REVIEW OPEN ACCESS

Mendelian Randomization Applied to Neurology

Promises and Challenges

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Abstract

The Mendelian randomization (MR) paradigm allows for causal inferences to be drawn using genetic data. In recent years, the expansion of well-powered publicly available genetic association data related to phenotypes such as brain tissue gene expression, brain imaging, and neurologic diseases offers exciting opportunities for the application of MR in the field of neurology. In this review, we discuss the basic principles of MR, its myriad applications to research in neurology, and potential pitfalls of injudicious applications. Throughout, we provide examples where MR-informed findings have shed light on long-standing epidemiologic controversies, provided insights into the pathophysiology of neurologic conditions, prioritized drug targets, and informed drug repurposing opportunities. With the ever-expanding availability of genome-wide association data, we project MR to become a key driver of progress in the field of neurology. It is therefore paramount that academics and clinicians within the field are familiar with the approach.

Introduction

Since the popularization of the term "Mendelian randomization" (MR) in 2003,[1] the number of theoretical and applied studies on MR has increased exponentially. The growing popularity of this "gene-based hack that is revolutionizing epidemiology"[2] is justified by 3 core attributes. First, the MR framework provides an opportunity to address causal questions using publicly available genetic summary data. Second, its application has successfully addressed multiple clinical inquiries across research areas, including the field of neurology. Third, MR provides a framework for prioritization of potential drug targets including for neurologic indications. Despite considerable methodologic advances in the past 2 decades, applied MR continues to be subject to common analytical and interpretive pitfalls. In this review, we present the MR framework, provide examples of its application in neurology, and discuss areas most vulnerable to bias and misinterpretation, where MR should be undertaken with caution.

Data Sources

This narrative synthesis of evidence was based on literature searches of PubMed tailored by the authors' expert knowledge and opinion. Episodically up to 24 July 2023, PubMed searches were conducted without date restriction using combination of "Mendelian randomization" and

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Glossary

AD = Alzheimer disease; **FXI** = coagulation factor XI; **GLP1R** = glucagonlike peptide 1 receptor; **GWAS** = genome-wide association study; **IDUA** = alpha-L-iduronidase; **IL** = interleukin; **IV** = instrumental variable; **MR** = Mendelian randomization; **MS** = multiple sclerosis; **PD** = Parkinson disease; **RCT** = randomized controlled trial.

the following clinical related terms: "ischemic stroke," "Parkinson," "multiple sclerosis," "migraine," "Alzheimer disease," and "drug development."

What Is MR?

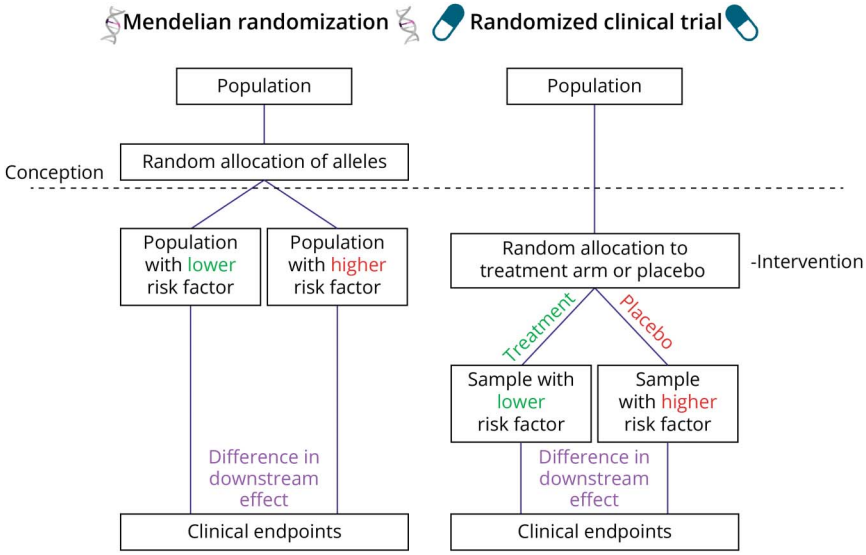
A traditional observational study faces 2 main challenges to assess causality: bias due to reverse causality and confounding. Reverse causality bias occurs when the observed association between an exposure and an outcome is due to a causal effect of the outcome on the exposure. Bias due to confounding, on the contrary, occurs when an association is explained by a third factor that causes both the exposure and the outcome. These biases make it difficult to know for certain whether an association from an observational study represents a cause-and-effect relationship.

MR is an epidemiologic method aimed at overcoming these limitations to draw causal inferences from observational data. To do this, MR relies on the logic of the instrumental variable (IV), an established statistical paradigm aimed specifically at overcoming these biases.[3] In MR, genetic variants serve as IVs to investigate whether a causal link exists between an exposure (e.g., low-density lipoprotein cholesterol) and an outcome (e.g., ischemic stroke). Because germline genetic variants are inherited at conception and are generally stable over time, they cannot be modified by the development of a disease, thereby eliminating reverse causality bias. Furthermore, because germline genetic variants are inherited randomly at conception, they are largely independent of environmental and lifestyle confounding factors (Figure 1). For example, a patient's body mass index in adulthood does not influence the variant they carry at any genomic location.

For MR to draw valid causal inference, the genetic instruments must meet 3 core assumptions. First, the genetic instrument must be associated with the exposure variable (i.e., relevance). Second, the genetic variant must not be associated with confounding factors of the exposure-outcome association (i.e., exchangeability). Third, the genetic instrument must have no direct effect on the outcome, but instead only affect the outcome through the exposure (i.e., exclusion-restriction).[4] If these 3 assumptions hold, then under further technical assumptions (e.g., linearity), the causal effect of the risk factor on the outcome can be calculated by dividing the association estimate of the genetic variant with the outcome by the association estimate of the genetic variant with the risk factor (Figure 2).

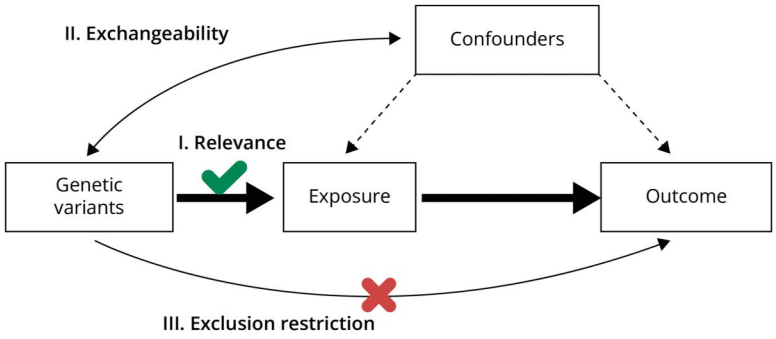
In practice, only the first assumption is verifiable. Assumption 2 is believed to generally be adequately satisfied by controlling for genetic ancestry but may still be violated by uncontrolled population stratification or by "assortative mating," whereby the traits or genetic variants under study influence partner selection. The third assumption is the strongest assumption

Figure 1 A Parallel Between Randomized Clinical Trials and Mendelian Randomization



The random allocation of alleles at conception offers a parallel to a "naturally randomized clinical trial." Under certain assumptions, the random distribution of alleles in Mendelian randomization studies allows for the separation of individuals into groups that differ only in terms of the risk factor of interest. While the random allocation of alleles in Mendelian randomization studies happens at conception and leads to lifelong effects, the randomization in randomized controlled trials typically happens later in life and lasts a shorter time. Created with BioRender.com.

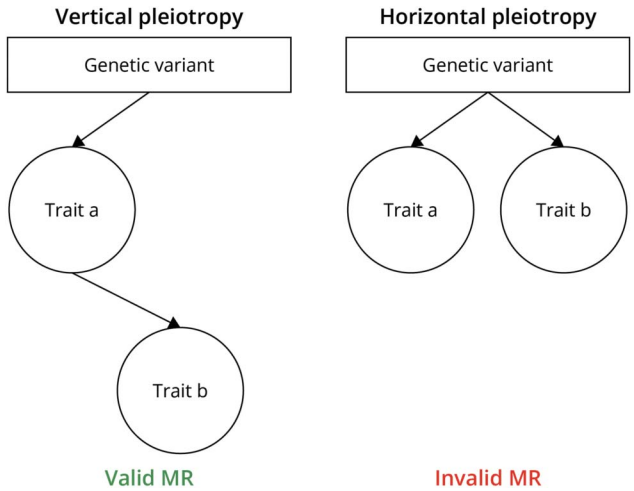
Figure 2 The 3 Main Assumptions of Mendelian Randomization



First, the relevance assumption states that the genetic instrument must have a significant effect on the exposure variable. Second, the exchangeability assumption states that the genetic variant must not be associated with confounding factors. Third, the exclusion restriction assumption states that the genetic instrument must only affect the outcome through its effect on the exposure. Created with BioRender.com.

and the most likely to be invalidated by pleiotropy. Pleiotropy in this context means that a genetic variant influences multiple traits (Figure 3). Vertical pleiotropy describes a scenario where a genetic variant has a direct effect on the exposure and an indirect effect on the outcome that is mediated by the exposure. This does not induce bias and the IV assumptions for the MR analysis hold. By contrast, horizontal pleiotropy describes a scenario where a genetic variant exerts direct effects on both the exposure and other parallel pathways that may influence the outcome. This does induce bias and threatens validity of causal inference from an MR analysis. Cautious genetic instrument selection and appropriate MR study design are paramount to minimizing the influence of this bias.

Figure 3 The Distinction Between Horizontal and Vertical Pleiotropies



Vertical pleiotropy occurs when the genetic variant has a direct effect on the exposure, and an indirect effect on the outcome that is mediated by the exposure. Horizontal pleiotropy occurs when the genetic variant has an effect on the outcome that does not wholly occur through the exposure. Vertical pleiotropy does not invalidate Mendelian randomization (MR) results, but horizontal pleiotropy invalidates MR results because it transgresses the exchangeability assumption. Created with BioRender.com.

Instrument Selection and Sensitivity Analyses (Statistical vs Biological Approaches)

The most critical decision in an MR investigation is the choice of which genetic variants to use as IVs. Broadly, there are 2 strategies to select genetic instruments: a biologically driven approach and a statistically driven, biologically agnostic approach.[5] In a biologically driven approach, variants are selected from genomic regions with a known biological link to the exposure. This method often uses fewer genetic instruments (sometimes just 1), which are less likely to be pleiotropic. For example, a *cis*-MR approach leverages genetic variants located in the genetic region encoding for the protein or gene transcript of interest. These genetic variants may influence protein levels more directly and are therefore more likely to be valid IVs. The disadvantage of this method is that it requires preexisting biological knowledge, often offers fewer IVs than a genome-wide approach, and precludes the use of certain sensitivity analyses that require multiple genetic variants.

By contrast, a statistically driven approach typically includes all the variants robustly associated with the exposure (often using a p value below the genome-wide significance threshold of 5×10^{-8}) across the entire genome, even if their biological function is unknown. Eligible genetic variants are subsequently pruned to remove correlated variants (i.e., variants that are inherited together). The statistically driven approach decreases reliance on a single genomic region and includes more variants, thus potentially increasing statistical power. However, it also increases the odds of including invalid, horizontally pleiotropic instruments, which might introduce bias into the analysis.

Statistical sensitivity analyses can be used to compensate for the inclusion of invalid instruments by performing robust MR methods that require less strict model assumptions to make valid causal inference. For example, the weighted median method can provide a valid causal estimate even if up to half of the information for the analysis comes from genetic variants

comprising the IV violate 1 or more requisite assumption.[6] Generally, it is recommended to perform a variety of robust MR methods that operate in statistically distinct ways and rely on different assumptions about the underlying nature of pleiotropy. The most commonly used robust methods are MR-Egger, weighted median, weighted mode, and MR-PRESSO methods.[7] Furthermore, the contamination mixture was found to have the lowest mean squared error across a range of methods in a simulation study.[8] A typical workflow could be to perform the inverse variance weighted as the primary MR analysis, and several of the afore mentioned methods as sensitivity analyses that make distinct assumptions regarding the nature of any included pleiotropic variants. Directionally consistent causal estimates across different robust MR methods provide confidence about the validity of the observed causal link. A detailed overview of the frequently used robust MR methods and their core assumptions is available[8] and can be used to assist in the interpretation of MR results.

Because biologically driven approaches often use few genetic variants, they are not always amenable to statistical sensitivity analyses that require multiple uncorrelated variants. When a single genetic variant is used, it is recommended to perform colocalization as a sensitivity analysis, such as is implemented in the method *Coloc*.[9] Coloc is a Bayesian algorithm that evaluates the posterior probability of 2 traits sharing a single causal variant.[10] Coloc is particularly useful to evaluate a specific form of confounding where the causal genetic variant for the exposure and the causal genetic variant for the outcome are different, but inherited together. A strong colocalization posterior probability of a shared causal variant provides additional evidence for a causal association by indicating that confounding by linkage disequilibrium is unlikely and the MR inference is unbiased.

The research question informs whether it is better to use more genetic variants and more complex methods (i.e., statistically driven approach) or a more curated set of variants and simpler methods (i.e., biologically driven approach). When investigating proximal gene products such as proteins or gene expression levels as the exposure, a biologically driven approach will likely yield a more valid inference. When investigating polygenic traits such as body mass index, statistically driven approaches may be more appropriate, particularly because such traits are heterogeneous with multiple distinct underlying pathways. Both instrument selection methods have advantages and disadvantages, and their combined use can help triangulate the true causal estimate.[11]

MR studies may be conducted in a single population, the so-called one-sample MR. Alternatively, if the genetic association estimates for the exposure and the outcome are taken from separate populations, then this is termed "two-sample MR." One-sample MR has the advantage of ensuring homogeneity in the population used to generate genetic associations for the exposure and the outcome because these are taken from the same population. However, in recent years, MR studies have increasingly used the two-sample design partly because these can offer greater statistical power. One potential limitation is the overlap of population samples. In the two-sample MR setting, sample overlap will bias the MR estimate toward the observational estimate in an order of magnitude inversely proportional to the strength of instruments (which can be estimated through the use of *F*-statistics).[12] In practice, when instruments are strong, the bias due to sample overlap will be minimal. However, when instruments are weak and overlap is substantial, performing the analysis with and without sample overlap may be a useful sensitivity analysis to help explore the extent of such bias.

MR Study of Drug Targets

Bringing a new drug to the market can cost, on average, anywhere from hundreds of millions to more than a billion dollars.[13] The cost of drug development is exacerbated by the high failure rate (up to 90% of drug candidates never make it to market, even after a successful phase 1 clinical trials).[13] The most common reason for failure is a lack of drug efficacy, which accounts for 40%–50% of terminated clinical trials. This means that even after promising results from preclinical studies and early-phase clinical trials, the drug does not demonstrate the intended therapeutic effect in humans and is therefore discontinued, highlighting the poor translatability of findings from animal models to humans.[14]

The MR paradigm is a promising strategy to help accelerate and reduce the cost of the drug development pipeline. Indeed, MR studies are relatively inexpensive to conduct—particularly compared with large randomized controlled trials (RCTs)—and can provide evidence that can be used to prioritize targets worth investigating in RCTs. Drug-target MR relies on the previously described *cis*-MR method, where genetic IVs are obtained from the genomic region of the target of interest.

For efficacy, MR can test whether a particular target is causally linked to a specific disease or condition. For safety, the MR framework can be implemented to test the effect of the target on thousands of clinical traits from electronic health care repositories. Second, drug-target MR can inform on drug repurposing opportunities by leveraging the genetic predictors of targets of medications with published safety profiles to identify novel indications for the drug.[15] Finally, with individual-level data, it is possible to test for the interactions between 2 drug targets using MR methods, as in a 2 × 2 factorial RCT.[16]

Applications of MR to Neurology

The MR paradigm has been used to investigate clinical hypotheses across all subspecialties of neurology. The guidance offered by MR analyses regarding disease pathophysiology,

causal effects, and potential drug targets are particularly important in neurology given the long slow progression of many neurologic diseases (e.g., Alzheimer disease [AD] and related dementias), which poses unique challenges to clinical trials. As in all specialties, time and money are limited, and tools to predict the success of clinical interventions and pharmacologic therapies are necessary. In this study, we highlight examples of applications of MR across several subspecialties of neurology.

Ischemic Stroke

MR has revolutionized the exploration of risk factors of different ischemic stroke subtypes. Large trials typically do not have the scope to phenotype stroke cases down to their etiologic subtypes. However, because of the recent availability of genome-wide association study (GWAS) data for stroke subtypes,[17] it is now possible to dissect their risk factor profiles with MR. For example, MR analyses have shown that elevated low-density lipoprotein cholesterol selectively increases the risk of large-artery atherosclerotic stroke, but not the risk of cardioembolic or small vessel stroke.[18] This finding addressed a clinical knowledge gap because the largest clinical trial of statins in stroke was conducted without stroke subtyping and excluded cases of cardioembolic stroke.[19]

In terms of drug target prioritization, the benefits of lowering blood pressure[20] and cholesterol[18] for stroke prevention were retrospectively identified by MR studies after large RCTs were conducted. For example, 1 MR study compared the effects of genetically proxied calcium channel blockade vs beta blockade on the risk of stroke.[20] This investigation found that genetically proxied blood pressure reduction through calcium channel blockade significantly lowered the risk of ischemic stroke, while genetically proxied beta blockade had no effect on stroke risk. These observations align with the current clinical guidelines for the treatment of hypertension that advise against the use of beta-blockers as first-line treatments for hypertension due to their inferior efficacy for reducing ischemic stroke risk.[21]

Several MR studies provided evidence for a causal effect of interleukin (IL)–6 signaling on ischemic stroke, particularly large artery, and small vessel stroke,[20,22] supporting the candidacy of IL-6 signaling as a target for prevention. Trials targeting IL-6 signaling are under way, so definitive evidence is yet to come. Recent MR data indicate that 67% of the effects of genetically downregulated IL-6 signaling on large artery atherosclerotic stroke could be mediated by a reduction in circulating CXCL10 levels.[23] These results suggest CXCL10 to be a potential causal mediator of atherosclerosis and as such might serve as a promising, potentially more specific drug target than IL-6 signaling.

Finally, MR has provided support for a novel drug target to be used for stroke prevention: the coagulation factor XI (FXI). Higher genetically predicted circulating levels of FXI were shown in an MR analysis to increase ischemic stroke risk.[24] Subsequent studies recapitulated this finding and demonstrated that these effects were specific to cardioembolic stroke.[8,9,25,26] Promisingly, genetically predicted FXI inhibition does not seem to increase the risk of intracranial or extracranial bleeding, further supporting the hypothesis that FXI inhibition may be a safer therapeutic strategy compared with established anticoagulants such as coagulation factor Xa inhibitors.[25] MR has also been used to investigate the outcomes of intracerebral hemorrhage and subarachnoid hemorrhage, and results from these studies are partially summarized elsewhere.[27]

Alzheimer Disease

Investigating the pathophysiologic basis of neurologic conditions, such as AD, ideally requires access to brain tissue, but this has historically not been feasible at a large scale. New GWAS of brain protein levels now permits detailed evaluation of the brain pathophysiology of neurologic conditions. For example, Robins et al.,[28] performed a GWAS of 8,356 proteins measured by liquid chromatography coupled to mass spectrometry in the dorsolateral prefrontal cortex of 330 postmortem brain donors. Using this dataset, they performed a brain proteome-wide MR and identified 13 genes whose cis-regulated brain protein abundance was associated with AD.[29] Similarly, Yand et al. performed a GWAS of 1,305 protein levels in the CSF (n = 971) and parietal cortex (n = 458) using an aptamer-based platform. Using MR, the authors identified 3 proteins implicated in AD risk in the CSF and 7 in the parietal lobe.

Parkinson Disease

MR analyses have been particularly useful in distinguishing causal from noncausal risk factors of Parkinson disease (PD). Observational studies have consistently shown that serum levels of vitamin D and urate are inversely associated with the risk of PD.[30] However, MR analyses have not supported a causal effect of either of these biomarkers on PD risk, suggesting that these observational associations may be confounded or attributable to reverse causation.[30,31] MR analyses have also been used to identify novel risk factors of PD, such as plasma levels of alpha-L-iduronidase (IDUA). Leveraging data from a hypothesis-free scan of the plasma, CSF, and brain proteome, IDUA was identified alongside 34 other proteins as having a potentially causal association with PD.[32] IDUA is responsible for the degradation of glycosaminoglycans in the lysosome, an organelle that is implicated in at least monogenic forms of PD.[32]

Multiple Sclerosis

The MR framework has been used to weigh in on longstanding epidemiologic controversies related to multiple sclerosis (MS). For example, it has not been repeatedly shown using MR that higher levels of vitamin D causally lower the risk of MS.[33] In addition, MR analyses have shown that the effect of adiposity on MS risk varies across the life course. Specifically, MR findings indicated that genetically proxied adiposity in childhood, but not adulthood, is causally associated with an increased risk of MS.[33] By contrast, MR analyses

have not supported an effect of these risk factors on MS severity.[34]

Regarding drug repurposing opportunities, 1 MR analysis identified interleukin-6 signaling as a potential causal risk factor of MS.[35] Subsequent mediation analysis revealed that altered interleukin-6 signaling explained more than 40% of the observed effect of body mass index on MS risk.[35] This example demonstrates how MR may simultaneously provide mechanistic insights for disease and identify novel causal risk factors and therapeutic targets.

Migraine

Migraine is a common disabling neurologic disorder that remains poorly understood. In an exploration of the causal relevance of prior epidemiologic findings linking poor sleep to migraine,[36] MR analyses have identified both insomnia and difficulty awakening as potentially causal risk factors.[37] This substantiates the prioritization of treating sleep disturbances when treating migraine. Another therapeutically relevant advance in migraine research is the potentially causal relationship between multiple coagulation factors and migraine risk, identified using MR analyses.[38] This work showcased the dual role that MR can play by indirectly providing evidence that microemboli may play a role in the etiology of migraine and by prioritizing certain coagulation factors as potential drug targets.

These select examples highlight the myriad applications of MR across subspecialties of neurology. Many neurologic diseases have yet to be investigated in MR analyses due to the paucity of large GWAS for certain phenotypes. This limitation precludes the detailed investigation of many disease subtypes (e.g., migraine with aura), disease severity (e.g., migraine frequency), and disease progression or recovery (e.g., motor recovery in stroke). The increasing availability of genetic data obtained from nervous system tissue, such as the CSF and neuronal cells, also presents opportunities for the application of MR to neurology. These large-scale data will allow more precise mechanistic insights and predictions regarding the efficacy of brain drug targets. Finally, the increasing availability of sex-stratified genetic data will facilitate a clearer understanding of how risk factors and mechanisms of disease may differ by sex.

Common Pitfalls of Applied MR Analyses

In this section, we discuss some common pitfalls in applied MR analyses. This is by no means an exhaustive list of all the potential challenges in applied MR but rather a highlight of some frequently encountered issues that we encourage practitioners to consider carefully.

Exposure and Outcome Specification

It is essential that the exposures being instrumented and the outcomes of interest are defined fully and accurately. Exposure definition is particularly important in MR studies of binary traits (e.g., case/control).[39] In these cases, MR estimates the effect of disease liability (e.g., risk of stroke) as the exposure of interest. This is distinct from conventional epidemiologic studies where one may directly estimate the association of disease status with an outcome. MR studies considering binary or dichotomized exposure should be careful to communicate results as the association of genetic liability to the exposure with the considered outcomes, rather than the effect of the exposure.

Instrument Selection

Genetic variants used as IVs should reflect variation in the exposure being studied. This issue created controversy in an MR study that aimed to investigate the effects of the antidiabetic drug metformin by selecting genetic instruments through their association with circulating levels of growth differentiation factor 15.[40] As several letters in response to this manuscript pointed out,[41] this MR study was in fact investigating the causal effect of circulating growth differentiation factor 15 levels rather than metformin use per se and the 2 are not equivalent nor interchangeable.

Minimizing the biasing effect of pleiotropy is an important consideration when selecting instruments for MR analyses. For exposures that are influenced by environmental and lifestyle factors, confounding by pleiotropic pathways are inherent to instrument selection and should be addressed appropriately. For example, in an MR study aimed at investigating educational attainment, there is likely to be pleiotropy through associations of the genetic IVs with intelligence.[42] Where such pleiotropic pathways are known, and relevant genetic association data are available, multivariable MR—an MR technique evaluating more than 1 exposure—may be used to adjust for potential bias.[43]

Finally, biological insight may be used to inform instrument selection. For example, when studying the effect of caffeine, it is more appropriate to instrument these effects using genetic variants that predict plasma caffeine levels (i.e., the physiologic presence of the bioactive component of interest) than variants affecting caffeine consumption (i.e., the behavioral trait). The latter is likely less biologically relevant and is more vulnerable to biasing pleiotropy through relation with pleiotropic pathways such as dietary preferences, socioeconomic status, and lifestyle.[44] For plasma caffeine levels specifically, instrument validity can be further optimized by selecting genetic variants that are located in genes related to caffeine metabolism instead of a genome-wide approach.

Shared Etiology vs Causal Effects

Similarly, biological understanding is required to distinguish shared etiology from causal effects. For example, if the genetic variants used as IVs for an exposure relate to an upstream trait that causes both the exposure and the outcome under consideration, then any apparent MR association may be attributable to a shared etiology for the exposure and the outcome, rather than a causal effect of the exposure on the outcome.

This phenomenon may be at play for MR analyses investigating the causal effect of perivascular space burden on the risk of cerebral small vessel disease[45] or of white matter hyperintensity volume on the risk of stroke or AD.[46] In these cases, common risk factors may be underlying the exposures and the outcomes. If a common shared etiologic factor is suspected, its confounding effect can be tested using multivariable MR.

Studies of Disease Progression

MR studies investigating the effects of an exposure on the risk of disease progression (or recovery) are vulnerable to collider bias if the exposure also affects disease risk.[47] Collider bias is a subtle form of bias that may emerge when analyses are performed in a "case-only" dataset. Following the release of the genome-wide association summary data for functional recovery after stroke,[48] several MR studies have attempted to unravel potential interventions that favorably affect stroke recovery.[49] Results from these investigations should be cautiously interpreted because the described collider bias can produce false-positive findings. Although there are methods tailored to detection and correction for collider bias,[47] their practical application is not always feasible, and they remain sparsely implemented. The limited feasibility of MR investigating the determinants of disease progression or recovery is of relevance to the field of neurology because the clinical objective in practice is typically to manage and treat chronic neurologic diseases once they have occurred. Indeed, the prevention of disease onset falls more within the realm of public health and primary care, rather than neurology per se.

Other Commonly Observed Limitations

Population stratification is a subtle form of bias in MR analyses, which occurs, for example, when a particular population subgroup has a higher level of a phenotypic trait and a higher frequency of particular genetic variants, thus creating a correlation between the phenotypic traits and genetic variants. In some instances, genetic ancestry can introduce such population stratification. To mitigate this, MR analyses often consider populations of the same genetic ancestry. However, due to the overrepresentation of European ancestry individuals in GWAS, most published MR analyses have been limited to this population group. This can hinder the generalizability of MR findings to other ancestries. As GWAS become more diverse, it will be of importance to perform MR in different ancestries. For example, the GWAS for stroke were recently reported for different ancestry groups (European, East Asian, African, Hispanic or Latin American, and South Asian) and pooled (trans-ancestry).[17] Similarly, a recent plasma proteome GWAS has identified protein quantitative trait loci in both European and African ancestry groups.[50] Other potential limitations of MR include the potential for publication bias, where findings with positive studies are more likely to be published, even if those represent chance associations in the analyzed data rather than true causal effects. To this end, it is important to incorporate power calculations in advance of undertaking MR analyses[51] to ensure that sufficient statistical power is available for the hypothesis being investigated. Following consensus standards, such as those set out in the Strengthening the Reporting of Observational Studies in Epidemiology-MR guidelines,[52] can also help maintain the quality and standards of analyses and their reporting.

Considerations for Drug-Target MR

There are various pitfalls that are specific to MR analyses aimed at investigating drug target perturbation. For example, some drug targets have multiple modes of action. This point is well illustrated by glucagon-like peptide 1 receptor (GLP1R), which is likely exerting effects on bodyweight and glycemic control through distinct mechanisms.[9] As such, selecting genetic variants in the *GLP1R* region based on their statistical association with type 2 diabetes mellitus liability may only allow for the investigation of effects mediated through mechanisms of glycemic control and exclude those related to other GLP1R effects, such as lowering body weight.

A particular note of caution for drug-target MR is that this approach cannot be used to study compound-specific pharmacologic effects but only the effect of perturbing the drug target. To illustrate this point, MR analyses have supported the effects of perturbing cholesteryl ester transfer protein on reducing risk of cardiovascular disease.[53] However, whether this finding translates to clinical trials will be affected by their study design, including the target population, duration and intensity of treatment, and pharmacologic compound being studied and its magnitude of effect on the target.

Finally, as with all MR analyses, it is imperative to communicate that the analytic paradigm reflects the cumulative effect of small lifelong genetically predicted differences in the exposure. This is in contrast to clinical interventions that take place over a shorter period and may result in much larger changes in the level of the exposure of interest (e.g., complete inhibition of a protein). Thus, results from MR analyses will not precisely predict the size of the effect of an intervention in clinical practice—these are typically of larger magnitude and over a much shorter period.[54]

Future Perspectives

New opportunities for deeper understanding of neurologic disease mechanisms come from the falling cost and increasing scalability of proteomics technologies, transcriptomics technologies, and imaging modalities. GWAS on brain protein levels,[28,55] cerebrospinal protein levels,[55] brain gene expression,[56] brain magnetic resonance imaging,[57] and brain single-cell gene expression[58] are, or will soon be, publicly available. These datasets can be leveraged to inform on pathophysiology of neurologic condition. Access to large volumes of public summary data also creates challenges, including the potential execution of large number of hypothesis-free tests and potential violation of the requisite assumptions of MR in some of

these tests. These issues should be transparently reported in such hypothesis-generating studies, and multiple testing should be adequately accounted for to limit the risk of type 1 errors.

The increasing power of GWAS will breach new frontiers in the study of rarer neurologic conditions where etiologic risk factors are not as well understood as for more prevalent conditions. GWAS have already uncovered the first loci for less common neurologic conditions such as dystonia, autoimmune encephalitis, Guillain-Barré syndrome, and hereditary ataxia. The application of MR to these disease outcomes may result in the identification of novel modifiable risk factors.

Finally, MR methods are rapidly evolving. For example, the application of MR to disease progression while correcting for collider bias is an area of intense methodologic development. Similarly, methodologies to mitigate bias due to horizontal pleiotropy are continuously being developed and will result in more reliable causal inference.

Conclusion

The integration of MR into neurology-oriented research has offered a solid foundation for identifying causal risk factors, advancing our understanding of the pathophysiology of neurologic diseases, and assisting in the prioritization of drug targets. To reap its full benefit, researchers must conduct MR investigation carefully and include biological insights through all stages of analysis. As the field of MR in neurology continues to evolve, it is our hope that academics and clinicians will fully embrace its potential and use it constructively in efforts to serve patients.

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Iyas Daghlas, MD	Department of Neurology, University of California San Francisco	Drafting/revision of the article for content, including medical writing for content; study concept or design
Loukas Zagkos, PhD	Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, United Kingdom	Drafting/revision of the article for content, including medical writing for content; study concept or design
Muralidharan Sargurupremraj, PhD	Glenn Biggs Institute for Alzheimer's & Neurodegenerative Diseases, University of Texas Health Sciences Center, San Antonio	Drafting/revision of the article for content, including medical writing for content; study concept or design
Marios K. Georgakis, MD, PhD	Broad Institute of MIT and Harvard, Cambridge, MA; Institute for Stroke and Dementia Research (ISD), University Hospital, LMU Munich, Germany	Drafting/revision of the article for content, including medical writing for content; study concept or design
Christopher D. Anderson, MD, MMSc	Broad Institute of MIT and Harvard, Cambridge, MA; Center for Genomic Medicine, Massachusetts General Hospital, Boston; Department of Neurology, Brigham and Women's Hospital, Boston, MA	Drafting/revision of the article for content, including medical writing for content; study concept or design
Helene T. Cronje, PhD	Department of Public Health, Section of Epidemiology, University of Copenhagen, Denmark	Drafting/revision of the article for content, including medical writing for content; study concept or design
Stephen Burgess, PhD	MRC Biostatistics Unit, and Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, United Kingdom	Drafting/revision of the article for content, including medical writing for content; study concept or design

Appendix *(continued)*

Name	Location	Contribution
Benoit J. Arsenault, PhD	Quebec Heart and Lung Institute, and Department of Medicine, Faculty of Medicine, Université Laval, Québec, Canada	Drafting/revision of the article for content, including medical writing for content; study concept or design
Dipender Gill, BMBCh, PhD	Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, United Kingdom	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

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