principal strata (under various assumptions)

- Allows for identification of pleiotropic effects and thus clues as to genetic variant to phenotype pathway.

1. Development of strategies to deal with heterogeneity of effects when pooling bounds across multiple cohorts
2. pooling bounds requires assumption of no effect modification of effect by study on relevant scale (because this requires that the distribution of effect modifiers is equal or at least balanced out across cohorts, it ends up being close to the assumption of no effect modification in distribution)
3. this assumption is likely unreasonable in most contexts
4. Could potentially use IP weighting/transportability methods developed by Dahabreh 2020 to weight distribution of effect modifiers in population to match either other populations or target population (requires individual level data on sample of target population)

🡪 has not been extended to bounds context yet

🡪 in the context of instrumental variables (especially mendelian randomization) this might be especially complex if the effect modifier is downstream of the proposed instrument

1. this weighting might also allow for more reasonable use of union bounds in addition to intersection bounds?
2. Development of methods that allow for time-varying exposures
3. pregnancy is somewhat unique case, where we might plausibly believe an individual is exposed to a constant level of an exposure for a fixed period of 9 months (the MR lit review shows this is not really the case, but deviates less from the truth than typical individual-level MR)
4. However, expansion of bounding and use of the instrumental inequalities into time-varying exposure cases will be key to use of these methods in the broader MR sphere
5. Some expansion of MR point estimation into time-varying case (look into Joy’s work)
6. Need for development of bounds for time-varying case
7. Alternatively, bounds based more on the MR-Genius framework, that allow for bounding of sharp effect at specific time even if there are violations because of time-varying exposure status (tchetgen tchetgen, sun)
8. Expansion of teaching examples and educational articles to expand the use of the instrumental inequalities and bounding approaches in mendelian randomization and instrumental variables more broadly
9. both the inequalities and the bounds have existed for a long time
10. however, neither has been broadly used
11. in contrast, methods like MR-Egger have obtained a high degree of use in a relatively short period of time

🡪 I could definitely make a joke here about a lie running around the world before the truth has got its boots on

1. One reason for this is likely both the accessibility of tools for these methods and the work of the original authors in expanding teaching about MR-Egger and related methods
2. One strength of this dissertation is the inclusion of adaptable R functions for the use of the inequalities and the bounds in the context of MR with multiple proposed instruments
3. further work is needed to improve the efficiency of these functions, especially in the context of large numbers of proposed instruments and large datasets (this will likely involve parallelizing the code and potentially incorporating other methods to improve the processing speed)
4. creation of an R package, creation of stata and sas code to implement the bounds and inequalities
5. While we took efforts to make this dissertation as approachable as possible, it likely isn’t reaching the largest audience of clinician-scientists and applied researchers interested in MR
6. to improve the reach of these methods, we need to increase the number of approachable educational articles on this topic in clinical journals with a wide reach, as well as articles that tend to focus on MR, such as international journal of epidemiology
7. work on expanding the use of these methods could also include the creation of teaching materials and toy datasets to allow for easy integration of these techniques into epidemiologic teaching
8. hopefully, one of the things that will expand the reach of these methods is further research into their properties, and how they behave in real data.
9. further simulation research on the inequalities in particular may help applied researchers feel more confident in a type of analysis they don’t fully understand.

Conclusions

1. MR studies of the prenatal environment, especially with regards to psychiatric outcomes, requires strong assumptions, and should be treated with more caution than is commonly employed

a. The prenatal environment may impact offspring health, including psychiatric health in a variety of ways. However, approaches based on confounder adjustment may be biased when exposure-outcome confounders are complex or difficult to measure.

b. For this reason, MR has become an increasingly popular alternative

c. However, MR, like other observational methods, relies on strong, unverifiable assumptions

🡪 like any observational study, these assumptions are specific to study context

d. In the context of prenatal exposures, MR is subject to some unique biases that infrequently discussed

e. at least in the applications included in this dissertation, there is strong evidence that the MR assumptions are more often violated than has been previously acknowledged, and that these violations may be severe

2. Where possible, the assumptions required to conduct prenatal MR studies should be minimized, researchers should attempt to falsify them where possible, and studies should make clear how much a particular conclusion depends on an assumption

a. bounding approaches allow for identification of direction of effects without homogeneity assumptions

b. bounding approaches also allow researchers to clarify how strongly a point estimate depends on homogeneity assumptions

c. when applied across multiple possible combinations of SNPs, bounding approaches can also show the degree to which the conclusions of a model change depending on which SNPs are proposed as instruments

d. this can also be applied in the context of pooling information across multiple studies

e. this study also provides a concrete example of how MR models can be falsified through the use of the instrumental inequalities

3. potentially also say something about heterogeneity of prenatal effects here

a. definition is sometimes more complex

b. conclusions of MR may be strongly reliant on homogeneity, though there are many cases where this is known not to hold because of biological knowledge, and is often implausible

c. effects may also be heterogenous across populations – as most prenatal MR studies are conducted within European ancestry populations, it is highly likely that such effects (and potentially the validity of the MR model itself) may be heterogenous across populations

d. more attention should be paid to this issue, both when constructing cohorts, conducting analyses, and discussing results.