A Mendelian Randomization dictionary: Useful definitions and descriptions for undertaking, understanding and interpreting Mendelian Randomization studies

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Standfirst

As Mendelian randomization (MR) becomes more commonplace in clinical guidelines and drug development, the aim of the MR Dictionary is to provide useful definitions and descriptions for undertaking, understanding and interpreting MR studies to a wide, inter-disciplinary audience – both those new to MR and those who are experienced in its use but who want to remain up to date.

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1 Introduction

2 Mendelian randomization (MR) is the use of genetic variants as instrumental variables
3 (IVs) to interrogate the causal effects of modifiable risk factors on disease or other outcomes. It
4 was first formally proposed in 2003 (1), following archetypal examples that used genetics, or
5 suggested their use, to infer causality in epidemiological associations (2,3). Over the last 15
6 years, it has been increasingly used to improve understanding of the causal effects of lifestyle
7 risk factors (4-7), biomarkers (8,9) and drug treatments (10-12) on human health. Alongside
8 these applications, considerable efforts have been undertaken to develop tools for exploring the
9 underlying assumptions of MR and the robustness of results from MR studies (13-21). Methods
10 for using MR in different contexts, including using both individual- and summary-level data and
11 testing the joint effects of two or more risk factors, understanding mediating mechanisms and
12 long-term effects of risk factors measured in pregnant women on their offspring, have also been
13 developed and applied (22-27). The rapidly advancing field of MR methodology has generated a
14 new language that reflects the multi-disciplinary contributions to the field, with terms
15 originating from epidemiology, econometrics, genetics and biostatistics. As MR becomes more
16 commonplace in clinical guidelines, drug development and the identification of targets for
17 lifestyle interventions (28,29), there is a need for researchers and practitioners from multiple
18 disciplines to understand the existing and evolving 'language' of MR.

19 There are several publications that describe the underlying principles of MR and/or
20 provide up-to-date descriptions of emerging novel methods, several of which include glossaries
21 of useful terms in MR (29-33). However, many of these are tailored to specific audiences and we
22 are not aware of an extensive "MR Dictionary" that provides useful definitions and descriptions
23 for undertaking, understanding and interpreting MR studies to a wide, inter-disciplinary
24 audience. Such a resource should be valuable to clinicians, other clinical and public health
25 practitioners as well as to researchers from difference disciplines, including those new to MR, as
26 well as those who are experienced in its use but want to remain up to date. Previous papers and
27 glossaries do not extensively cover the increasing number of different statistical methods that

can be used in MR, nor do they have the commitment and a process for regular updating, as we plan to do here.

The aim of this dictionary is to provide definitions and key references for terms and concepts that are necessary to undertake MR studies, and to critically appraise and appropriately interpret findings from MR studies.

Layout of the dictionary

The MR Dictionary is split into 11 chapters: the first ten contain tables that define terms in broad areas, providing a definition for each term, additional information (e.g., relevance to MR or assumptions of statistical methods) and key references, websites or figures for further clarification. These ten chapters cover the following areas:

- **Chapter 1** – Genetic terms useful for understanding MR.
- **Chapter 2** – Defining MR and different types of MR studies.
- **Chapter 3** – Pertinent study designs, such as genome-wide association studies, or approaches that are related to MR.
- **Chapter 4** – Common terms used when describing sources of bias or limitations specific to MR.

Following on from these, we have chapters describing the key statistical methods that can currently be used to conduct MR analyses and estimate causal effects:

- **Chapter 5** – Standard one-sample MR methods.
- **Chapter 6** – Pleiotropy-robust one-sample MR methods.
- **Chapter 7** – Standard methods for two-sample MR.
- **Chapter 8** – Pleiotropy robust two-sample MR methods.
- **Chapter 9** – Model selection and averaging approaches for two-sample MR.
- **Chapter 10** – Heterogeneity and outlier detection methods for two-sample MR.

The idea behind these separate chapters is that we envisage different users of this dictionary to have different broad areas of interest or needs in relation to undertaking or interpreting MR studies. However, for those who wish to quickly search for a specific term, the index (at the end of the dictionary) provides a complete list of all terms in any of the tables in alphabetical order. In addition, within each of the chapters, terms are presented alphabetically and, to help readers identify terms that are described in any of the chapters, we have cross-referenced (using hyperlinks, indicated in italics) any terms that are defined in any of the chapters included in the MR Dictionary. For example, in the first entry of Chapter 1, the terms *gene*, *locus*, *chromosome* (and several others) are in italics. Holding the cursor over these terms (or any others that are italicised in any of the chapters) whilst pressing control and clicking take you to the main definition of that term.

All figures are numbered by chapter and order (e.g., Figure 1.1 is the first figure in Chapter 1). Chapter 2 (types of MR study) includes seven figures (Figure 2.1 to Figure 2.7), which are also referred to (and hyperlinked) in other chapters.

In the final chapter, **Chapter 11**, we provide links to some databases that support MR analyses, a link to an animation that describes MR and a flow diagram (Figure 11.1) that summarises how to undertake an MR study.

Help us to keep the MR Dictionary sustainable and up to date

We realise that it is difficult (nigh impossible) to ensure that this initial version of the Dictionary includes every term that all individuals interested in MR might wish to be included. We are also aware that new and relevant methods are certain to emerge after publication of this paper. We have tried hard to minimise any errors, but it is also likely that some readers may feel some entries need corrections. To manage these issues and ensure the Dictionary is sustainable and up to date, we will:

- Undertake an annual update of the MR Dictionary by the lead author group in August of each year.

- Develop an open, collaborative and interactive online MR Dictionary tool that we hope to release on the *BMJ* website in August 2020. This will support more rapid and extensive linked searches and more immediate updates and online discussion. The online platform will differ (and be an improved version) from the current full online word document version of the Dictionary in the following ways:
 1. There will be a user-friendly interface that will allow users to enter and search for any term. The search will take them to the main definition but will also highlight all other ‘chapters’ in which the term appears, so that users can see the broad relevance of that term and go to and read those sections also. The search will also provide links to any references in the definition of the term that are available on PubMed and provide full references for books/chapters or other referenced articles that are not on PubMed. This compares to the current version, where hyperlinks usefully take readers to the main definition of any term (from anywhere that it appears in the Dictionary) but does not cross references all uses of the term.
 2. The online version will support continuous edits, updates and comments (without users having to wait for the next annual update in August). All such edits and entries will be dated and linked to the person making them and their institution. The tool will allow discussion of existing terms and definitions and of corrections and edits in an open and transparent way. The tool will request that those making edits and new entries indicate whether they would like to join the lead author group. Those who request this will be expected to make an appropriate contribution to the next (and subsequent) annual update(s) of the Dictionary and its online tool. Annual reviews in August will continue and, at each of these, a new ‘clean’ version of the online MR Dictionary will be announced, with previous versions (including edits and discussions) archived.

109 Our intention is that this open and collaborative approach will ensure the sustainability
110 and relevance of the Dictionary.

111 In the mean-time (i.e., up to August 2020 and the launch of the interactive tool), if you
112 think key terms are missing or you notice errors/inconsistencies in what we have written in
113 this initial version, then please let us know by emailing mr-dictionary@bristol.ac.uk, such that
114 we can include relevant suggestions in the next version with an appropriate acknowledgement.
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Chapter 1: Useful genetic terms

Term	Brief definition	Relevance to MR	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Allele	A variant form of a <i>single nucleotide polymorphism (SNP)</i> , a specific polymorphic site or a whole <i>gene</i> detectable at a <i>locus</i> . Most <i>SNPs</i> are biallelic, meaning that there are two values (forms) that are possible (e.g., the <u>rs234</u> <i>SNP</i> denotes that individuals could have either an A or G allele at position 105,920,689 on <i>chromosome 7</i> on the positive strand). At a given <i>SNP</i> , the most common allele is referred to as the ‘major allele’; whereas, the least common (or rarer) allele is referred to as the ‘minor allele’. The minor allele frequency is therefore the frequency at which the minor allele occurs within a population.	In <i>MR</i> , most commonly, the genetic <i>instrumental variable (IV)</i> is one or more <i>SNP(s)</i> that are robustly associated with the risk factor/exposure of interest. The number of ‘trait-increasing’ alleles is used analogously to the lifetime ‘dose’ of the risk factor. The commonest unit of the genetic <i>IV</i> -risk factor or genetic <i>IV</i> -outcome associations are ‘per-allele’ – i.e., assuming a linear additive change across the three <i>genotype</i> categories. See <i>DNA</i> below.	(34) Figure 1.1

Chromosome	A <i>deoxyribonucleic acid (DNA)</i> molecule that carries a portion of all genetic material. Two homologous chromosomes carry the same collection of <i>gene</i> , but each <i>gene</i> can be represented by a different <i>allele</i> on the two homologues (a <i>heterozygous</i> individual). A gamete (egg or sperm) will receive one of these homologues, but not both. Humans have 22 pairs of autosomal homologous chromosomes and 1 pair of sex chromosomes.	Used to identify the position of a <i>genetic variant</i> within <i>MR</i> studies.	(34) Figure 1.1
Copy number variation (CNV)	Relatively large sections of the <i>DNA</i> are sometimes deleted or repeated one or more times, such that in those sections of <i>DNA</i> , the number of copies of the section varies between individuals (rather than, for example, a single <i>DNA</i> base differing between individuals, as in <i>SNPs</i>).	So far, copy number variation has not commonly been used in <i>MR</i> owing to a) the majority of natural variation in humans taking the form of <i>SNPs</i> , 2) genotyping technology being principally designed to assay <i>SNPs</i> and 3) a relative paucity of robustly associated copy number variants with phenotypes of interest.	(35,36)

Deoxyribonucleic acid (DNA)	<p>DNA is a molecule that contains the genetic instructions used in the development and functioning of all living organisms. The main role of DNA is the long-term storage of information. It contains the template needed to construct other components of cells, including ribonucleic acid (RNA) and protein molecules, as well as regulatory mechanisms. DNA has four nucleotide bases: Adenine (A), Thymine (T), Guanine (G) and Cytosine (C). The two strands of DNA in the double-helix structure are complementary (sense and anti-sense strands) such that A binds with T and G binds with C.</p>	<p>Natural variation in DNA sequences is assayed and tested for association with phenotypes. Variants (most commonly <i>SNPs</i>) are used as <i>IVs</i>. DNA has a double helix structure, formed of two linked strands of nucleotides that run in opposite directions - either from the five-prime (5') to the three-prime (3') (also known as sense or forward/positive direction) or from 3' to 5' (also known as anti-sense or reverse), as shown in Figure 1.1. The importance of this to <i>MR</i> is in relation to harmonizing <i>alleles</i> encoded differently (on either forward or reverse strand) in different studies for <i>two-sample MR with summary (aggregate) data</i>.</p>	<p>(34)</p> <p>Figure 1.1</p>
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Gene	A gene comprises a <i>DNA</i> sequence, including introns, exons and regulatory regions, related to transcription of a given RNA. They provide the template for production of a specific protein.	Some <i>genetic variants</i> that are used in <i>MR</i> are within the coding region of a known gene. When <i>MR</i> is used to test the effect of a product of a known gene (e.g., adiponectin or C-reactive protein) on an outcome, variants in the genes coding that protein are used as <i>IVs</i> . Bias due to <i>horizontal pleiotropy</i> is less likely than with variants whose function is unknown or where the risk factor being tested is complex and not a direct gene product (e.g., body mass index [BMI]).	(34)
Genetic variant	A position in the <i>DNA</i> sequence at which there are at least two forms in the population. A genetic variant that is within a region of the genome that codes for a protein (i.e., is in an exon), can be categorised as either synonymous (i.e., both forms code for the same amino acid and the form that might be	The genetic variants used as <i>IVs</i> in <i>MR</i> studies are usually <i>SNPs</i> . However, the term “genetic variant” can refer to other variations within the genome (e.g., <i>chromosome abnormalities, copy number variation, insertions/deletions</i> or other	(34,36)

	<p>considered a mutation has no downstream effect on the protein structure that is synthesized) or non-synonymous (i.e., one form (the mutation form) changes the amino acid and protein structure). Synonymous variants can still have robust associations with phenotypes through <i>gene</i> regulation.</p>	<p>sequence variations). Whilst most genetic variants occur outside coding regions, these are completely valid for <i>MR</i> analyses, even though their function or how they influence the exposure of interest is unknown.</p>	
Genotype	<p>The genotype of an individual refers to the <i>allele</i> inherited at a specific <i>locus</i> – if the <i>alleles</i> are the same, the genotype is <i>homozygous</i> and, if different, <i>heterozygous</i>. Because humans are diploid (i.e., we inherit two pairs of <i>chromosomes</i>, one from each parent), study participants can be categorised into one of three genotype groups – <i>homozygous</i> for the ‘reference’ <i>allele</i> (<i>homozygotes</i>), <i>heterozygous</i> (<i>heterozygotes</i> - i.e., having one of each of the ‘reference’ and ‘non-reference’ <i>allele</i>) and <i>homozygous</i> for the ‘non-reference’ <i>allele</i> – usually</p>	<p>See <i>Allele</i> above.</p>	<p>(34)</p> <p>Figure 1.1</p>

	<p>coded 0, 1 and 2, for possible genotypes. A genotype is usually denoted with two letters representing the two nucleotide bases on the forward <i>DNA</i> strand at that <i>locus</i> (i.e., for the <u>rs234</u> <i>SNP</i> that has two common <i>alleles</i> A and G, AA, AG or GG would denote three possible genotype combinations, representing a <i>homozygote</i> for the A <i>allele</i>, a <i>heterozygote</i> and a <i>homozygote</i> for the G <i>allele</i>, respectively). Genotypes are often encoded in 'additive' or 'dosage' format, which represent the count of the non-reference <i>allele</i>, where AA = 0, AG = 1 and GG = 2.</p>		
Haplotype	<p>A haplotype is a version of a section of a <i>chromosome</i>. It describes the particular sequence of <i>alleles</i> of linked <i>genetic variants</i> found on a single <i>chromosome</i>.</p>	<p>Whilst <i>SNPs</i> are most commonly used in <i>MR</i>, haplotypes have also been used in <i>one-sample MR</i>.</p>	(34,37,38)
Heterozygous/heterozygote	<p>Where the <i>alleles</i> at a particular genetic <i>locus</i> are different (i.e., the AG <i>genotype</i> stated in the examples for <i>genotype</i> and <i>allele</i>).</p>	<p>See <i>Allele</i> above.</p>	(34)

Homozygous/homozygote	Where the <i>alleles</i> at a particular genetic <i>locus</i> are the same (i.e., either the AA or GG <i>genotype</i> stated in the examples for <i>genotype</i> and <i>allele</i>).	See <i>Allele</i> above.	(34)
Linkage disequilibrium (LD), also known as gametic phase disequilibrium	LD is the non-random occurrence of <i>genetic variants</i> in the population, produced due to the chromosomal sections of genetic material inherited, such that <i>alleles</i> for <i>genetic variants</i> close together are always co-inherited unless randomly segregated by recombination. This induces correlation between <i>genetic variants</i> because the <i>allele</i> of one <i>locus</i> is disproportionately co-inherited with an <i>allele</i> at another <i>locus</i> . LD is typically high amongst variants close together on the same <i>chromosome</i> , but long-range LD including across multiple <i>chromosome</i> can be induced due to various population processes. The term LD describes a state that represents a departure from the hypothetical situation in which	In <i>MR</i> , the use of multiple <i>IVs</i> is akin to aggregating information from multiple independent trials. Therefore, ideally independent genetic <i>IVs</i> are used as to not 'double-count' the contribution of any particular variants, which would lead to under-estimation of the standard error of the estimate. This is done by either 1) clumping, whereby only a <i>SNP</i> is retained per <i>locus</i> based on LD within that population (or by using a representative reference panel); or 2) the estimation procedure is performed conditional on the LD matrix between <i>genetic variants</i> .	(34)

	all <i>loci</i> exhibit complete independence (linkage equilibrium, LE).		
Locus	A locus is a section of <i>DNA</i> sequence and can be a <i>SNP</i> , a <i>gene</i> or a larger region of <i>DNA</i> sequence.	See <i>Chromosome</i> above.	(34) Figure 1.1
Palindromic SNP	A sequence of <i>DNA</i> is a palindrome if the 5' to 3' (sense or forward) sequence is the same as the 3' to 5' (antisense or reverse). For example, the sequence ACCTAG (reading 5' to 3') is a palindrome because its complement (3' to 5') is GATCCA. A palindromic <i>SNP</i> (also known as an 'ambiguous' <i>SNP</i>) is a <i>SNP</i> in which the possible <i>alleles</i> for that <i>SNP</i> pair with each other in the double helix structure. When this occurs, <i>alleles</i> on the forward strand are the same as on the reverse strand (e.g., C/G on forward is G/C on the reverse, as shown in Figure 1.1).	In <i>two-sample MR with summary (aggregate) data</i> , it can be difficult to harmonize palindromic <i>SNPs</i> if it is unclear which way (5' to 3' or vice versa) the <i>alleles</i> are presented in each <i>genome-wide association studies (GWAS)</i> . For some palindromes, it is possible to infer which is the effect <i>allele</i> using the <i>allele</i> frequency information. Where this is not the case, the options are to (i) assume all <i>SNPs</i> in both <i>GWASs</i> have been presented in the 5' to 3' way based on knowledge of other <i>SNPs</i> that are non-palindromic or	(39) Figure 1.1

		(ii) drop all palindromic <i>SNPs</i> for which it is not possible to infer the direction.	
Polygenic risk score (PGRS), also known as a genetic risk score, allele score, risk score, genetic score, genetic values, breeding values	A single variable that is produced by aggregating information from several <i>SNPs</i> that associate with a trait/phenotype and that can be used in prediction or causal analyses (i.e., <i>MR</i>). Usually, genetic risk scores tend to comprise <i>SNPs</i> that have been identified in <i>GWASs</i> at a <i>genome-wide significance</i> p-value threshold to include <i>SNPs</i> that are most robustly associated with a particular trait. PGRSs are increasingly being used and tend to include genetic information on the whole genome (or a much less stringent threshold of inclusion). In an unweighted PGRS, the number of 'effect' <i>alleles</i> each person in a study has are simply added together. For example, if a score was composed from 5 <i>SNPs</i> for a trait and an individual had <i>genotypes</i> of 0, 1, 1, 2 and 0, referring to the number of trait increasing <i>alleles</i> , their score	PGRSs have commonly been used in <i>one-sample MR</i> . They can also be generated and used in <i>two-sample MR</i> . In comparison to using each <i>genetic variant</i> as a separate <i>IV</i> , combining them into a single <i>IV</i> can increase <i>statistical power</i> but if some of the variants contributing to the score are invalid (i.e., violate <i>IV</i> assumptions) results are likely to be biased.	(40,41)

	<p>would be 4. Weighted scores multiply the number of 'effect' <i>alleles</i> by the magnitude (usually the beta) of the <i>GWAS</i> association value for each <i>SNP</i>. In the above example, if the differences in mean level of the trait per <i>allele</i> for each of the 5 <i>SNPs</i> were 0.5, 1.0, 0.5, 2.0 and 0.6, respectively, then the weighted score for the same individual would be $0 \times 0.5 + 1 \times 1.0 + 1 \times 0.5 + 2 \times 2.0 + 0 \times 0.6 = 5.5$. Weighted scores are also known as weighted <i>allele</i> scores (WAS).</p>		
Polymorphism	<p>A polymorphism is a <i>genetic variant</i>. The term is usually limited to variants that are moderately common (it is unusual for the term to be used when referring to <i>rare variants</i>).</p>	<p>Moderately common <i>genetic variants</i> tend to have modest effects on traits; therefore, the variance explained in that trait by common polymorphisms (including <i>SNPs</i>) is relatively small. Thus, <i>MR</i> studies using a small number of moderately common polymorphisms will require large sample sizes or the combination of many polymorphisms/</p>	(34)

		<i>genetic variants</i> (within a <i>polygenic risk score (PGRS)</i>) for adequate <i>statistical power</i> .	
Rare variants	Rare variants occur at low frequencies (usually defined as a <i>genetic variant</i> for which the rare <i>allele</i> occurs in <1% of a population). The majority of <i>genetic variants</i> analysed in <i>GWAS</i> are common <i>SNPs</i> due to the relative ease in assaying them. Improved <i>imputation</i> methods, and reduced costs of sequencing mean that more genetic associations will be found for rare variants in the future.	<i>Statistical power</i> to detect a genetic association chiefly depends on the sample size and the variance explained by the <i>genetic variant</i> . While the majority of genetic associations with common variants have very small effects, rare variants can only explain sufficiently large variation for detection if they have large effect sizes. The utility of rare variants in <i>MR</i> , therefore, is that they provide an opportunity to evaluate if the causal effect estimate increases linearly with larger intervention sizes.	(42,43)
Single nucleotide polymorphism (SNP)	<i>SNPs</i> are <i>genetic variants</i> in which one base in the <i>DNA</i> is altered, e.g., a G instead of an A in the	See <i>Allele</i> and <i>Genetic variant</i> above.	(34)

	<p>example above (see <i>Genotype</i> above). The term</p> <p>single nucleotide variant (SNV) is also widely used to</p> <p>include both <i>rare variants</i> and common</p> <p><i>polymorphisms</i>.</p>		
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Figure 1.1: Schematic representation of Chromosomes, DNA and genetic variants in a diploid cell. From Hartwig *et al.* (16), with permission.

Chapter 2: Definition and types of Mendelian randomization (MR)

Term	Brief definition	Summary assumptions / key sources of bias	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Bidirectional MR	An <i>MR</i> analysis that tries to differentiate whether factor A is a cause or a consequence of factor B or whether there is a true bidirectional causal effect between A and B.	<i>Genetic variants</i> for ‘risk factor’ A and ‘outcome’ B need to fulfil the same assumptions as <i>IVs</i> . This requires <i>genetic variants</i> for both variables (A and B) to test each of the three possibilities: A causes B; B causes A; there is a true bidirectional causal relationship between A and B. In comparing results in both directions, caution is required to take account of differences in <i>statistical power</i> related to the <i>IV</i> -risk factor association.	(44,45) Figure 2.1

Binary exposure MR	When <i>MR</i> is used to test the effect of a binary exposure, such as hypertension, obesity, or insomnia, results can also be difficult to interpret. At face value, the effect estimate is a ratio of a ratio. Additional considerations regarding violation of <i>IV</i> assumptions may be required in comparison to continuous exposures.	The <i>exclusion restriction</i> assumption may be violated by variation in the underlying or latent continuous trait (e.g., blood pressure or BMI), which will vary within the two categories (e.g., hypertensive versus normotensive). In <i>MR</i> analyses (as with multivariable analyses), one should consider whether dichotomising underlying continuous variables is appropriate. Where binary exposures are used, a cautious interpretation of the ‘genetic liability’ to an exposure might be warranted. Scaling the results, for example describing the effect estimate as a risk ratio (of a binary outcome) of difference in mean (continuous outcome) per doubling of the liability to exposure has been recommended.	(46-48)
Factorial MR	An <i>MR</i> method that explores the separate and joint causal effects of two (or potentially more) risk factors on an outcome and is analogous to factorial randomized controlled trial (RCT) designs.	Genetic <i>IVs</i> for the two (or potentially more) risk factors are the categories defined by <i>genetic variants</i> . These categories need to fulfil the key <i>IV</i> assumptions. Factorial <i>MR</i> may have limited <i>statistical power</i> (in	(22) Figure 2.2

	<p>Participants in a factorial <i>MR</i> study are categorised into different levels of each risk factor based on their genetic <i>IVs</i>. Where the genetic <i>IV</i> involves multiple <i>SNPs</i>, categories of high vs. low exposure might be defined by being above and below the median of a <i>polygenic risk score (PGRS)</i> of those <i>SNPs</i>. For a genetic <i>IV</i> that includes a <i>SNP</i>, the categorisation will be one group of <i>homozygotes</i> versus a group of the <i>heterozygotes</i> and other <i>homozygotes</i> together, or three groups of <i>homozygotes</i> for one <i>allele</i>, <i>heterozygotes</i>, and <i>homozygotes</i> for the other <i>allele</i>. Regression analyses are then conducted with the genetic <i>IV</i> categories.</p>	comparison to factorial RCTs and <i>MR</i> of single risk factors).	
Instrumental variable (IV)	<p>An IV is a variable (characteristic) that is robustly associated with the risk factor of interest and can be used to explore the unconfounded causal effect of that risk factor on an outcome, under the assumptions in the next column.</p>	<p>There are three core assumptions of IV analyses:</p> <ol style="list-style-type: none"> 1. The IV is robustly associated with the risk factor of interest (i.e., <i>replication</i> of its association has been demonstrated). In 	(30,49-52)

		<p>econometrics, this is known as the <i>relevance</i> assumption.</p> <p>2. The IV is not associated with confounders of the risk factor-outcome association. In econometrics, this is known as the <i>independence</i> or exchangeability assumption.</p> <p>3. The IV is only associated with the outcome through the risk factor. In econometrics, this is known as the <i>exclusion restriction</i> assumption.</p> <p>Additional assumptions are required to identify a well-defined causal parameter. These assumptions can be grouped as follows:</p> <ul style="list-style-type: none"> • <i>Homogeneity assumptions</i>: assuming either that the association between the IV and the exposure is homogeneous (i.e., is the same for everyone in the population) or that the effect of the exposure on the outcome is homogeneous. If true, this implies 	
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		<p>that the IV estimate is consistent with the average causal effect (ACE).</p> <ul style="list-style-type: none"> • No <i>effect modification</i>: if the IV does not modify the effect of the exposure on the outcome within levels of the exposure and for all levels of the exposure, then the IV estimate is consistent with the ACE even if the effect of the exposure on the outcome is heterogeneous. • Deterministic <i>Monotonicity</i>: often referred to simply as “<i>monotonicity</i>”, this assumes a monotonic relationship between the IV and exposure. In other words, a (genetic) IV could not increase the exposure in some people and decrease it in others. This allows the assumption that the effect of the IV on the exposure is homogeneous to be relaxed. If <i>monotonicity</i> holds, then the IV estimate is consistent with the ACE among compliers (under deterministic 	
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		<p><i>monotonicity</i>, this corresponds to the subgroup of the sample affected by the IV). This causal estimate does not straightforwardly apply to continuous exposures.</p> <ul style="list-style-type: none"> • <i>Stochastic Monotonicity</i>: this is a relaxation of deterministic <i>monotonicity</i>, because this only requires that a monotonic increasing association between the IV and the exposure exists conditionally on a set of covariates (which may or may not be measured). If this holds, then the IV estimate is consistent for a weighted average of treatment effects, such that more weight is given to treatment effects among subgroups where the effect of the IV on the exposure is greater. This assumption applies to both binary and continuous exposures but allows identifying an estimate that is less useful and harder to clearly define than in the ACE among compliers. 	
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Mendelian randomization (MR)	A method that uses <i>genetic variants</i> as <i>IVs</i> to explore causal effects of risk factors on outcomes in observational epidemiological studies.	Genetic <i>IVs</i> must fulfil the same assumptions as <i>IVs</i> . In most MR studies, the assumption that is most likely to be violated is the <i>exclusion restriction</i> assumption due to <i>horizontal pleiotropy</i> .	(1,30)
Multivariable MR	<i>MR</i> analyses in which genetic instruments for more than one exposure are used. Multivariable MR can be used to estimate mediating effects in <i>two-step MR</i> , to adjust for possible <i>pleiotropy</i> (e.g., to isolate specific lipid effects) bias due to <i>horizontal pleiotropy</i> of a specific effect, or to adjust for potential <i>confounding</i> .	All of the genetic instruments should fulfil the <i>IV</i> assumptions. In addition, each instrument should be associated with more than one of the exposures included in the analyses; each instrument does not need to be associated with all exposures but each one does need to be associated with more than one exposure. When using multivariable <i>MR</i> to adjust for <i>confounding</i> or <i>horizontal pleiotropy</i> , it is important to make sure that an exposure on the causal path between the primary exposure of interest is not being adjusted for.	(4,53,54) Figure 2.3
One-sample MR	<i>MR</i> analyses in which the genetic <i>IV</i> -risk factor association and genetic <i>IV</i> -outcome association are from the same sample.	Genetic <i>IVs</i> must fulfil the same <i>IV</i> assumptions. The <i>gene</i> -outcome model must be correctly specified, but the <i>gene</i> -exposure model can be miss-specified. One-	(45) Figure 2.4

		sample <i>MR</i> is more prone to data <i>overfitting</i> than <i>two-sample MR</i> . Though, <i>two-sample MR</i> may be similar to one-sample if there is overlap of participants in the two samples. In one-sample <i>MR</i> , <i>weak instrument bias</i> will tend to bias towards the confounded multivariable regression estimate.	
Two-sample MR	<i>MR</i> analyses in which the genetic <i>IV</i> -risk factor association and genetic <i>IV</i> -outcome association are generated from different (<i>non-overlapping</i>) samples. NOTE: there are 'grey areas' between <i>one-sample</i> and two-sample <i>MR</i> (e.g., where two samples have some overlap, as shown in Figure 2.5).	Genetic <i>IVs</i> must fulfil the same <i>IV</i> assumptions. The <i>gene</i> -exposure and <i>gene</i> -outcome models must be correctly specified. In two-sample <i>MR</i> , <i>weak instrument bias</i> will tend to bias towards the null. Correct harmonization of <i>alleles</i> across the two samples is essential. The two samples should be from the <i>same underlying population</i> . The two samples should be (largely) <i>non-overlapping</i> .	(17,45,55) Figure 2.5, Figure 2.6
Two-sample MR with individual participant data (IPD)	<i>Two-sample MR</i> in which IPD is available. NOTE: some <i>MR</i> studies use a mixture of aggregate and IPD (where the genetic <i>IV</i> -risk factor association	Assumptions and key sources of bias are as for <i>two-sample MR</i> . There is more potential (than with aggregate data) for assessing the genetic <i>IV</i> association with observed confounders of the risk factor-outcome	(17,45) Figure 2.5, Figure 2.6

	may be from aggregate data and the genetic <i>IV</i> -outcome association may be from IPD – or vice versa).	association, exploring interactions or subgroup effects and removing overlapping samples.	
Two-sample MR with summary (aggregate) data	<i>Two-sample MR</i> in which summary (aggregate) results are used for the genetic <i>IV</i> -risk factor and genetic <i>IV</i> -outcome associations. These are usually summary statistics from <i>GWASs</i> that are publicly available.	Assumptions and key sources of bias are as for <i>two-sample MR</i> . With aggregate data, it may be difficult to ensure the two samples are from the <i>same underlying population</i> . It is difficult to explore whether the genetic <i>IVs</i> are related to confounders of the risk factor-outcome association. It is not possible to detect <i>non-linear effects</i> unless there are aggregate data results for <i>non-linear effects</i> . Where the two samples overlap, the greater the overlap, the more the study properties become like <i>one-sample MR</i> .	(17,39) Figure 2.5, Figure 2.6
Two-step/Mediation MR	An <i>MR</i> framework that aims to quantify whether a risk factor-outcome association (that is either established or has supportive evidence from <i>MR</i> analyses) is mediated by another risk factor. In many published examples, initial <i>MR</i> will assess evidence for a causal	The <i>IV</i> assumptions must be met for the two steps of the <i>MR</i> analyses of the (i) risk factor on mediator and (ii) mediator on outcome. This methodology can be extended to networks of relationships (i.e., network <i>MR</i>).	(4,24,25,56) Figure 2.7

	<p>effect of the risk factor on an outcome and then go on to undertake two-step <i>MR</i>. First, genetic <i>IVs</i> associated with the risk factor are used to determine the causal effect of the risk factor on the potential mediator (step one). Secondly, genetic <i>IVs</i> associated with the potential mediator and independent of those used for step one are used to determine the effect of the potential mediator on the outcome of interest (step two). Where evidence is found for a causal effect of a risk factor on an outcome, a risk factor on a mediator and a mediator on an outcome, methods such as <i>multivariable MR</i> or product-of-coefficients can be used to explore the extent of this mediation.</p>		
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Figure 2.1: Bidirectional Mendelian randomization. Adapted from Richmond *et al.* (44).

Bidirectional MR involves exploring both possible directions of effect between two traits (e.g., between body mass index and physical activity) by undertaking two separate MR analyses (shown in (A) and (B)). In figure (A), a genetic instrument (Z_1) is used to assess the causal effect of the first trait (T_1 , physical activity) on the second trait (T_2 , body mass index). In figure (B), an independent genetic instrument (Z_2) is used to assess the reverse effect – i.e., a possible causal effect of body mass index on physical activity.

Figure 2.2: Factorial MR Mendelian randomization Adapted with permission from Carter *et al.* (23).

*In this example, Factorial MR is used to investigate the combined effects of BMI and alcohol on liver disease and liver disease biomarkers. Disease and biomarker levels are compared across the four categories of genetically instrumented combinations of low and high BMI and alcohol levels. Low and high BMI were defined as below or equal to and above the median of a weighted polygenic risk score (PGRS) for BMI, respectively. For low and high alcohol categories, a single candidate genetic instrumental variable (*ADH1B*) was used as an instrument and homozygotes for the allele associated with higher alcohol consumption (high) were compared to those who were heterozygotes or homozygotes for the allele associated with lower alcohol consumption.*

Figure 2.3: Multivariable Mendelian randomization. Adapted from Zheng *et al.* (32).

Multivariable MR uses multiple instruments ($Z_1, \dots, Z_n$) associated with multiple, potentially correlated exposures (e.g., X_1, X_2 and X_3) to jointly estimate the independent causal effect of each of the exposures on a particular outcome (Y). It can also be used to explore mediation following two-step MR.

Figure 2.4: One-sample Mendelian randomization

(A) MR relies on the following three core assumptions (see Chapter 2 for other assumptions): (1) the genetic variant(s) being used as an instrument (Z) is associated with the exposure (X); (2) the instrument is independent of measured and unmeasured confounders (U) of the association between the exposure (X) and outcome (Y); and (3) there is no independent pathway between the instrument (Z) and outcome (Y) other than through the exposure (X) – otherwise known as horizontal pleiotropy or the exclusion restriction assumption (see Chapter 4 for definition). (B) MR can be perceived as being analogous to a randomized controlled trial (RCT), whereby the random assortment of alleles at conception is equivalent to the randomization method with an RCT. This randomization process produces groups of individuals who differ with respect to the intervention (in the case of MR, genetic variation) and between which confounders are equally distributed. Therefore, any differences observed in the outcome of interest between these randomly allocated groups should be due to the exposure with which the genetic variant(s) are associated. (C) In the most simple of scenarios, the causal estimate of the association between the exposure (X) and outcome (Y) can be derived using the instrumental variable ratio method (otherwise known as the Wald ratio), where β_{IV} is the causal estimate derived from instrumental variable (i.e., MR) analyses, β_{YZ} is the association between the instrument (Z) and the outcome (Y) and β_{XZ} is the association between the instrument (Z) and the exposure (X) – see Chapter 5 – Chapter 8 for the variety of methods used to derive causal estimates.

Figure 2.5: Two-sample Mendelian randomization. Adapted from Hemani *et al.* (39)

(A) In two-sample MR, the associations of the instrument(s) with the exposure and outcome are derived from two independent (i.e., non-overlapping) samples. In this example, there are three SNPs acting as genetic IVs for the hypothetical exposure (i.e., SNP1, SNP2 and SNP3). (B) Manhattan plots showing the SNP-exposure estimates for each of the three SNPs are derived from a genome-wide association studies (GWAS) of the exposure variable. (C) The estimates of association between these three same SNPs and the outcome variable are then obtained from the outcome GWAS (results that are also depicted in a Manhattan plot). (D) Effects are harmonized to ensure that the 'effect' estimates in both the exposure and outcome GWASs correspond to the same allele (i.e., one that consistently either increases or decreases the exposure variable) for each SNP. (E) Once effects are harmonized, MR analyses can be performed. Visually, a scatter plot can be generated to represent the results, whereby the slope of the line is equivalent to the causal estimate. For example, the when using the inverse-variance weighted method, the intercept is held at zero (see Chapter 8 for two-sample methodology).

Figure 2.6: Relationship of one- and two-sample Mendelian randomization: populations and samples

In all examples, the green box represents the same underlying population from which samples are drawn; the black circles represent the samples and the text in these summarises the source of association of genetic instrument with exposure (β_{ZX}) and association of genetic instrument with outcome (β_{ZY}). In one-sample MR (A) where β_{ZX} and β_{ZY} are estimated within the same population, there may be over-fitting of the data because the predicted (by genetic IV) values of X are then used to predict Y in the same sample. In this study type, weak instrument bias will be expected to bias towards the confounded result. In one-sample MR, it is not necessary to have exposures measured on all sample participants. For expensive exposures, these could be measured in a subsample (B). The properties and sources of bias will be broadly similar to those in (A), where exposures are measured in all participants, but the likelihood of weak instrument bias may be greater. When β_{ZX} is obtained in a one-sample MR study but with external weights (i.e., the

association magnitudes taken from a GWAS to which the sample being used for the MR did not contribute), as shown in (C), over-fitting of the data is minimised. Ideally, in two-sample MR, both samples are drawn from the same underlying population but there is no overlap of participants between the two samples, as shown in (D). In this situation, data will not be over-fitted and any weak instrument bias would be expected to bias towards the null. As GWAS get larger, and with more cohorts contributing to them, the potential for overlap between samples in summary data two-sample MR becomes increasingly likely, as shown in (E). The more overlap there is between the two samples, the more effects of over-fitting and weak instrument bias become similar to those seen in one-sample MR. In figure (F), the two samples are drawn from two different underlying populations. This might occur when using MR for testing developmental origins, when β_{ZX} is estimated in pregnant women and β_{ZY} is estimated in their offspring. In that situation, it is important to consider (and ideally test) whether the β_{ZX} association in pregnancy is the same as in non-pregnant females and males (i.e., as in the offspring sample). Similarly, when using aggregate data in two-sample MR and when the outcome of interest can only occur in one sex (e.g., cervical or prostate cancer), ideally one would want aggregate β_{ZX} estimates to be sex-specific. If that is not possible, then drawing on other external evidence to consider the extent to which β_{ZX} is likely to be similar in females and males is important.

Figure 2.7: Two-step Mendelian randomization for exploring mediation

(A) In the first step of two-step MR, a genetic variant (Z_1) is used as an instrument for the exposure of interest (X) to estimate the causal impact of the exposure on a hypothesized mediator (M) of the association between the exposure (X) and outcome (Y). (B) In the second step, an independent (of Z_1) genetic variant (Z_2) is used as an instrument for the mediator (M) to establish the causal impact of the mediator (M) on the outcome (Y). If there is evidence for a causal effect of X on M and M on Y (as well as X on Y), the estimates from these two steps can be combined to provide evidence for or against the mediating role of a variable on the exposure-outcome effect using e.g., multivariable MR.

Chapter 3: Study designs and terminology related to MR

Term	Brief definition	Summary of strengths and limitations or other relevant comments	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Candidate gene study	Studies that assess the association of one or a small number of <i>genetic variants</i> with a trait. 'Candidate' <i>genes</i> in such studies are commonly selected based on hypothesised functional relevance for the trait being assessed. Candidate <i>genes</i> might then be used as <i>IVs</i> in <i>MR</i> studies testing the effect of that trait on an outcome.	<p>Potential limitations include:</p> <ul style="list-style-type: none"> • There is an assumed effect/robust association with the trait (risk factor of interest in <i>MR</i> analysis) but this is may not be the case if <i>replication</i> for the candidate <i>gene</i> has not been demonstrated in independent studies. • Single or a few genetic <i>IVs</i> might increase the likelihood of <i>weak instrument bias</i>. • It may not be feasible to use some <i>IV</i> methods, such as <i>MR-Egger</i> and <i>weighted median</i>-based methods, that provide different means of 	(57,58)

		<p>exploring instrument validity, with single or few genetic instruments.</p> <p>Potential strengths:</p> <ul style="list-style-type: none"> • Having knowledge of how genetic <i>IVs</i> function to influence the risk factor of interest can be valuable in terms of considering <i>IV</i> assumptions and the extent to which these are likely to be violated. 	
Directed acyclic graph (DAG)	<p>A DAG is a visual representation of potential causal relationships between variables. These relationships are demonstrated by nodes (representing variables) and arrows (a.k.a. edges or arches) between variables. The relationship between two variables in each DAG must be directed (i.e., there cannot be a bidirectional relationship). Relationships between all variables must be acyclic (i.e., a variable cannot have an</p>	<p>DAGs are useful tools for explicitly demonstrating the underlying assumptions of a proposed analysis. Arrows are drawn between any two variables according to the following criteria:</p> <ul style="list-style-type: none"> • An arrow from one variable to a second indicates that you assume that it is plausible that the first variable causes the second. • Where there is no arrow between one variable and a second, this indicates that you assume 	<p>(59)</p> <p>Figure 2.1, Figure 2.3, Figure 2.4, Figure 2.5A, Figure 2.7, Figure 4.1 and Figure 4.2 are examples of DAGs</p>

	<p>impact on itself through any number of other variables).</p>	<p>that there is no causal relationship between the first and second variable.</p> <p>Thus, the three key assumptions of <i>IV</i> analyses are illustrated by an arrow from the <i>IV</i> to exposure; an absence of an arrow from the <i>IV</i> to confounders of exposure and outcome; and an absence of an arrow from the <i>IV</i> directly to the outcome.</p>	
<p>Gene-Environment (G×E) interaction study</p>	<p>Studies that assess interaction between (typically) one <i>genetic variant</i> and a non-genetic factor with respect to a given outcome. These can be useful in several <i>MR</i> contexts. For example, some <i>genetic variants</i> for smoking, alcohol and other risk factors act on a metabolite or mechanism that is caused by the risk factor but does not directly affect levels of the risk factor. In these circumstances, if the risk factor causes the outcome of interest, we would only expect the <i>genetic variant</i> to associate with the outcome in</p>	<p>Sometimes, G×E can be used to investigate mechanisms. For example, if the <i>genetic variant</i> blocks the postulated mediating mechanism from the exposure to the outcome, then the exposure would not be expected to be associated with the outcome among carriers of the mediator-blocking <i>genotype</i>. G×E can be exploited to mitigate bias due to <i>horizontal pleiotropy</i> in <i>MR</i>. Adequate <i>statistical power</i> typically requires very large samples. Many published studies did not properly adjust for potential confounders.</p>	<p>(60,61)</p>

	<p>those who have ever smoked / drunk alcohol or been exposed to the risk factor. In those who have never been exposed, we would not expect an association between the <i>gene</i> and the outcome.</p> <p>Thus, a $G \times E$, where E is ever versus never exposure, would provide <i>MR</i> evidence for a causal effect.</p>		
Genetic colocalization	<p>The phenomenon whereby genetic factors at a particular <i>locus</i> are shared between two or more traits (not to be confused with declaring the exact causal variant). Tests for genetic colocalization try to separate between two scenarios: (i) there is a causal variant for trait A that is distinct from the causal variant for trait B, whilst being at the same <i>locus</i>, and (ii) the causal variant for trait A and trait B are shared. A variety of algorithms exist for distinguishing between these scenarios, typically</p>	<p>Genetic colocalization of a <i>locus</i> between two traits is necessary but not sufficient for a causal relationship, and it can be thought of an <i>MR</i> analysis performed with a <i>SNP</i> but with an added sensitivity analysis in which we are trying to rule out the possibility that there are two distinct causal variants in the same region. The approach is regularly used to infer putative causal relationships between 'omic variables and complex traits. Often an assumption of these</p>	(62-64)

	by looking for concordance of effects across all <i>SNPs</i> at the <i>locus</i> at both traits.	algorithms is that there is only one causal variant in the region, which may not be appropriate.	
Genome-wide association studies (GWAS)	<p>GWASs are hypothesis-free study designs in which a panel of (hundreds of thousands or millions) of <i>genetic variants</i> are each systematically tested for association with a single trait or disease outcome. GWASs have the primary objectives of identifying specific variants that can be used for prediction of the trait, and for highlighting <i>gene</i> or <i>loci</i> that are relevant to the aetiology of the trait or disease. Most genetic effects are small, so GWASs need to be performed using large biobanks or collaborations between many studies in which the GWAS results from all studies are meta-analysed. Control for <i>confounding</i> due to population structure, the use of strict significance thresholds and <i>replication</i> of findings in independent samples are key features of reliable GWASs. Commonly,</p>	<p>Potential limitations:</p> <ul style="list-style-type: none"> • <i>Genome-wide significance</i> is based on a Bonferroni correction that leads to small genetic effect estimates being biased upwards due to <i>winner's curse</i>. The use of these ('discovery') effect estimates in <i>MR</i>, rather than those obtained from independent <i>replication</i> studies can lead to biased effect estimates. • The function of <i>genetic variants</i> identified from GWASs (known genome-wide hits) may be unknown. Particularly for complex traits (such as BMI), where there is likely to be a chain of effects or associations from the <i>gene</i> to the trait, there could be a strong potential for <i>horizontal pleiotropy</i>. 	<p>(65-67)</p> <p>GWAS catalogue</p> <p>www.ebi.ac.uk/gwas/</p>

	<p>GWAS results are visualised using a Manhattan plot, which shows the $\log_{10}(\text{p-value})$ of each <i>SNP</i> by the location of that <i>SNP</i> in the genome by <i>chromosome</i>. All variants that associate with the trait at <i>genome-wide significance</i> are then commonly used as genetic instruments in <i>MR</i> studies.</p>	<ul style="list-style-type: none"> • With sample sizes growing ever larger, the risk of subtle <i>population stratification</i> or <i>dynastic effects</i> leading to false positive or biased GWAS findings is growing. <p>Potential strengths when used to identify genetic <i>IVs</i>:</p> <ul style="list-style-type: none"> • Most GWAS will highlight <i>genetic variants</i> that replicate in (large) independent studies. • Where several variants are identified as potential instruments for a trait, several statistical methods each with differing assumptions can be used and <i>triangulation</i> of results across them. 	
Genome-wide significance	<p>Genome-wide significance is widely defined as having a statistical association in which the p-value $\leq 5 \times 10^{-8}$ in a <i>GWAS</i>.</p>	<p>As with all p-value thresholds for ‘statistical significance’, the value is arbitrary. The threshold of $\leq 5 \times 10^{-8}$ was a Bonferroni correction of the commonly used $p \leq 0.05$, based on the estimated number of independent tests in the <i>GWAS</i> if all</p>	(68,69)

		<p>common <i>SNPs</i> in the first version of HapMap were tested with direct genotyping or <i>imputation</i> (i.e., $0.05/10^6$). More stringent thresholds should be used if the study also included <i>rare variants</i>. The major feature of a reliable genetic association; however, is <i>replication</i> in an independent sample.</p>	
Imputation of genetic variants in GWAS	<p>Genotyping assays are a cost-effective way of recording <i>genotypes</i> on hundreds of thousands of positions across the genome and across many individuals. However, there are in excess of 10 million common <i>genetic variants</i> in human populations and drastically more <i>rare variants</i>. This can be treated as a missing data problem, where there are a few hundred thousand known variables and many million unknown variables in a dataset. Genetic imputation makes probabilistic inference of the unknown variables by matching</p>	<p>Jointly analysing <i>GWAS</i> results from multiple cohorts is key to improving sample sizes to the point that small effects can be detected at stringent <i>genome-wide significance</i> levels. However, different cohorts often use different genotyping platforms, which means that combining the data in totality is not possible. Genetic imputation has been crucial for enabling very large <i>GWAS</i> sample sizes because if each cohort imputes to the same reference panel then they then have a set of common variables across the genome. Genetic imputation is now very</p>	(70)

	<i>haplotypes</i> within the sample with <i>haplotypes</i> from a sequenced reference dataset.	effective at inferring common variants, but <i>rare variants</i> tend to have low imputation accuracy.	
Replication	Refers to demonstrating consistency of results between independent studies. It is one method for guarding against the promotion of ‘false’ results. In relation to <i>MR</i> , <i>genetic variants</i> that are used as <i>IVs</i> should be shown to have consistent results across two (or ideally more) independent studies.	Replication has commonly been defined as associations across studies being ‘statistically significant’. This assumes similar <i>statistical power</i> across studies and showing consistency of direction and magnitude of association might also be valuable.	(71)
Triangulation	Triangulation, in epidemiology, refers to integrating and comparing results from several studies (or several different methods applied to the same study data) that use different approaches to test causal effects, where each approach has different (and unrelated) key sources of bias. Ideally with the different key sources of bias in different studies resulting in bias in different directions.	If results from <i>MR</i> ‘triangulate’ with those from other approaches (with different key sources of bias), this increases confidence that those similar results are the correct causal effect. The use of different <i>MR</i> methods (with different assumptions and key sources of biases) can also be valuable to increase confidence in <i>MR</i> results, where consistent results across methods are observed.	(72)

Chapter 4: Terms commonly used when describing sources of bias or limitations in Mendelian randomization and specific situations where Mendelian randomization may have additional limitations

Term	Brief definition	Comments on relevance to MR / how these might be explored	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Assortative mating	Occurs when people choose their partners based on particular characteristics, such as height, intelligence, rather than at random. Single-trait assortative mating is characterised by assortment on a single trait (e.g., taller women tend to partner with taller men; mid-height men tend to mate with shorter women). Cross-trait assortative mating occurs when, for example, men with high values of one trait tend to partner with women with either higher or lower values of another	Cross-trait assortment on the exposure and outcome leads to a spurious genetic correlation between parents, which renders the exposure and outcome phenotypes genetically correlated in the offspring. In some cases, single-trait assortative mating can lead to the same phenomenon. Such spurious genetic correlation can lead to bias in <i>MR</i> . If available parent's genetic data can be used to detect, and adjusted for, any bias from assortative mating.	(26,73)

	trait (e.g., more educated men tend to partner with taller women).		
Canalization	The process by which potentially disruptive influences on normal development from genetic (and environmental) exposures are damped or buffered by compensatory developmental processes. This results from animals (including humans) being relatively 'plastic' during development periods. For example, hypothetically fetuses with <i>genetic variants</i> that result in on average higher glucose levels might develop other compensatory systems in such a way that higher glucose levels do not adversely affect them.	It is not directly possible to determine. If canalization has occurred, then genetic <i>IVs</i> may still relate to the risk factor by the same magnitude (as in the absence of any canalization) but the effect on a potential outcome of that risk factor could be dampened. Biological evidence for canalization having a marked impact on genetic associations is lacking. This will not impact <i>MR</i> results when assessing intrauterine effects (i.e., via maternal genetic <i>IVs</i> for maternal pregnancy risk factors) on offspring outcomes, as the mother's <i>genotype</i> will not have been influenced by canalization in the offspring (though this has additional potential problems – see <i>MR for testing developmental origins</i> below).	(1,30)
Collider	In an epidemiological setting, a collider is a variable that is caused by both the exposure and the outcome	Adjusting for a collider in any epidemiological setting can induce an association between the exposure and	(59) Figure 4.1

	of interest. The term ‘collider’ refers to the fact that, in a <i>DAG</i> , arrow heads showing directional associations from the two exposure and outcome variables <i>collide</i> into a third variable (the collider).	outcome (see <i>collider bias</i>). In an <i>MR</i> context, <i>collider bias</i> can be induced if adjusting for a collider (e.g., the exposure would be a collider of the genetic instrument and <i>confounding</i> factors).	
Collider bias	Collider bias occurs when a model is adjusted for a <i>collider</i> or the descendant of a <i>collider</i> . This can occur when there is selection bias (e.g., because of a very low response into the study, loss to follow-up or missing data). It can also occur when analyses are restricted to subgroups of a population, such as the Nurse’s or Physician’s Health studies, other occupational based cohorts, or when studying people with a specific disease (e.g., when looking at the effect of a risk factor or treatment on prognosis/disease progression). The resulting bias in the exposure-outcome causal effect estimate can be in either direction, can mask an effect so that it falsely appears null, or can induce an effect when none exists.	In <i>MR for testing developmental origins</i> /intrauterine effects, adjustment for potential violation of <i>IV</i> assumptions via fetal <i>genetic variants</i> by adjusting for the fetal <i>genetic variants</i> can generate a spurious association between mother’s and father’s <i>genetic variants</i> (because maternal and paternal <i>genetic variants</i> collide on fetal/offspring <i>genetic variants</i>). If it is not possible to adjust for paternal <i>genetic variants</i> (this is often the case) and the paternal phenotype affects the offspring outcome, then the <i>MR</i> result is likely to be biased. New methods for separating maternal and fetal genetic effects and availability of genetic data on trios can be used to mitigate this. <i>MR</i> in people with diseases to explore its progression or	(26,27,59,74,75) Figure 4.1

		<p>results to treatments may be affected by collider bias.</p> <p>For example, if a hypothesised risk factor and disease progression both influence being diagnosed or selected into the study, they are colliding on study selection and this will generate a spurious association between them. The extent to which this has a major effect in different studies is a subject of active research. In <i>two-sample MR</i>, where summary data have been adjusted (e.g., the <i>GWAS</i> of waist-hip ratio adjusted for BMI) the use of these adjusted data can introduce collider bias.</p>	
Confounding	<p>The traditional definition of a confounder is that:</p> <ul style="list-style-type: none"> i) It is associated with the exposure ii) It is associated with the outcome conditional upon the exposure iii) It is not on the causal pathway between the exposure and outcome. 	<p>Associations of <i>genetic variants</i> with outcomes are generally less prone than associations of non-genetic characteristics to confounding by many socio-demographic, lifestyle and clinical characteristics from across the lifecourse. The '<i>independence</i>' <i>IV</i> assumption states that <i>IVs</i> are not associated with confounders of the risk factor-outcome association. In</p>	(26,76)

	<p>However, because this set of rules does not generalize to complex modelling structures, the modern interpretation uses Pearl's directional-separation rules. In this context, the modern definition of confounding is the existence of an open “backdoor pathway” between the exposure and outcome - where one or more characteristics influence the risk factor and outcome of interest and generate a spurious (confounded) association between risk factor and outcome. Confounding can generate a positive or negative association when there is no causal effect of risk factor on outcome and can exaggerate or attenuate a true causal effect.</p>	<p><i>MR</i> studies, demonstrating that genetic <i>IVs</i> are not related to observed risk factor-outcome confounders is important (though we cannot show they are not related to unobserved confounders). In <i>two-sample MR with summary (aggregate) data</i>, it may not be possible to explore associations with observed risk factor-outcome confounders in the outcome sample (sample 2). However, summary-level data from <i>GWAS</i> for any known or hypothesised confounders may be available and if so these should be used to explore whether there are associations with the risk factor-outcome confounders.</p>	
Dynastic effects	<p>Dynastic effects are the potential for <i>MR</i> causal estimates to be confounded by characteristics that are transmitted across generations. For example, an <i>MR</i> study of the effect of height on coronary heart disease might be biased by a dynastic effect through a</p>	<p>It is difficult to detect the magnitude of any bias resulting from this. It is less likely to occur in <i>MR</i> studies of ‘own’ risk factors (as opposed to intrauterine exposures from maternal pregnancy risk factors), that are not ‘visible’ and, hence, not likely</p>	(26,77)

	<p><i>confounding</i> path linking genetic instruments for height to coronary heart disease via the correlation between own and mother's height <i>genes</i> and the possibility that maternal height influences fetal growth and development in utero, which influences future offspring (own) heart disease.</p>	<p>copied from parents by offspring. <i>Triangulation of MR</i> findings with those from other methods that have different and unrelated potential causes of bias can help improve confidence about whether <i>MR</i> results are markedly biased by these effects.</p>	
<p>(no) Effect modification assumption) (Additional IV assumption)</p>	<p>This is one of a set of additional (to the core) <i>IV</i> assumptions that are required for a well-defined causal parameter.</p>	<p>If the genetic <i>IV</i> does not modify the effect of the exposure on the outcome within levels of the exposure and for all levels of the exposure, then this assumption holds and <i>IV</i> estimate is consistent for the ACE even if the effect of the exposure on the outcome is heterogeneous (see <i>Homogeneity</i> assumption). As noted in Chapter 3, when there is biological understanding, a <i>Gene-Environment (G×E) interaction study</i> can provide evidence of causality. In more general, <i>MR</i> studies biological evidence may provide evidence against effect modification being plausible.</p>	<p>(50-52) <i>See Instrumental variable (IV)</i> above.</p>

		<p><i>Monotonicity</i> (a weaker assumption) might be used but for some <i>MR</i> causal estimates. It is difficult in practice to know how important violation of these additional <i>IV</i> assumptions are in <i>MR</i> studies. Large <i>GWAS</i> collaborations increasingly combine results from many studies (though mostly from European original populations) and show consistency of association across these studies for variants defined as being associated with the exposure at <i>genome-wide significance</i> (variants used in most <i>MR</i> studies), suggesting homogeneity may exist for several <i>MR</i> studies in European populations. <i>Non-parametric methods</i> that provide bounds of causal effect estimates requiring only the core <i>IV</i> assumptions may be applicable for some <i>MR</i> studies <i>Triangulation</i> of results with other (non-<i>MR</i>) methods is likely to improve causal inference from <i>MR</i> studies.</p>	
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Exclusion restriction (IV3 core assumption)	Known as the third <i>IV</i> assumption, this states that the <i>IV</i> is not related to the outcome other than via the risk factor of interest.	In general, <i>MR</i> this may be violated by <i>horizontal pleiotropy</i> . In <i>MR</i> exploring effects of maternal (or paternal) exposure on offspring, this may be violated by fetal (offspring <i>genotype</i>) (see also <i>MR for testing developmental origins</i>).	(26,29,30,78) See <i>Instrumental variable (IV)</i> above.
Harmonization failure (in two-sample MR)	When one or more <i>genetic IVs</i> have different effect <i>allele</i> between exposure and outcome datasets of summary association results. This is of greater concern for <i>palindromic</i> variants with high minor <i>allele</i> frequency, especially when strand issues are a possibility (Figure 1.1).	To overcome errors due to harmonization, it is advised to harmonize using automated scripts that have been thoroughly tested (for example, those used in <i>MR-Base</i>) and check the correlation between effect <i>allele</i> frequencies before and after harmonizing. It is also useful to provide pre- and post-harmonization datasets to allow assessment of the quality of the harmonization and perform sensitivity analysis to evaluate the influence of variants difficult to harmonize (e.g., <i>palindromic</i> variants with high minor <i>allele</i> frequency).	(16,17,39)

Homogeneity Assumption	This is one of a set of additional (to the core) <i>IV</i> assumptions that are required for a well-defined causal parameter.	<p>The assumption is that either:</p> <ul style="list-style-type: none"> the association between the genetic <i>IV</i> and the exposure is homogeneous (i.e., is the same for everyone in the population) or the effect of the exposure on the outcome is homogeneous. <p>If this assumption holds then the <i>IV</i> estimate is consistent with the average causal effect (ACE) for the population to which inference is being made.</p> <p>Increasing biological knowledge of <i>genetic variant</i> functionality may provide evidence for some genetic instruments fulfilling this assumption. 'Weaker' assumptions (no <i>effect modification</i> and <i>monotonicity</i>) can alternatively be evoked. It is difficult in practice to know how important violation of these additional <i>IV</i> assumptions are in <i>MR</i> studies. Large <i>GWAS</i> collaborations increasingly combine results from many studies (though mostly from European original</p>	<p>(50-52)</p> <p>See <i>Instrumental variable (IV)</i> above.</p>
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		<p>populations) and show consistency of association across these studies for variants defined as associated with the exposure at <i>genome-wide significance</i> (variants used in most <i>MR</i> studies), suggesting homogeneity may exist for several <i>MR</i> studies in European populations. <i>Non-parametric methods</i> that provide bounds of causal effect estimates requiring only the core <i>IV</i> assumptions may be applicable for some <i>MR</i> studies. <i>Triangulation</i> of results with other (non-<i>MR</i>) methods is likely to improve causal inference from <i>MR</i> studies.</p>	
Horizontal Pleiotropy (a key way in which <i>MR</i> may violate the exclusion restriction assumption)	Also known as “genuine” or “true” <i>pleiotropy</i> . This is when a <i>genetic variant</i> affects other traits which influence the outcome independently of the hypothesised exposure.	This can result in biased <i>MR</i> estimates because of violation of the <i>exclusion restriction</i> assumption. For example, if some or all of the genetic <i>IVs</i> that robustly associate with the risk factor of interest also, independently of the association with the risk factor, associate with other risk factors for the outcome, then the <i>MR</i> estimate will be the combined effect of all of	(32,45) Figure 2.4, Figure 4.2

		these (independent) risk factors – not the effect of the risk factor of interest alone. Methods such as <i>MR-Egger</i> , <i>weighted median</i> - and <i>mode-based MR</i> methods have been developed to explore and account for the impact of horizontal <i>pleiotropy</i> in <i>MR</i> studies (see Chapter 4). This can also be informative about the trait's aetiology (see <i>MR-TRYX</i>).	
Independence (IV2 assumption)	Known as the second <i>IV</i> assumption (and sometimes also referred to as the “exchangeability” assumption) this states that <i>IVs</i> are not associated with confounders of the risk factor-outcome association	As <i>genetic variants</i> are determined at conception it is not possible for them to be affected by confounders of risk factor-outcome associations, but they could influence these confounders. If they do and these confounders are not measured / not controlled for in the <i>MR</i> analysis then a path from the genetic <i>IV</i> via these confounders could bias the causal effect estimate. As noted above under <i>confounding</i> in both <i>one-sample</i> and <i>two-sample MR</i> , associations of the genetic <i>IV</i> with potential confounders should be	(52,76)

		undertaken and methods such as <i>multivariable MR</i> used to control for these where possible.	
InSIDE assumption (in Two-sample MR using aggregate data)	The Instrument Strength Independent of Direct Effect (InSIDE) assumption a further assumption for <i>MR-Egger</i> and related <i>MR</i> methods (additional to core and additional <i>IV</i> assumptions outlined in Chapter 2).	The assumption states that the association between the genetic instrument and exposure is not correlated with path from the genetic instrument to the outcome that is independent of the exposure of interest. If unbalanced <i>pleiotropy</i> is present and the InSIDE assumption is violated, then this is likely to result in a biased <i>MR-Egger</i> effect. Violation of the InSIDE assumption can occur if several <i>genetic variants</i> influence the outcome via the same pleiotropic path or if several variants are related to the same risk factor-outcome (unmeasured) confounder(s), as then the genetic instrument-exposure association is in part via that (those) confounders and it is also related to the outcome via the same confounder(s).	(15,79)

Monotonicity (IV4 assumption)	This is one of a set of additional (to the core) <i>IV</i> assumptions that are required for a well-defined causal parameter.	<p>Two types of monotonicity may be inferred to make causal inference in <i>IV</i> (including <i>MR</i> studies)</p> <ul style="list-style-type: none"> • Deterministic monotonicity: often referred to simply as “monotonicity”, which assumes a monotonic relationship between the <i>IV</i> and exposure. In other words, a genetic <i>IV</i> could not increase the exposure in some people and decrease it in others. If this monotonicity holds, then the <i>IV</i> estimate is consistent with the ACE among compliers, where compliers are the subgroup of the sample affected by the genetic <i>IV</i>. Defining who this subgroup might be is unclear. • Stochastic monotonicity: this is a relaxation of deterministic monotonicity, because this only requires that a monotonic increasing association between the <i>IV</i> and the exposure exists conditionally on a set of covariates (which may or may not be measured). If this holds, then the <i>IV</i> 	<p>(50-52)</p> <p>See <i>Instrumental variable (IV)</i> above.</p>
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		<p>estimate is consistent with a weighted average of treatment effects, such that more weight is given to treatment effects among subgroups where the effect of the <i>IV</i> on the exposure is greater. This assumption applies to both binary and continuous exposures but allows identifying an estimate that is less useful than the ACE among compliers.</p> <p>It is difficult in practice to know how important violation of these additional <i>IV</i> assumptions are in <i>MR</i> studies. Large <i>GWAS</i> collaborations increasingly combine results from many studies (though mostly from European original populations) and show consistency of association across these studies for variants defined as being associated with the exposure at <i>genome-wide significance</i> (variants used in most <i>MR</i> studies), suggesting homogeneity may exist for several <i>MR</i> studies in European populations. <i>Non-parametric methods</i> that provide bounds of causal effect estimates</p>	
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		requiring only the core <i>IV</i> assumptions may be applicable for some <i>MR</i> studies. <i>Triangulation</i> of results with other (non- <i>MR</i>) methods is likely to improve causal inference from <i>MR</i> studies.	
MR for testing critical or sensitive periods	As <i>genetic variants</i> are fixed at conception, in general, they instrument a life-course predisposition to a particular risk factor. This makes <i>MR</i> less prone to <i>regression dilution bias</i> , but such <i>genetic variants</i> might be unsuited to testing the effects of risk factors that are believed to act during critical (a time period in which the risk factor is hypothesised to solely have its effect) or sensitive (a period during which the risk factor is hypothesised to have the greatest effect) periods.	If the research question is about determining an effect in a critical or sensitive period (i.e., showing an exposure only affects an outcome when it occurs during a specific age range and not at other life times or has a stronger effect when it occurs during a specific time of life), <i>MR</i> may not be a useful method as the result it will provide is the mean effect of the exposure across all/most of the life course. As <i>GWASs</i> of trajectories (e.g., change in weight or height across infancy childhood and into early adulthood) are undertaken, they may provide genetic <i>IVs</i> that could be used in <i>MR</i> to compare change in an exposure between two ages with change in the same exposure between two later ages and compare these to see if	(26,72)

		<p>there are differences in the effect at different ages.</p> <p>This is likely to need very large sample sizes.</p>	
MR for testing developmental origins	<p>The Developmental Origins of Health and Disease (DOHaD) hypothesis suggests that risk factors during intrauterine or early infancy development periods have a causal effect on future offspring health. <i>MR</i> has been used to test intrauterine effects by using genetic instruments for maternal risk factors during pregnancy and exploring their effects on offspring outcomes.</p> <p>Note: DOHaD does not necessarily assume a <i>critical or sensitive periods</i> but rather that exposures in utero are hypothesised to have a lasting effect. Some DOHaD hypotheses do propose that an exposure only has an effect during the intrauterine period (or that any exposures has a stronger effect then); for those the considerations for <i>MR for testing critical or sensitive periods</i> apply.</p>	<p>As <i>genetic variants</i> are usually identified from <i>GWAS</i> of men and (non-pregnant) women, it is assumed that these relate to the risk factor in women during pregnancy in the same way as they do in the <i>GWAS</i> populations. This should be explored where possible.</p> <p>Offspring <i>genetic variants</i> are a potential source of violation of the <i>exclusion restriction</i> but adjustment for offspring <i>genotype</i> can introduce a spurious association between maternal and paternal <i>genotype</i> (i.e., <i>collider bias</i>). If there is no information on paternal <i>genotype</i> (so it cannot be adjusted for), this can bias the causal effect of maternal pregnancy risk factor on offspring outcome. Adjustment for offspring and paternal <i>genotype</i> (where available), non-transmitted <i>allele</i> analyses, structural-equation</p>	(26,27)

		modelling approaches and simulation studies can help explore the extent of bias via offspring <i>genotype</i> .	
Non-linear effects	Most <i>MR</i> studies assume a linear (dose-response) effect of the risk factor on outcome. Methods for exploring non-linear effects in <i>one-sample</i> and <i>two-sample MR</i> have been developed but require very large numbers to be adequately powered.	There may be lack of <i>statistical power</i> to detect non-linear effects.	(80,81)
Non-overlapping samples (in two-sample MR)	The strengths of <i>two-sample MR</i> in terms of <i>weak instrument bias</i> being towards the null and minimising, <i>overfitting</i> of the data assume that the two samples are completely independent of each other (i.e., there is no overlap of participants between the two samples).	In practice, when aggregate data from publicly available data are used, it may not be possible to determine whether there is overlap between samples. This is because many <i>GWASs</i> are conducted on consortia of many studies. Thus, there is potential for overlap between some of the studies that contribute to these consortia (such as the GIANT consortia <i>GWAS</i> of BMI, waist-hip ratio and height, and the DIAGRAM consortia <i>GWAS</i> of diabetes). Careful reading of consortia websites and supplementary material should be undertaken to determine which studies contribute	(16,17) Figure 2.6

		to each of the ‘samples’ used for the genetic <i>IV</i> -risk factor and genetic <i>IV</i> -outcome samples and, if possible, sensitivity analyses undertaken with overlapping studies removed.	
Overfitting	This refers to exaggerated (bias away from the null) results occurring when <i>MR</i> studies are done within the same <i>GWAS</i> from which the genetic <i>IVs</i> are selected.	Ideally, selecting genetic <i>IVs</i> from the same study in which the <i>MR</i> analysis is being conducted should be avoided to reduce this overfitting. Using weighted <i>polygenic risk score (PGRS)</i> in <i>one-sample MR</i> where the weights of the genetic <i>IV</i> -exposure association are taken from an independent <i>GWAS</i> minimises bias due to over fitting. <i>Two-sample MR</i> , where genetic <i>IV</i> -outcome associations are obtained from an independent <i>GWAS</i> (i.e., to the <i>GWAS</i> of the exposure) and there is little or no overlap between the two samples avoids overfitting.	(30,32) Figure 2.6
Pleiotropy	Refers to the potential for <i>genetic variants</i> (including <i>SNPs</i>) to associate with multiple phenotypes.	The relevance of a <i>SNP</i> being pleiotropic to an <i>MR</i> study is context specific. If the <i>SNP</i> is pleiotropic because it influences the outcome only through an	(32,45) Figure 4.2

		<p>exposure (trait A), this is known as <i>vertical pleiotropy</i> and is the exact phenomenon that <i>MR</i> seeks to detect.</p> <p>The same variant could associate with another exposure (trait B) while only influencing the outcome through (trait A). In the <i>MR</i> analysis of trait B on the outcome, the variant is exhibiting <i>horizontal pleiotropy</i> resulting in violation of the <i>exclusion restriction</i> and, hence, bias of the <i>MR</i> effect estimate.</p>	
Population stratification	<p>Population stratification occurs when there exist population subgroups that experience both different phenotypic distributions and have different <i>allele</i> frequencies for <i>genetic variants</i> that might be used in <i>MR</i>. This can result in spurious (confounded) associations between <i>genotype</i> and phenotype (i.e., a trait that might be used as either the risk factor or outcome in <i>MR</i>).</p>	<p>If genetic <i>IVs</i> used in an <i>MR</i> study were derived from <i>GWAS</i> in a population in which there were sub-groups (e.g., different ethnicities) that differed with respect to the distributions of both the genetic <i>IVs</i> and the phenotype (risk factor of interest in the <i>MR</i> study), then the genetic <i>IV</i>-risk factor association could be biased by <i>confounding</i>. If the genetic <i>IV</i>-outcome associations were determined in studies in which there were sub-groups with differing genetic and outcome distributions, the genetic <i>IV</i>-outcome</p>	(30)

		association could be biased by <i>confounding</i> . Most <i>GWASs</i> and <i>MR</i> studies try to minimise this by including homogeneous groups of participants (e.g., of just one ethnicity) and/or by adjusting for genetic principal components that reflect different sub-groups within that population sample.	
Regression dilution bias (attenuation by errors)	Refers to the expected attenuation towards the null of an association due to random measurement error in a risk factor. This is particularly likely to occur with risk factors that have naturally and widely fluctuating levels, such as blood pressure and (non-fasting) glucose.	Because <i>genetic variants</i> instrument for lifetime (or long-term) levels of a risk factor, regression dilution bias is less likely in <i>MR</i> studies compared with other methods, such as multivariable regression, commonly used with observational data.	(1,30)
Relevance (IV1 assumption)	This is the first of the core <i>IV</i> assumptions which states that the <i>IV</i> must be associated with the risk factor of interest.	In <i>MR</i> the aim is to select <i>genetic variants</i> that have been shown to be robustly associated with the risk factor of interest. In most <i>MR</i> studies, this means <i>genetic variants</i> that are associated with an exposure at <i>genome-wide significance</i> and replicated in several independent study samples from the population of	(29,30)

		interest. The statistical strength of the genetic instrument – risk factor association if ‘weak’ can result in <i>weak instrument bias</i> .	
Reverse causality	The phenomenon where an association in the direction of a hypothesised causal relation between a risk factor and outcome is observed but is due (at least in part) to (potentially undiagnosed or precursors of) prevalent outcome influencing the risk factor.	In the presence of valid genetic <i>IVs</i> for both the ‘risk factor’ and ‘outcome’, this can be tested in <i>bidirectional MR</i> .	(1,30) Figure 2.1
Same underlying population (in two-sample MR)	It is important that the genetic <i>IV</i> -risk factor and genetic <i>IV</i> -outcome associations in <i>two-sample MR</i> are from the same underlying population or, at a minimum, there is evidence that the <i>gene IV</i> -risk factor association is similar in the population used for the second sample (genetic <i>IV</i> -outcome association).	Many <i>GWAS</i> that are used to identify genetic <i>IVs</i> for <i>MR</i> (and for the genetic <i>IV</i> -risk factor associations) are conducted in women and men combined. If these findings are then combined with genetic <i>IV</i> -outcome associations in women only (for example, if the outcome of interest were breast or ovarian cancer), the assumption is that the genetic <i>IV</i> -risk factor association does not differ between women and men. Ideally, we would want to have the two samples from the same underlying population. If this is not possible	(16,17) Figure 2.6

		(e.g., because aggregate data being used and the populations differ in the two samples), some attempt should be made to find some data that provides evidence that the genetic <i>IV</i> -risk factor association is similar in the population used for the genetic <i>IV</i> -outcome association, as in the original <i>GWAS</i> .	
Statistical power/efficiency	In many situations, <i>MR</i> will be less biased than conventional multivariable regression (or similar) approaches to exploring causal associations with observational data but will have less statistical power.	Large consortia used for <i>MR</i> studies and the increasing availability of publicly available summary statistics from <i>GWAS</i> that can be used in <i>two-sample MR</i> can provide adequate statistical power.	(32,82)
Vertical Pleiotropy	Also known as “spurious” or “false” <i>pleiotropy</i> . This is when a <i>genetic variant</i> affects other traits (which influence the outcome) via its effect on the exposure.	This is the very phenomenon that <i>MR</i> seeks to detect (i.e., the chain of traits to the outcome is what gives rise to an unbiased non-null causal effect estimate).	(32,45) Figure 4.2
Weak instrument bias	Instrument strength is determined by the magnitude and precision of association of the genetic <i>IVs</i> with the risk factor of interest.	The R^2 and F-statistic for the association of the genetic <i>IV</i> with the risk factor provide an indication of instrument strength (the higher the value of both, the stronger the instrument and the greater <i>statistical power</i> in <i>MR</i> analyses). For reference, genetic <i>IVs</i> were	(30,32)

		traditionally considered to be sufficient if the corresponding F-statistic was >10. In <i>one-sample MR</i> , weak instruments tend to bias towards the confounded risk factor-outcome association; whereas, in <i>two-sample MR</i> (with <i>non-overlapping samples</i>) the bias is expected to be towards the null.	
Winner's curse	This refers to the phenomenon that the 'first' (discovery) results for an association tend to be exaggerated (away from the null), with <i>replication</i> studies tending to be more modest.	In <i>MR</i> , if the genetic <i>IV</i> -risk factor association is based on results (or weights) of the discovery <i>GWAS</i> (or a single study) rather than combined discovery and <i>replication</i> results or <i>replication</i> results only, then the <i>IV</i> -risk factor association may be exaggerated. As this is the denominator of the <i>Wald ratio</i> (used to estimate the <i>MR</i> effect), the <i>MR</i> result would tend to be biased towards the null. This may be particularly likely in large biobank studies in which both the primary <i>GWAS</i> and <i>one-sample MR</i> analyses are undertaken in the same sample.	(32,83)

Figure 4.1: Collider bias

This figure illustrates collider bias in an MR study exploring the effect of maternal pregnancy body mass index (X: Mat. BMI) on offspring body mass index in later adult life (Y: Off. BMI). There is a clear violation of the assumption that the genetic instrumental variable (Z: Mat. BMI genetic IVs) is not related to the outcome (Y) other than via the exposure (X). It is clear that maternal BMI genetic IVs will influence offspring BMI genes (Off. BMI genes), which in turn will influence offspring BMI. This path can be blocked by adjusting for offspring genetic variants (this is illustrated by the box around offspring BMI genes). However, both maternal and paternal BMI genes (Z: Pat. BMI genes) ‘collide’ on offspring BMI genes and this generates a spurious association between maternal and paternal BMI genes. As only maternal and paternal BMI genes determine offspring BMI genes once we condition maternal genes on offspring genes, we know to some extent what paternal genotype is (e.g., if mother is a homozygote for a BMI increasing allele at a gene and offspring is heterozygous at the same gene, then dad must be heterozygous or homozygous for the alternative (not BMI increasing) allele). If it is not possible to condition on paternal genotype (which is often the case) and paternal BMI genes directly influence offspring BMI, this collider bias will bias the MR analysis of maternal pregnancy BMI on subsequent offspring BMI (26).

Figure 4.2: Vertical and Horizontal Pleiotropy. Adapted from Hemani *et al.* (84) and Holmes *et al.* (85).

(A) Classic horizontal pleiotropy, whereby the instrument (Z) for the exposure of interest (X) is independently associated with the outcome (Y) either directly or indirectly through other trait(s) – denoted “?”. Here, this would violate the third assumption of MR and would bias results from an MR study. (B) Indirect horizontal pleiotropy, whereby another SNP (Z_2) in linkage disequilibrium (LD) with the instrument (Z_1) for the exposure of interest (X) is associated with the outcome (Y) and, due to this correlation between SNPs, the instrument is therefore not independent of the outcome of interest. This is another reason to use independent genetic variants as instruments in an MR analysis and to have some biological knowledge about the mechanisms by which the SNPs are associated with the exposure. (C) A depiction of vertical pleiotropy, whereby the genetic instrument (Z) for the exposure (X) is associated with other trait(s) – denoted “?” – however, this reflects the downstream effects of the exposure that is likely on the causal pathway linking the exposure to the outcome (Y). This is the very essence of MR and is not something that needs to be accounted for in analyses. Measured and unmeasured confounders in all diagrams as represented by “ U ”, “ U_1 ” and “ U_2 ”.

Chapter 5: Key statistical methods used in MR for a standard one-sample context

Term	Brief definition	Summary of assumptions and restrictions of use in different types of MR / with number of genetic IVs	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Generalized Method of Moments (GMM) estimator	Generalized Method of Moments (GMM) is an estimation technique that is helpful where the distribution of the data maybe unknown and therefore alternative estimation techniques such as <i>maximum likelihood</i> are not applicable. For a given model, we specify a certain number of moment conditions, which are functions of the model parameters and the data, such that their expectation is zero at the parameters' true values. The GMM estimator of a linear <i>IV</i> model has been	In the context of <i>IV</i> estimation, we usually specify 1 moment condition per <i>IV</i> . The GMM method then minimizes a certain norm of the sample averages of the moment conditions. For identification we require at least as many <i>IVs</i> as there are parameters to be estimated. For binary outcomes, GMM estimation of structural mean models can be used to estimate parameters such as the causal risk	(86,87)

	applied to individual-level data in Economics since the 1980s.	ratio in <i>MR</i> studies using individual-level data.	
MR with a time-to-event outcome	Two-stage approach for causal estimation with time-to-event outcomes, where 2 nd stage regression is substituted with either a Cox proportional hazard, or additive hazard regression.	<i>Genetic variants</i> must satisfy the <i>IV</i> assumptions. Outcome must be rare for Cox model to be valid. Censoring must be independent of mortality. Model specification must be correct (i.e., additive hazard). <i>confounding</i> acts linearly on additive hazard scale. However, there is no consensus about the gold standard way of dealing with time-to-event data within an <i>MR</i> context.	(90,91)
Non-parametric methods with bounds of causal effect	A non-parametric method that estimates upper and lower bounds for an <i>MR</i> causal effect. The bounds are not the same as confidence intervals they	<i>Genetic variants</i> must satisfy the <i>IV</i> assumptions. It does not require any of the additional <i>IV</i> assumptions. This method can only	(88,89)

	reflect the upper and lower limits of an estimated distribution of causal effects.	be used in the situation of binary (or categorical with few levels) risk factor, <i>IV</i> and outcome. A method for implementing these in Stata has been developed.	
Polygenic risk score approach	A method which combines multiple genetic <i>IVs</i> into a single univariate score, which is then used as a single <i>IV</i> .	The univariate score must be a valid instrument. More robust to <i>weak instrument bias</i> than <i>two-stage least squares (TSLS)</i> . Hard to test for instrument validity.	(92)
Semi-parametric structural mean model (SMMs) for binary outcomes	SMM approach to <i>MR</i> that avoids having to specify a first stage regression model.	<i>Genetic variants</i> must satisfy the <i>IV</i> assumptions. No need to specify first stage <i>gene-exposure</i> association model. Can be extended to handle case control data.	(47)
Two-stage least squares (TSLS)	The canonical method to estimate the causal effect of a continuous exposure on a continuous outcome using an <i>IV</i> .	<i>Genetic variants</i> must satisfy the <i>IV</i> assumptions. <i>Gene-outcome</i> model must be correctly specified.	(93)

TSLS with binary outcome	Ad-hoc method for causal estimation with binary outcomes, where 2 nd stage regression is substituted with a logistic regression.	<i>Genetic variants</i> must satisfy the IV assumptions. <i>Genes</i> each exert a small effect on the exposure in order to approximately identify the same causal effect. Non-collapsibility of odds ratio means that population average rather than individual-level causal effects are estimated.	(46-48)
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Chapter 6: Pleiotropy-robust one-sample MR methods

Term	Brief definition	Summary of assumptions and restrictions of use in different types of MR / with number of genetic IVs	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
MR GENIUS	MR G-Estimation under No Interaction with Unmeasured Selection.	Exploits the assumption that a <i>genetic variant</i> is a valid instrument with respect to altering the variance rather than the mean. Genetic instrument must strongly affect the variance.	(94)
MR GxE	MR-GxE is an extension to <i>TSLs</i> that exploits <i>gene-covariate</i> interactions to test for correct for invalid instruments within a linear interaction model framework.	Requires a strong <i>gene-environment interaction</i> and the pleiotropic effect to be constant across covariate subgroups.	(18,95,96)
PRMR	<i>Pleiotropy</i> robust MR estimates the causal effect in the presence of <i>pleiotropy</i> by identifying a patient subgroup for which the <i>genetic variant</i> does not affect the exposure. The pleiotropic	Requires a perfect 'no-relevance subgroup'. The pleiotropic effect must be constant across covariate subgroups.	(21)

	effect estimated from this subgroup is then subtracted as an offset from the <i>TSLS</i> estimate.		
SISVIVE	Some Invalid Some Valid Instrumental Variable Estimation (SISVIVE). An extension to the <i>TSLS</i> procedure which incorporates LASSO penalization.	Can identify consistent estimates of the causal effect if the majority of <i>SNPs</i> are valid instruments. Currently, only developed for continuously measured outcomes.	(20)
Two-stage Hard Thresholding (TSHT) with voting	<i>TSLS</i> applied to a subset of <i>SNPs</i> which are judged to be estimating the same causal effect.	Assumes that valid <i>SNPs</i> form the largest set of <i>SNPs</i> which identify the same (true) causal effect. Requires strong genetic instruments.	(19)

Chapter 7: Two-sample MR methods Note: these methods were mostly developed for use with summary GWAS data

Term	Brief definition	Summary of assumptions and restrictions of use in different types of MR / with number of genetic IVs	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Inverse variance weighted (IVW) fixed effect estimate	Combines <i>Wald ratios (or Ratio estimates)</i> together in fixed effect meta-analysis, where the weight of each ratio is the inverse of the variance of the <i>SNP</i> -outcome association. Here, each instrumenting <i>SNP</i> is treated as an independent 'study', and the <i>Wald ratios</i> estimated for each <i>SNP</i> are meta-analysed under a fixed effects model.	The fixed effects meta-analysis assumes that the only source of differences between estimates across the studies is due to sampling variation (i.e., the true causal effect estimate is identical across all studies). In the <i>MR</i> context this translates to each <i>SNP</i> exhibiting no <i>horizontal pleiotropy</i> . <i>Genes</i> must be valid instruments. If all <i>SNPs</i> exhibit <i>horizontal pleiotropy</i> , then the effect estimate is asymptotically unbiased, but the standard error will be overly precise. Uses weights that assume the <i>SNP</i> -exposure association	(15,97)

		is known, rather than estimated, with no measurement error (i.e., known as the NOME assumption). Causal effect estimates from the IVW approach exhibit weak <i>instrument bias</i> whenever <i>SNPs</i> used as <i>IVs</i> violate the NOME assumption, which can be measured using the F-statistic with IVW methods.	
Maximum likelihood-based MR	Similar <i>IVW (fixed effect)</i> but approached using a maximum likelihood method where the genetic effects on the exposure and outcome are modelled directly as a bivariate normal distribution.	The method requires a term which represents the proportion of sample overlap between the exposure and outcome datasets. Requires an iterative estimating procedure that can occasionally fail to converge. This method is largely equivalent to <i>IVW</i> and is no longer commonly used.	(92)
Summary MR (SMR)	A method often applied to <i>MR</i> estimates which is related to the <i>Wald ratio</i> and often applied in	See <i>Wald ratio</i> .	(98)

	analysis of <i>gene</i> expression on complex traits and used in conjunction with the <i>HEIDI</i> method.		
Unweighted regression	Similar to the <i>IVW (fixed effects)</i> in concept, but all <i>SNPs</i> are weighted equally.	The intention of the method is that in some cases a <i>GWAS</i> will return one extremely large effect on the exposure and many small effects, and that single large effect will dominate the analysis. The method is seldom used in practice though, as alternative approaches such as leave-one-out analysis can be used for sensitivity instead.	
Wald ratio (or Ratio) estimate	Causal estimate obtained for a single <i>genetic variant</i> by dividing its <i>gene</i> -outcome association by its <i>gene</i> -exposure association.	<i>Genetic variants</i> must satisfy the <i>IV</i> assumptions. Equivalent to <i>TSLS</i> (and its approximate <i>binary/time-to-event</i> outcome) variants with one instrument.	(99)

Chapter 8: Pleiotropy-robust MR methods for a two-sample MR context Note: these methods were mostly developed for use with summary GWAS data

Term	Brief definition	Summary of assumptions and restrictions of use in different types of MR / with number of genetic IVs	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Generalized summary MR (GSMR)	The name given to a method implemented in a particular piece of software which is similar to standard <i>IVW</i> but also allows for correlation between variants and seeks to remove outliers.	The ' <i>MendelianRandomization</i> ' package also provides utility for including the <i>LD</i> matrix to provide overly precise standard errors if instruments are correlated. Performance of the method is dependent on accurate estimation of the <i>LD</i> matrix for the samples being used.	(100)
IVW (random effects)	Combines <i>Wald ratios (or Ratio estimates)</i> together in an inverse variance weighted meta-analysis, adjusting for heterogeneity.	All genetic <i>IVs</i> can be invalid due to <i>pleiotropy</i> as long as the <i>pleiotropy</i> is balanced (it has a zero mean and satisfies the <i>Instrument Strength Independent of Direct Effect – InSIDE</i> - assumption). This means that the association of genetic	(101)

		<p>instrument with exposure of interest</p> <p>should not be associated with the path</p> <p>between instrument and outcome that</p> <p>does not go via the exposure of interest.</p> <p>Asymptotically equivalent to <i>TSLS</i> with uncorrelated variants.</p>	
Mode-based estimate	Defined as the mode of an <i>inverse variance weighted</i> empirical density function of the <i>Wald ratio (or Ratio estimates)</i> .	Requires that the largest subset of instruments which identify the same causal effect to be valid instruments.	(102)
MR-Egger regression and extensions	Combines <i>Wald ratio (or Ratio estimates)</i> together into a meta-regression (with an intercept and slope parameter) to estimate the causal effect adjusted for any directional <i>pleiotropy</i> .	All <i>SNPs</i> can be invalid due to <i>pleiotropy</i> as long as the <i>pleiotropy</i> satisfies the <i>InSIDE</i> assumption. Can be implemented in a Bayesian framework. Standard implementation requires <i>gene</i> -exposure associations to be orientated in positive direction. <i>Radial MR</i> formulation avoids this step. The intercept of a MR-Egger regression provides an indication of	(14,103-105)

		<i>pleiotropy</i> when it is not null. Similar to <i>IVW</i> methods, the NOME assumption still holds and, if violated, there may be <i>weak instrument bias</i> . This can be tested with the I^2 statistic with the MR-Egger method.	
MR-RAPS	MR using a Robust Adjusted Profile Score. Maximises the profile likelihood of the <i>Wald ratio (or Ratio estimates)</i> , accounting for <i>weak instrument bias, pleiotropy</i> and extreme outliers.	All <i>SNPs</i> can be invalid due to <i>pleiotropy</i> as long as the <i>pleiotropy</i> is balanced.	(106)
Multivariable MR (MVMR) and extensions	<i>Multivariable MR</i> extension of the <i>IVW</i> approach applied to the summary data context. Used to learn about the causal effect of two or more exposures on an outcome.	All genetic <i>IVs</i> must be associated with at least one exposure. All genetic <i>IVs</i> can be invalid instruments due to <i>pleiotropy</i> as long as the <i>pleiotropy</i> is balanced. Method can be combined with <i>MR-Egger</i> regression to allow for directional <i>pleiotropy</i> .	(107,108)

Weighted median	Defined as the 50 th percentile of an <i>inverse variance weighted</i> empirical density function of the <i>Wald ratio (or Ratio estimates)</i> .	Requires that at least 50% of the weight in the analysis stems from variants that are valid instruments.	(13)
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Chapter 9: Model and instrument selection and averaging approached for a two-sample MR context Note: these methods were mostly developed for use with summary GWAS data

Term	Brief definition	Summary of assumptions and restrictions of use in different types of MR / with number of genetic IVs	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Bayesian model averaging for IVW and MR-Egger	Uses Bayesian Model Averaging to infer the posterior probability that the <i>IVW</i> or <i>MR-Egger</i> regression models (with or without heterogeneity) is the true data generating model.	Requires the specification of priors for the regression model parameters and/or the probability that each model is 'true'.	(109)
MR-MoE	MR Mixture of Experts uses machine learning to choose the best performing model out of 28 different <i>pleiotropy</i> robust estimation methods.	Based on meta-learning, in which simulations are used to train a machine which selects the method most likely to return a reliable <i>MR</i> estimate.	(110)
Rucker framework for IVW and MR-Egger	Uses goodness-of-fit heterogeneity statistics to decide whether the <i>IVW</i> or <i>MR-Egger</i> regression models (with or without heterogeneity) is best supported by the data.	Requires p-value thresholds to be specified to guide model choice.	(101)

Steiger filtering	<p>Large-scale <i>GWAS</i> have the power to detect very small effects. Steiger filtering is a method for selecting valid genetic <i>IVs</i> from very large <i>GWAS</i> with <i>statistical power</i> to identify <i>genetic variants</i> that are related to the trait of interest at a <i>genome-wide significance</i> threshold because it is downstream of many other traits that the <i>genetic variant</i> influences. For example, a sufficiently large <i>GWAS</i> of a biomarker (A) may identify numerous <i>genetic variants</i> that influence another biomarker (B) because A is influenced by B.</p>	<p>If those variants are included in an <i>MR</i> study of the effect of A on B without knowledge of their biology (as is typical from <i>GWAS</i> results), the result could (erroneously) suggest that A causes B (when the opposite is true). These reverse causal <i>IVs</i> will bias the <i>MR</i> estimate. In these situations, Steiger filtering uses a statistical test to identify the stronger of bidirectional effects (e.g., A on B or B on A) to select valid <i>IVs</i> from very large <i>GWAS</i> that can be used in <i>MR</i>. Steiger filtering assumes that a valid <i>IV</i> should explain more variance in the exposure than the outcome and removes those <i>genetic variants</i> that do not satisfy this criterion. If A has much more measurement error than B, then the variance explained by the <i>SNP</i></p>	(110,111)
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		<p>on A might be smaller than on B even though it primarily influences A. Some patterns of <i>confounding</i> can also lead to this problem.</p>	
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Chapter 10: Heterogeneity and outlier-detection methods for a two-sample MR context Note: these methods were mostly developed for use with summary GWAS data

Term	Brief definition	Summary of assumptions and restrictions of use in different types of MR / with number of genetic IVs	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
Cochran's Q statistic	Heterogeneity statistic for <i>IVW</i> model.	If Q much larger than its degrees of freedom (No. of <i>IVs</i> minus 1), this provides evidence for heterogeneity and invalid instruments. Assumes genetic <i>IVs</i> are uncorrelated. Individual contribution of each <i>IV</i> to Q can be used to detect individual invalid <i>IVs</i> . An extended version of Q statistic necessary when genetic instruments are weak.	(15,112)
HEIDI (heterogeneity in dependent instruments)	A method which seeks to address if many <i>SNPs</i> in a single region give <i>Wald ratio (or Ratio estimates)</i> estimates that are more different	This is essentially a <i>genetic colocalization</i> method, and a number of similar methods exist (e.g., R/coloc and S-predixscan).	(62,63,98)

	from each other than expected by chance under the assumption that there is a single causal variant and each <i>SNP</i> only exhibits an effect due to <i>LD</i> with the causal <i>SNP</i> .		
MR-PRESSO	This is an extension to <i>IVW</i> , which attempts to perform the same form of outlier removal as in <i>GSMR</i> and <i>Radial MR</i> .	<i>Genetic variants</i> are removed based on their contributions to heterogeneity. It is achieved through a simulation approach. See notes on <i>outlier removal</i> .	(113)
MR-TRYX (Treasure your exceptions)	A framework for exploiting <i>horizontal pleiotropy</i> . If a <i>SNP</i> appears to be an outlier in an <i>MR</i> analysis, then it is hypothesised that it is additionally associating with the outcome through another trait. Searching through <i>GWAS</i> databases to find candidate traits, then modelling the original exposure-outcome association accounting for the candidate traits allows a) detection of new putative risk factors	Depends on the ability to accurately estimate the effect estimates on the path through candidate traits. Automated using available <i>GWAS</i> databases such as <i>MR-Base</i> , which do not have complete coverage of the human phenome and therefore unlikely to be able to completely account for the heterogeneity.	(114)

	and b) reducing heterogeneity and/or bias in the original estimate.		
Radial MR (MR pleiotropy residual sum and outlier)	Similar to <i>IVW</i> but uses a simulation-based approach to detect outlying variants and flags them for removal in order to re-estimate the original exposure-outcome relationship. It has been shown that this outlier detection framework is closely related to the analytical approach of applying <i>IVW</i> with so-called “modified second order weights”.	Outlier removal methods can effectively reduce bias in <i>MR</i> estimates, but caution must be made because they will necessarily reduce the standard error and could lead to over-fitting. Other outlier removal methods have also been implemented elsewhere in <i>GSMR</i> and <i>RadialMR</i> .	(104)

Chapter 11: Useful software and tools

Name	Link	Useful journal reference(s) or references to figures shown later in the chapter, if appropriate
GSMR	http://cnsgenomics.com/software/gsmr/	An R-package that implements the <i>Generalized summary MR (GSMR)</i> method to test for putative causal association between a risk factor and a disease using summary-level data from <i>GWAS</i> (100).
LD Hub	https://github.com/bulik/ldsc http://ldsc.broadinstitute.org/ldhub/	A database of summary-level <i>GWAS</i> results and a web interface for undertaking <i>LD</i> score regression – a method that estimates the <i>SNP</i> heritability of complex traits and diseases, partitions this heritability into functional categories, and estimates the genetic correlation between different phenotypes (115).

MendelianRandomization	https://cran.r-project.org/web/packages/MendelianRandomization/index.html	R-package to perform <i>MR</i> analyses with <i>summary (aggregate) data</i> , which requires summary-level data on the associations of <i>genetic variants</i> with the exposure and outcome to be obtained from large consortia by the user (116).
MR-Base and TwoSampleMR	http://www.mrbase.org/ https://github.com/MRCIEU/TwoSampleMR	A database and analytical platform for undertaking <i>two-sample MR</i> using summary <i>GWAS</i> data. It runs a large number of <i>two-sample MR</i> analyses (including <i>pleiotropy</i> robust methods) and produces numerical and graphical results (39). The data and analytical platform can be accessed both online and with the TwoSampleMR R-package.
MR-TRYX	https://github.com/explodecomputer/tryx	R-package for undertaking <i>MR-TRYX</i> analyses (114).
PhenoScanner	http://www.phenoscanter.medschl.cam.ac.uk/	A curated database of publicly available summary-level <i>GWAS</i> results and an analytical

		platform that facilitates ‘phenome scans’ – the cross-comparison of <i>genetic variants</i> with many phenotypes, to help aid understanding of disease pathways and biology (117).
PhenoSpD	https://github.com/MRCIEU/PhenoSpD	An integrated toolkit for phenotypic correlation estimation and multiple testing correction using <i>GWAS</i> summary-level statistics (84).
PHESANT	https://github.com/MRCIEU/PHESANT	A tool for performing automated hypothesis-free <i>MR</i> phenome-wide scans in UK Biobank (118).
RadialMR	https://github.com/WSpiller/RadialMR	Fitting and visualising <i>Radial MR</i> from <i>IVW</i> and <i>MR-Egger</i> methods (104).
Sample size and power calculators	http://cnsgenomics.com/shiny/mRnd/ (<i>one-sample</i> setting) https://sb452.shinyapps.io/power/ (<i>two-sample</i> setting)	Example calculators for required sample size given a desired level of power or for <i>statistical power</i> given a specific sample size in both one- and <i>two-sample</i> settings. These calculators can

		be used for both binary and continuous outcomes (32,82).
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Figure 11.1: Flow diagram for undertaking one-sample and two-sample Mendelian Randomization

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