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Integrating genomics with biomarkers and therapeutic targets to invigorate cardiovascular drug development

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Abstract | Drug development in cardiovascular disease is stagnating, with lack of validated targets a major roadblock to innovation. Human genetics can provide compelling evidence of causation through approaches such as Mendelian randomization (MR). Such genetic support can increase the probability of a clinical trial succeeding. MR is being widely applied to quantitative traits (e.g. adiposity and blood lipids) with the aim of identifying risk factors for disease that are both causal and amenable to therapeutic modification. However, there are important differences between genetic investigations of a biomarker (such as HDL-cholesterol) and a drug target that seeks to modify the same biomarker of interest (such as CETP), with implications on the methodology, interpretation and applications of MR to drug development. Differences include the comparative nature of the genetic architecture, i.e. that of a biomarker

typically being polygenic, whereas that for a protein drug target being monogenic. and the potential for drug targets to show pleiotropic disease associations which may differ to those of the biomarker it is designed to modify. In this review, we compare and contrast the role of MR as applied to quantitative traits versus drug targets, describing differences between approaches and the function each plays. Our review will put into context findings emerging from this burgeoning field of scientific investigation, with the aim of clarifying the aetiological roles of biomarkers in disease, prioritizing candidate drug targets based on genetic-guided credibility of causation and in so doing, improve the odds of success in the drug development pipeline.

Introduction

Cardiovascular disease (CVD), comprising ischemic heart disease, stroke and peripheral vascular disease, represents the leading cause of death globally, accounting for 15.2 million deaths (or ~ 1 in 3 deaths) in 2016 alone [1]. While non-pharmacological prevention of CVD is a key component of public health approaches to preventing onset and sequelae of CVD, a cornerstone to disease prevention and treatment is the use of pharmacological therapies.

In order for drugs to become licensed and available to patients, they must undergo rigorous investigations, including early studies of tolerability, followed by phase II trials (typically dose-ranging in nature) and pivotal phase III outcome randomized controlled trials which serve to show clinical efficacy for the intended outcome (in this case, ideally a reduction in risk of cardiovascular disease) while showing lack of clinically-meaningful adverse events. Such phase III trials typically represent the final hurdle prior to applying for a marketing

authorization. If the drug is found to be efficacious for the primary outcome and safe, the regulators are likely to grant the applicant a license to market the drug. For interventions for cardiovascular disease, the process, from date of first testing in humans (clinical entry) through to marketing authorization takes a median of 10 years[2], with this duration remaining largely unchanged since the 1970s [2].

A notable trend observed over recent decades is that the number of drugs being developed for cardiovascular disease has stagnated. [3] While the average number of new drug approvals for any indication have fluctuated between 25/year (from 2000-2009) to 41/year (from 2010-2018)[4], the corresponding values for cardiovascular indications were 2.6/year and 3.2/year[2]. This has occurred on the background of a rapid increase in R&D expenditures in real terms [3] (with the median cost of bringing a drug to market between 2009-2018 being approximately $1bn [5]), which together with large numbers of generic drugs being approved, collectively impedes the return on investment for new drug development. One of the major issues in developing drugs is failure due to lack of efficacy (accounting for ½ of phase II and III failures) [6], with therapies targeting risk factors that are not causal representing a major roadblock to innovation [3]. Another major hurdle is safety (accounting for ¼ of failures [6]), with therapies being abandoned due to both dose-related and idiosyncratic adverse events. It is largely for these reasons that fewer than 1 in 10 drugs succeed in running the gauntlet from phase I through to marketing authorization, with the success rate being disease-specific [7]. Of note, the comparative probability of success (i.e. of a drug being launched) when a candidate drug enters each phase of clinical trials is lower when the therapeutic indication is cardiovascular disease as compared to other therapeutic areas[8]. Between 2010-2017, there was

a 4% probability of launch for cardiovascular drugs entering phase I RCTs versus 9% for anticancer therapies. For drugs entering phase II RCTs the comparable probabilities of launch were 10% for those with cardiovascular indications and 23% for anticancer indications and for drugs entering phase III, values were 46% for cardiovascular versus 59% for anticancer.[8]

Human genetics can provide a tool by which the causal role of an exposure (such as a complex phenotype and/or a drug target) can be characterized [9]. Performed well, such studies called Mendelian randomization (MR) analyses, can provide rigorous evidence of causation[9,10]. The presence of genetic evidence to support causation increases the probability several-fold of a therapeutic targeting the same biomarker being successful in a phase III outcome trial and obtaining a marketing authorisation [11 12]. With increasing availability of large-scale phenotyping (and genotyping), MR is being widely applied to quantitative traits (e.g. markers or adiposity, such as body mass index[13-16]; blood lipids such as HDL-cholesterol[17-19]; inflammation-related proteins such as interleukin-6 receptor[20,21 12]) with the aim of discovering modifiable risk factors for disease that are causal and amenable to therapeutic modification. Such risk factor biomarkers are typically modified pharmacologically through drug targets (the terms biomarkers and drug targets are described below). However, important differences exist between genetic investigations of a quantitative trait (such as HDL-cholesterol) as compared to a drug target (such as CETP[22-25]), with major implications on their interpretation, role and applications to drug development.

In this Review, we compare and contrast the role of MR as applied to quantitative polygenic traits versus (typically monogenic) drug targets, describing the main differences between the approaches and the function each plays in guiding the process of drug

development. Our Review puts into context findings emerging from this burgeoning field of scientific investigation. We aim to build on our previous review [26] which described challenges in interpreting estimates from MR in cardiovascular disease but which did not contrast between therapeutic targets and complex biomarkers. [26] We draw themes across historical[27 28 29] and more contemporary [30 31 32] articles that describe the application of human genetics to therapeutic targets. We comparatively appraise the motivation, application, methodologies and interpretation of MR as applied to drug-targets versus complex biomarkers, including an evaluation of when findings from the two aetiological approaches (i.e. MR of a drug target versus MR of a complex biomarker) may diverge. Our overarching ambition is to guide the investigator who is concerned with elucidating the aetiological roles of biomarkers in disease, prioritizing candidate drug targets based on credible genetic evidence of causation and in doing so, attempting to improve the success and thus efficiency of the drug development pipeline.

Mendelian randomization to elucidate causality

We can exploit the properties of the human genome (specifically, the random segregation of alleles and their non-modifiable nature) to make causal inferences about the relationships between risk factors and disease [10]. The process by which genetic variants are inherited in offspring from their parents occurs at random, owing to Mendel's law of independent assortment. Within families, this process ensures that genetic variants are unlikely to be associated with confounding factors that can results in bias in other study designs. For this reason, when MR was introduced by analogy with a randomized controlled trial, it was in the context of between-sibling studies, owing to the true randomization of genetic information

(outside those in linkage). [33] [9] However, most MR studies to date have used datasets of unrelated individuals and make the assumption that what is true within families (i.e. the random inheritance of genetic variants from parents to offspring), holds across the population (i.e. the random distribution of variants) [34]. In other words, the assumption is made that when individuals are grouped in the population by their genotype, they should be similar in all regards other than one group possesses one or more genetic variant(s) and the other group another set of variants – if those genetic variants influence an exposure of interest, the comparison of disease associations across the groups permits causal inference. However, there is increasing evidence that genetic variants associated with some phenotypes are not entirely randomly distributed across the population in relation to potential confounding factors, e.g. for social or behavioural phenotypes such as educational attainment, intelligence and smoking [35]. This is (at least partially) due to what have been called "dynastic effects" that relate to the environment provided by parents to their offspring [35] [36]. Of note, phenotypes that are more genetically proximal (e.g. gene expression, protein levels and many biomarkers; see **Figure 1**), are likely to be considerably less prone to these biases.

Many of the benefits and potential limitations of MR have been extensively described in prior articles [9,26,37,3836]. There have been a number of cases when MR has retrospectively found similar findings to that of randomized controlled trials (RCTs) (e.g. statins [39] and drug-target MRs using LDL-C lowering variants in/around *HMGCR* [40]; varespladib which pharmacologically targets secretory phospholipase A2-IIA[41] and genetic variants in *PLA2G2A* [42]) and there are cases where MR has predicted the outcome of trials too (such as use of genetic variants in *CETP*[23] predicting the outcomes of a clinical trial of anacetrapib, a cholesteryl ester transfer

protein (CETP) inhibitor) including findings that have yet transpire clinically (e.g. despite LDL-C lowering genetic variants in/around *PCSK9* showing associations with higher risk of T2D [43,44], an excess risk of T2D has yet to reliably transpire from patients treated with PCSK9 inhibitors [45]). Other examples of notable MR studies are described in **Box 1**. Concerns about the plausibility of the assumptions and overuse of MR have been raised.[46,36] For example, there are well-founded concerns over the challenges of interpreting the implications of life-long estimates derived from MR in the context of pharmaceutical interventions that are typically used later in life (e.g. the issue of 'time zero')[47], although pragmatically, well-conducted MR studies can (and often do) provide credible causal evidence[10]. There are multiple cases where MR can complement RCTs and examples where MR can enhance information that would require several phase III RCTs of multiple doses of drugs– e.g. providing evidence of dose-response relationships across the physiological distribution of a trait (described in **Box 2**). In a nutshell, MR can be used throughout the lifecycle of a drug, as illustrated in **Figure 2**.

Why focus on biomarkers?

Biomarkers are phenotypes that can be measured objectively and provide quantifiable indications of biological processes [48]. The study of the roles that biomarkers play in causing disease is of interest for several reasons. First, such information provides aetiological knowledge, which enriches our understanding of biological processes that underpin disease and can stimulate new avenues of investigation. For example, whilst inflammation in general has long been postulated to influence vascular disease[49,50], MR has been able to test whether particular elements in the inflammatory response cause[20] or are caused by[51] disease. This

evidence facilitates exploration of other inflammatory biomarkers, new therapeutic targets[52] and repurposing of existing drugs to new indications[53]. Second, an understanding of how biomarkers cause disease can help clarify the sequence of intermediaries that lie on a causal pathway from a risk factor through to disease, permitting development of therapies that act on risk factors along the causal pathway to ameliorate the consequences of harmful exposures (an example being drugs for hypertension that inhibit angiotensin converting enzyme and angiotensin II receptor antagonists, which act along the renin-angiotensinogen system pathway). This is illustrated in **Figure 3** using inflammation as an example, and serves to highlight the value of randomized evidence for prioritising where on the causal pathway to intervene (as pharmacological modification of C-reactive protein is unlikely to be beneficial to CHD [51], whereas randomized evidence demonstrates the value of targeting biomarkers further upstream, e.g. interleukin-6 receptor [21,54] or interleukin 1-beta [52]). A further example is the causal role that adiposity plays in CHD: adiposity increases systolic blood pressure[13,16] which mediates part of the effect of BMI on risk of CAD [55]. Mendelian randomization can elucidate such causal intermediaries and help develop new pharmacological approaches to ameliorating the effects of harmful exposures that are challenging to modify at the population level (see **Box 3** and **Figure 4**).

Third, when drug targets modify several traits, an exploration of the comparative role that such traits plays in disease aetiology through multivariable MR approaches can shed light on the likely causal pathway to disease[56]. For example, blood lipid traits (including apolipoprotein B, low-density lipoprotein cholesterol and triglycerides) each associate independently with risk of CHD. Conducting MR analyses individually on these traits shows that

each is related to a higher risk of CHD. However, when appraised together, the evidence supports apolipoprotein B as being necessary for lipid-mediated atherogenesis by LDL-C to occur.[57,58]. This can help pinpoint the trait(s) scientists should focus on when developing lipid-modifying drugs to treat CHD – i.e. all things being equal, apolipoprotein B can potentially be used as a reliable surrogate marker for the expected reduction in relative risk of CHD for a novel lipid-modifying therapy. Fourth, for environmental exposures (e.g. alcohol), knowledge of causation can help frame public health policy. In this case, a trial would be challenging and/or unethical to conduct, despite previous attempts, however MR provides credible information on causation that can inform public health policies.[59 60]

Why focus on drug targets?

While valuable from an aetiological perspective (and for public health), it is generally infeasible to pharmacologically modify a non-protein biomarker in isolation. This is because therapeutic targets tend to modify phenotypes through specific mechanisms that are unique and thus dissimilar to the totality of the genetic/environmental architecture of the trait (as in the example of cholesteryl ester transfer protein (CETP) inhibitors and high-density lipoprotein cholesterol (HDL-C) [22], illustrated in **Figure 5**).

Thus, the MR estimates of the effects of a complex biomarker may differ from the effects of a protein drug target that is under investigation on the basis that it modifies the same complex biomarker. For this reason, in order to potentially inform on the expected clinical effects of a therapy, we ideally need to conduct de novo MR studies of therapeutic targets that modify the biomarker of interest. In other words, MR of drug targets is useful because it uses genetic

variants that mimic as closely as possible the action of the therapeutic target of interest. This can potentially provide some of the most reliable evidence of the effects of modifying the biomarker, via a specific target, on long-term health outcomes. Beyond simply providing quantitative evidence of whether a therapy targeting a given drug target is likely to be efficacious and safe, drug target MR can provide a treasure trove of additional information that can feed into multiple facets of trial design (summarised in **Box 2**).

Biomarker versus drug target

There is an important distinction between a complex (polygenic) biomarker and an (oligogenic) drug target, which we outline in **Figure 1**. This has a bearing on the motivation, conduct, application and interpretation of MR studies as applied to complex biomarkers versus drug targets (**Table 1** and **Box 4**). The key question and motivation of a biomarker MR is typically whether the biomarker plays a role in disease, which is of interest from a biological perspective (as it may highlight new approaches to preventing disease) and from a public health perspective (as lifestyle modification mediated alterations in the biomarker may yield large benefits in public health [61]). In contrast, the key question and motivation of drug-target MR is whether modifying the drug target alters risk of disease and thus whether a phase III clinical trial of a therapy that modifies the drug target is likely to demonstrate clinical benefit to patients, enabling applications for marketing authorisations and health technology appraisals. Other features and distinctions between biomarker and drug target MR are described in **Table 1** and throughout this review.

We consider a complex biomarker to be a non-protein trait (e.g. metabolites and traits such as adiposity, blood pressure and lipids; **Figure 1**) that is not encoded for by any individual gene. Complex traits have polygenic architectures, with multiple genetic variants affecting the biomarker through discrete and/or overlapping pathways.

Most drug targets are proteins. Both conventional (small molecules) and newer therapies (e.g. biological monoclonal antibodies, antisense RNA inhibitors, and the consequences of gene editing) typically target proteins. Thus, if the therapeutic aim is to treat or prevent disease through the pharmacological modification of a complex biomarker, this is typically conducted by exploiting the vertically pleiotropic effects of the protein on the complex biomarker (see **Box 5** which describes pleiotropy as applied to drug target MR). Proteins are typically encoded for by a unique (single) gene making it theoretically possible to generate a genetic instrument for the protein of interest that is specific (so-called 'cis' protein instruments that are close to, or in, the relevant gene, see **Box 6**). Thus a genetic instrument utilising cis-acting variants for a drug target is typically monogenic but can sometimes be oligogenic if more than one region of the genome (as in SNPs from more than one locus) is involved in encoding the final protein product. For example, haemoglobin is a heterotetramer that is made up of alpha and beta globin polypeptides, each encoded by discrete genes[62]. Thus a drug-target MR of haemoglobin selecting cis-acting variants from across discrete protein-encoding genes would be oligogenic, in contrast to a monogenic instrument for CRP[51], encoded for by a single locus. Notably, if more than one SNP is used as an instrument from the same locus, this remains a monogenic instrument.

Cis variants typically associate with protein concentrations in the blood or gene expression in tissues (**Box 7**). Cis variants for proteins (cis-protein quantitative trat loci; cis-pQTL) are generally preferable for use as instruments in drug-target MR as compared to cis variants for mRNA expression (cis-expression quantitative loci; cis-eQTL) as the potential for pre-translational pleiotropy[32] (i.e. horizontal pleiotropy that arises prior to translation of mRNA into proteins) is potentially diminished when selecting variants based on their properties as cis-pQTLs versus cis-eQTLs. Another reason is that protein concentrations can act as a marker of drug efficacy, allowing a quantitative appraisal of effects of drug versus genetic instrument, which may be particularly valuable when the causal intermediaries (i.e. biomarkers downstream of the drug target which mediate the effect on disease risk) are unknown. Such cis-acting variants are likely to provide an accurate and reliable estimate of the causal effects of modifying that protein. However, there are specific caveats to the use of *cis* acting variants – for example the issue of *cis*-pQTLs altering epitope binding sites leading to potential false negative findings (as described in **Table 2**) and to protein MRs in general – e.g. the issue of targeting proteins (e.g. IL6) versus their receptors (e.g. IL6R). In this latter example, genetic variants used to mimic pharmacological inhibition of IL6R (and thus the effects of inhibiting pathways downstream of IL6) lead to a paradoxical increase in IL6 concentrations in the blood[21], with the interpretation of such findings described in more detail elsewhere[26]. While genetic variants genomically distant from the protein-encoding gene (so-called *trans* variants) may associate with protein levels at conventional GWAS significance thresholds, they are more likely to have horizontally pleiotropic effects (e.g. by affecting the expression of multiple genes), making the interpretation of MR estimates based on trans-acting variants challenging (**Figure 6**). An

example here is the use of SNPs to instrument C-reactive protein from regions of the genome other than the *CRP* locus, which might lead to the erroneous conclusion that C-reactive protein causes CHD [63] – when restricting to SNPs within the *CRP* locus, no such effect of C-reactive protein on risk of CHD is identified[51].

What does this mean in practice?

Consider pharmacological therapies that seek to lower risk of CVD through the modification of a biomarker, LDL cholesterol. Such pharmacological interventions work by targeting proteins and enzymes involved in the synthesis or metabolism of LDL cholesterol. LDL cholesterol lowering pharmacological drugs are thus unlikely to solely alter LDL cholesterol in physiological isolation, but rather, target a specific part of its synthesis and/or metabolism. Other than LDL apheresis [64] (an extracorporeal method for the elimination of LDL cholesterol, used in patients with familial hypercholesterolaemia) it is not possible to directly and specifically remove LDL cholesterol from the blood stream. Modifying a complex exposure (such as LDL cholesterol) through a drug target (e.g. HMGCR or PCSK9) is likely therefore to lead to a phenotypic modification of LDL cholesterol that may differ to that if LDL cholesterol were removed in isolation (e.g. by apheresis). This is evidenced by, for example, examining the metabolic effects of statins and *HMGCR* genotype on refined measures of blood lipids where widespread effects on multiple lipoproteins are seen, together with alterations to fatty acids. [65] I.e. statins have wide-reaching effects on the lipidome beyond that of LDL cholesterol, and some of these may lead to differences in disease risk which are discrete to those solely

attributable to LDL cholesterol. One example of this is ovarian cancer, where a protective effect of an instrument based on *HMGCR* is seen, in contrast to instruments for other LDL-cholesterol lowering agents[66]. Furthermore, discrete classes of drug targets that are designed to modify the same biomarker can display heterogeneous effects on blood lipid biomarkers, likely a reflection of the unique underlying mechanism(s) by which the drug targets exert their physiological effects. For example, HMGCR and PCSK9 differ in their effects on the cholesterol concentration of apolipoprotein-B containing lipoproteins [67], despite the motivation of both drugs being to prevent CHD through the lowering of LDL cholesterol. At a more extreme level, while both CETP inhibitors and HMGCR lower CHD through the pharmacological reductions of apolipoprotein B[22], their metabolomic signatures differ wildly[68]. Similar differences are also seen between ANGPLT3 and ANGPTL4 (enzymes which are involved in the metabolism of triglycerides) [69]. Thus, a drug target may have different effects as compared to a biomarker, and different drugs that modify the same biomarker may differ in their effects on risk factors and disease outcomes.

A similar situation is true for adiposity. BMI is a polygenic trait, and a genetic instrument for BMI consists of many SNPs that associate with BMI through multiple mechanisms. Therefore, the relationship of the BMI polygenic score (PGS) with disease likely recapitulates the effects of modifying BMI by an average of these polygenic effects. Environmental changes in BMI (e.g. through cosmetic liposuction or weight loss surgery) might lead to effects on cardiovascular risk factors and disease that are specific to each surgical procedure. Lowering BMI through conventional dieting might lead to different effects on disease as the environmental manipulation of BMI in this setting (as opposed to surgery) might have differing

roles in disease. In contrast, existing pharmacological approaches to lowering BMI - e.g. drugs acting on the central nervous system or peripherally (e.g. GLP-1 receptor agonists [70]), while lowering BMI, do so through very different mechanisms. Therefore, the effects on disease risk of different pharmacological (and surgical [71,72]) approaches to modifying BMI, may potentially differ. This can be empirically tested by conducting drug-target MR using instruments for GLP-1R (to approximate the effects of GLP-1R agonists) and comparing disease associations to the equivalent BMI-lowering derived from a BMI PGS that is depleted for *GLP1R* variants. This approach was recently applied in a study investigating the vascular effects of a bone mineral density increasing therapy[73]. Early data from phase III clinical trials suggested an excess of vascular disease events in those randomized to receive a monoclonal antibody inhibitor of sclerostin. The pharmaceutical company developing the drug submitted unpublished data to regulators, which led to an attenuation of the CHD risks arising from sclerostin inhibition. However, genetic evidence suggested that increasing BMD via sclerostin inhibition was likely to increase risk of CHD[73]. This was further investigated by comparing the effects on CHD of modifying BMD implied by genetic variants in the *SOST* gene (which encodes the drug target sclerostin) to the effects implied by other BMD associated genetic variants across the genome. Very similar disease associations were found suggesting that BMD increasing therapies in general, including sclerostin inhibitors, are likely to increase risk of CHD (rather than representing a case of target-mediated pleiotropy, i.e. an effect specific to sclerostin inhibition; see **Box 5** for definition).

When might findings from MR of a complex biomarker, MR of a drug target and findings from an RCT diverge?

There are several scenarios where the relationships of a biomarker with disease may differ to that which the drug target (either in MR or RCT) shows. These are described in **Table 2** together with illustrations, and below. We describe pleiotropy in **Box 5**.

Target-mediated pleiotropy (or, 'specificity' of target): A biomarker may be non-causal for disease, but the drug target may show clinical efficacy (scenario 3 in **Table 2**). The exemplar for this is CETP inhibitors that were primarily developed on the basis that they increased HDL-C (rather than for any effect on cholesterol in apolipoprotein B containing lipoproteins) [74]. It is perhaps ironic that CETP inhibitors exert their relatively modest cardioprotective effects on the basis that in addition to their HDL-C raising effects, more potent CETP inhibitors also lower apolipoprotein B [22,23,75]. Had CETP not exerted such apolipoprotein B lowering effects, then all trials of CETP inhibitors (including that of anacetrapib [75]) would have likely failed owing to lack of efficacy. Indeed, this is precisely what was seen with earlier CETP inhibitors (e.g. dalcetrapib [76]) that increased HDL-C but had little or no effect on LDL-C or apoB. These findings are in keeping with evidence from human genetics in East Asians, where *CETP* variants in the China Kadoorie Biobank that were strongly associated with HDL-C, but not with apolipoprotein B, did not associate with CVD [77].

Off-target effects of therapies. When a drug exerts properties that alter pathways that are discrete to the target of interest, and when these discrete pathways lead to altered risk of

disease, then the findings from MR and RCTs may not converge (scenario 4 in **Table 2**). An example of this is the blood pressure effects of CETP inhibitors [74]: while all CETP inhibitors show modest SBP increasing effects, this appears to be most pronounced for torcetrapib [78 74]. Of note, such SBP associations have not been identified in drug-target MRs using HDL-C raising variants in *CETP* [79 77], for reasons that are unclear. This discrepancy between drug-target MR and RCT highlights the vital need for clinical trials for elucidating the effects of treatments in patients – i.e. we do not advocate drug target MR as a substitute for clinical trials (other than perhaps when findings from MR provide strong evidence against a plausible causal effect, indicating that a trial would be unlikely to succeed), but rather, we see drug-target MR to be complementary to and enhance the design and interpretation of conventional randomized trials.

Disease onset vs progression: Most MR studies use either case control studies where the case has a first event and controls are disease free, or prospective cohorts of the general population with individuals followed up for incident events. In both circumstances, the SNP to disease association captures the risk of incident disease. Using these types of data, drug target MR would estimate whether modifying the biomarker or drug target affects the risk of developing a first event. However, in the setting of RCTs, often for fiscal reasons (to lower the number of trial participants, increases in statistical power, and also magnify absolute differences in effect between treatment groups in order to improve cost effectiveness analyses), and because prevention of disease progression is clinically important, trial participants often have prevalent disease at enrolment and drugs are tested to elucidate whether treatment slows disease

progression and/or prevent clinical manifestations of recurrent disease. A prime example of this in cardiovascular disease is that many trials are set in patients either at high risk of CVD (who likely have prevalent subclinical atherosclerosis at entry into the trial) or patients who have in the past experienced a CHD event. In this scenario, the trial typically estimates the effect of a drug on recurrent disease or disease progression.

If a risk factor is causal for first disease manifestation, but not for recurrent disease, findings from biomarker and/or drug target MRs for incident disease may be qualitatively different to estimates from an RCT on disease progression (scenario 7 in **Table 2**). Although in CVD, risk factors for onset (e.g. blood pressure, lipids) appear to be similar for first and recurrent disease, it is plausible that some risk factors for disease incidence and progression may differ. For example, there may be drug targets that are specific for plaque initiation and others that are specific for plaque rupture: if plaque initiation and rupture have differing roles in incident vs recurrent disease, then biomarkers (and drug targets) that modify plaque may demonstrate heterogeneity of disease associations between MR studies (of first events) and RCTs (of recurrent events), and vice versa. Another example is atrial fibrillation (AF) – for example a cause of AF may be hypertension, however once AF is established, its persistence is likely to be influenced to a greater extent by drugs involved in controlling the rate and rhythm, as opposed to drugs treating the initiating cause (e.g. lowering blood pressure).

Returning to the comparison of the aetiology for disease onset vs that of disease progression, how can this discrepancy be reconciled? Methods are under development for applying MR to disease progression, [80] which take into account issues such as index event bias [81]. Such endeavours are facilitated by the accumulation of large datasets (e.g. GENIUS-CHD,

which is a consortium of studies with established disease at study entry with follow-up for recurrent disease [82]).

Pleiotropy of genetic instrument. If genetic variants possess horizontally pleiotropic properties, then drug-target MR estimates may be invalid (scenario 5A in **Table 2**). While the susceptibility to such bias is likely to be reduced by selecting *cis*-acting variants as instruments (i.e. SNPs in/around the gene encoding the protein of interest; **Box 6**), it is plausible that local linkage disequilibrium can distort findings. Colocalization, which is a statistical approach that quantifies the extent to which two traits share the same underlying causal variant, and thus can be used to infer vertical pleiotropy [83-85], can be used to test for such bias[86], but can suffer from issues of lack of power and/or availability of suitable data from relevant tissues.

Adaptation: Individuals at high genetic risk of elevated LDL-C are more likely to be prescribed lipid lowering drugs, which is an environmental adaptation to genetic risk. Another example is individuals at higher genetic predisposition to obesity who smoke more [87,88], which could represent an attempt to self-administer smoking for its nicotinic effects on appetite suppression and weight loss [89]. When such adaptation occurs to genetic predisposition to traits, this can distort effect estimates (typically biasing them to the null).

Canalization: Also called developmental compensation[90], canalization is the process by which adaptation to a major perturbation owing to a genetic variant arises from a compensatory physiological or anatomical mechanism such that the phenotypic consequences are not as

pronounced as would be expected [9]. If present, canalization would represent a mechanism by which findings from a drug-target MR would diverge from those of an equivalent clinical trial. Reassuringly, in the case of drug-target MR, there is little evidence to support presence of such compensatory mechanisms – e.g. genetic variants with large phenotypic effects appear to have the expected dose-response association with disease as compared to genetic variants of smaller phenotypic effect [57 40].

Time-dependent effects: If the exposure to disease process occurs over many years, then effect estimates from Mendelian randomization may be much greater than the comparative estimates derived from phase III clinical trials (which are typically shorter in duration), when scaled to the same difference in exposure. Thus, it may be necessary to scale the MR estimate in order to obtain the equivalent effect estimate that would be predicted to be obtained in a phase III clinical trial for a given duration. The timing of causal effects for each exposure-disease pair is likely to differ. For example, the optimal timing for lower LDL-C is likely to be different to the optimal timing for BMI lowering interventions [91]. Approaches such as estimating the causal effects of a biomarker at different points in life using multivariable MR may help provide evidence about timing (as has been conducted for BMI[91]), however this approach has not been used for drug targets, and may not be possible owing to the limited repertoire of (particularly cis-acting) genetic instruments. Fortunately, however, it is possible, that in the absence of target mediated pleiotropy of individual drug targets, once the specific biomarker-outcome relationship is characterized, including an understanding of the differences between treatment effects originating from a treatment trial of a specified duration as compared to lifelong

exposure instrumented through MR, such properties are likely be similar for drugs that modify the same biomarker including drugs in discrete classes. This is what is seen with multiple LDL-C lowering drug classes: MR estimates of the effects of LDL-C lowering and risk of CHD are approximately 3-fold greater than estimates from RCTs [92], and as such, we can naively scale MR estimates of LDL-C lowering drug target MR studies by this same scaling factor in order to provide some indication of the possible magnitude of the effect estimate obtained in treatment trials. The process to establishing this is several-fold: first conducting MR of the complex biomarker (and ideally the drug-target) followed by a quantitative comparison to the phase III cardiovascular outcome trial (CVOT) result in order to estimate the comparable differences in magnitudes of effect owing to e.g. duration of exposure, linearity of dose-response and presence of time-specific effects; second, an appraisal of consistency of disease associations across multiple MRs of discrete drug classes scaled to the same difference in biomarker; and third, once a scaling factor for e.g. life-long estimates from MR as compared to short-term effects of RCTs is derived for an exposure-outcome pair, applying the same scaling factor to multiple drug classes to inform of likely treatment effects in prospectively-planned clinical trials[92,93]. A fourth check would be, for example using non-linear MR approaches [94], that the dose response of the biomarker and disease risk is log-linear to facilitate scaling of effects in drug-target MR[95]. Such dose-response can also be approximated using individual drug-target MRs of varying effect size on the exposure[96]. The approach outlined here to obtaining a scaling estimate has generally held true for lipid-lowering therapies and risk of cardiovascular disease[22,23,40,92] but there are several potential reasons why the derivation of such a scaling for other therapeutic target-indication pairs may pose a challenge. One such reason is that the

scaling factor is extremely likely to differ for each therapeutic target-indication pair, but such scaling is, as a rule, unlikely to be different when the therapeutic target modifies the same final common pathway (except in the case of target-mediated pleiotropy). Thus, in the absence of target-mediated pleiotropy, it is possible that a scaling factor for broad groups of target-indication pairs may be constructed, which provides value beyond more limited 'qualitative' inferences (e.g. presence/absence of causation) that can be inferred from MR.

Putting the pieces together

How then are we to design approaches which identify therapeutic targets for disease prevention? One such framework is outlined in **Figure 7**. Complex (polygenic) biomarkers are modified pharmacologically by targeting (monogenic) proteins that, through vertical pleiotropy, modify the complex biomarker of interest. A first step, typically through complex biomarker MR, is to establish causation of the complex biomarker and clarify whether modifying the biomarker may represent a valid therapeutic goal to modifying disease risk. Since, as described above, findings from a biomarker MR cannot be assumed to be identical to those derived from drug target MR performed on the basis that a drug target modifies the same biomarker of interest, the next step is usually to conduct a drug-target MR. Data from these various sources can be used to triangulate evidence and predict the likely outcome of a therapeutic trial. In comparing a *cis*-pQTL instrument (representing a therapeutic target) to a phenotypic PGS (comprised of all phenotype-associated genetic variants minus SNPs in/around the gene encoding the drug target) it may be possible to identify potential differences between the

485 expected effect of the biomarker on a disease, versus the effect by modifying it via a specific
486 drug target. These comparisons can identify if perturbation of the drug-target pathway of
487 interest has a differential effect from other potential targets that affect the same biomarker of
488 interest. If a biomarker is identified to have a particular relationship with disease (e.g. long
489 latency or time dependency), then individual drug targets that modify the same biomarker
490 ought to display the same attributes in their relationship with disease. Drug-target MR also
491 heralds multiple opportunities for additional exploration – for example when the precise
492 mediators and adverse effect profiles are not known between a drug target and disease
493 outcome, explorations through MR-PheWAS can provide new insights[66]. For example,
494 colchicine[53] and icosapent ethyl[97] have both demonstrated efficacy in reduction of CVD in
495 recent large-scale phase III trials, however the precise mechanisms by which these drugs act,
496 i.e. the causal intermediaries, remains elusive. A proteomic exploration of individuals treated
497 with colchicine or icosapent ethyl might identify proteins that these drugs modify. MR studies
498 of these proteins for CHD would characterize, for each protein, whether it is likely to be on the
499 causal pathway from therapeutic target to disease. This might permit development of more
500 specific drugs that could display fewer adverse events (as demonstrated in **Figure 3**) and
501 facilitate accurate titration to dose-response, with knowledge of the causal intermediary. Thus,
502 discrepancies between biomarker and drug-target [97] can be informative for elucidating the
503 aetiology of disease and with it, shed light on new approaches to treating and preventing
504 disease. Enhancements to drug-target MR described in **Box 8** can also address criticisms[98] often
505 levied at RCTs (with such criticism subsequently rebuked[99-101]) including that individuals
506 recruited into trials are usually highly-selected (thus hampering generalizability), and that

findings from RCTs apply to the group-level rather than to individuals, hampering progress in personalised medicines. Finally, in writing an article about the conduct of drug-target MR, it behooves us to stress that the scientific (and pharmaceutical) community has a responsibility in applying this scientific approach to promote the equitable development of therapies, ensuring that advances in drug development (including those catalysed by drug-target MR) benefit minority under-represented groups, as we describe in **Box 9**. In summary, the use of human genetics can facilitate drug development throughout the lifecycle.

Conclusions

In this review, we have contrasted MR of a biomarker with that of a drug target that seeks to modify the same biomarker. A biomarker is typically a complex phenotype that is optimally instrumented through MR using multiple genetic variants across the genome. In contrast, drug target MR typically focuses on a protein trait using *cis*-acting genetic variants in an individual gene that encodes the protein. The motivation for biomarker and drug target MR differs, with biomarkers informing the effects of potential public health measures and shedding light on causal risk factors for disease that motivate their pharmacological modification, while drug target MRs estimate the likelihood of a specific therapeutic (typically designed to modify a complex biomarker) showing efficacy in a clinical trial. In both scenarios, human genetics can be harnessed as an invaluable tool to elucidate causal roles in disease aetiology and through careful application, such studies can be used to guide the design and interpretation of clinical trials of medicines.

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Competing interests

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Key points

- Mendelian randomization offers unique opportunities to explore the likely causal role of biomarkers and drug targets in disease aetiology.
- A biomarker is typically a complex trait that has a polygenic architecture, whereas a drug target is usually a protein that has a monogenic architecture.
- The motivation, application and interpretation of Mendelian randomization as applied to complex biomarkers and drug targets differs,
    - These differences have implications for how each source of evidence contributes to aetiological insights in disease and in predicting the effects of therapies on clinical outcomes
- In this review, we describe these differences and offer insights into how such can be leveraged to comprehensively evaluate the causal role of a biomarker followed by an evaluation of which drug targets may be most tractable to the clinic.

Figure 1. DNA, proteins, complex biomarkers and disease. Proteins, encoded by genetic variants, are modified into complex biomarkers. Some proteins and complex biomarkers play a causal role in cardiovascular disease. If we wish to modify a complex biomarker that has a

putative causal role in CHD aetiology, this is typically done by therapeutic modification of an upstream protein trait. The figure illustrates the distinction between a protein trait (being typically monogenic) and a complex biomarker (being polygenic). It is this distinction that is critical to differentiating the motivation, conduct, interpretation and application of MR to drug targets (i.e. proteins) as compared to complex biomarkers.

Figure 2. Applications of human genetics to the lifecycle of drug development. Mendelian randomization can be used at each stage of the drug development pipeline. cIMT: carotid intima medial thickness; PheWAS: phenome-wide association study

Figure 3. Application of Mendelian randomization to clarify where on the causal pathway to intervene. Randomized evidence derived from treatment trials and human genetics for traits on a causal pathway can be used to construct evidence of the likely underlying mechanisms, and which trait to intervene on to optimally lower risk of disease. As the focus shifts upstream (i.e. from right to left in the plot above), there is greater potential for drug targets to display heterogeneous disease associations (including both those that may be adverse, and those that may be beneficial) arising from target-mediated pleiotropy, described in **Box 5**. Mendelian randomization using variants in/around the gene encoding C-reactive protein (CRP) does not support the hypothesis that CRP causes CHD [51]. Exploring inflammation-related proteins more upstream, genetic variants around the gene encoding interleukin 6 receptor (IL6R) shows an association with risk of CHD [21,54]. A clinical trial of a monoclonal neutralising antibody inhibitor to interleukin 1 beta (canakinumab) showed efficacy in a phase III cardiovascular outcome

trial[52]. Nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3 (NLRP3) inflammasome (upstream of IL1b) is under exploration as a potential therapeutic target [102].

Figure 4. Mendelian randomization for mediation. MR can be used to identify intermediates that lie on the causal pathway between polygenic (complex) biomarkers and disease. The figure depicts an exploration of traits that mediate the effect of body mass index (used as a measure of obesity) and risk of coronary heart disease (CHD). By identifying traits that BMI modifies and conducting de novo MR investigations, it is possible to identify causal intermediaries (i.e. traits that lie on the causal pathway from BMI to risk of CHD). Such may lead to the development of new therapies that stem the tide of harmful exposures (e.g. excess adiposity) that are proving challenging to modify at a population level. For further information, please see **Box 3**.

Figure 5. Biomarker versus drug target MR. For complex biomarker MR, a genetic instrument typically consists of multiple SNPs selected from across the genome (a polygenic instrument) associated with the biomarker of interest. A suite of sensitivity analyses can be used to test for evidence of unbalanced (directional) horizontal pleiotropy [37]. In contrast, a drug-target MR typically uses one or a handful of SNPs selected from the gene that encodes an individual protein (*cis*-acting variants) in a monogenic instrument. Even when a drug target is selected on the basis of modifying a given biomarker, the biomarker and drug target MR estimates may differ. For example, in the example illustrated, an MR of circulating HDL-C finds little-to-no evidence that HDL causally affects risk of CHD (once taking into account unbalanced horizontal

1042 pleiotropy [18,19,58]). However, using SNPs located in *CETP* associated with HDL-C at genome-wide
1043 significant levels in a drug-target MR suggests that modifying HDL-C via CETP, is likely to reduce
1044 risk of CHD [23,103]. Rather than being attributable to HDL-C , this CHD association arises due to
1045 target-mediated pleiotropy through apolipoprotein B.
1046
1047
1048 **Figure 6. Drug-target Mendelian randomization using *cis* vs *trans*-acting SNPs.** In this example,
1049 we have 4 *cis*-acting SNPs in the gene encoding the protein and 7 *trans*-acting SNPs from other
1050 parts of the genome. By virtue of the mechanism by which the *trans*-acting SNPs associate with
1051 protein levels, they are more likely to have pleiotropic effects (illustrated by the red arrow) that
1052 may modify disease risk independently of the exposure (thus violating one of the central
1053 assumptions of Mendelian randomization[95]). Care must therefore be taken in the application of
1054 *trans*-acting variants in drug target MR as their inclusion in the genetic instrument may lead to
1055 bias. An example relates to the use of trans-pQTL to instrument C-reactive protein, which can
1056 distort the CHD association, leading to an erroneous conclusion.
1057
1058 **Figure 7. A framework for identifying therapeutic targets.** How might we make progress on
1059 identifying treatment targets for disease, especially when causal risk factors are not fully
1060 characterised? GWAS of disease can be exploited by conducting phenome-wide association
1061 analyses (PheWAS) of disease-associated SNPs. The phenotypic signature of a genetic risk score
1062 (GRS) derived from SNPs reliably associated with disease will likely capture multiple signals,
1063 including associations arising due to causal pathways leading to disease, trait associations

arising due to disease and other elements[104]. Once equipped with the phenotypic signature of a disease GRS, de novo MRs of those traits can be conducted to establish disease causation (by means of a conventional biomarker MR). If a biomarker is identified to cause disease, de novo GWAS of the biomarker may identify SNPs in loci encoding therapeutic targets. An exploration through available resources such as OpenTargets or ChEMBL[105] can provide additional information to establish whether individual loci are likely to represent tractable therapeutic targets. Drug-target MR of protein-encoding loci identified as being of relevance to risk factors can establish the predicted effects of pharmacological modification in clinical trials and be used to guide clinical development (as shown in **Figure 2**). As discussed in the text, care needs to be placed in interpreting the magnitudes of effect given that MR of an exposure represents, naively, lifetime exposure and depending on the exposure-outcome pair, this may lead to a magnification of the effect estimates arising from MR. Of note, it may be possible to proceed from disease GWAS through to drug-target MR without elucidating causal risk factors, but this is likely to have less power to do so.

1079 **Table 1.** Key features of Mendelian randomization of a biomarker and a drug target

Type of MR	Biomarker MR	Drug target MR
Question	Does this biomarker cause disease?	Does modifying this drug target alter risk of disease?
Motivation	Public health (e.g. implications of obesity on disease) Biological understanding to discover biomarkers to intervene on	New therapies for the prevention of incident and recurrent disease, and disease-related sequelae
SNP selection	GWAS-derived preferable (unless protein exposure)	*Cis*-acting SNPs where possible
MR methods	Heavily reliant on sensitivity analyses to show lack of unbalanced horizontal pleiotropy	Might be less vulnerable to pleiotropy but should ideally check consistency of independent SNPs in same locus
Vertical pleiotropy	Can point to pharmacological approaches to intervention (e.g. proteomic mediators of adiposity and BMI/CAD)	Can shed light on differences in treatments (e.g. PCSK9 and HMGCR) on intermediaries (e.g. blood metabolic markers[106]), which can then be used to explore whether such differences are clinically meaningful[92]
Horizontal pleiotropy	Need to formally evaluate, e.g. through robust MR approaches [37]	Confounding by linkage disequilibrium can be assessed through colocalization [84]
Causal inference	Evidence on whether the biomarker causes incident disease and progression of disease	Evidence on whether modifying a drug target is likely to alter risk of (i) disease onset (when the study is set in general population); or (ii) disease progression (when the study is set in individuals with established disease)
Role of PheWAS	Unknown effects of biomarker on other biomarkers and diseases	Unknown (both unintended: ADRs, and beneficial: repurposing) target-mediated effects
Limitations	Public health interventions may not reciprocate effects (as challenges in purely 'modifying' biomarker)	Cannot predict *off-target* effects of individual therapies

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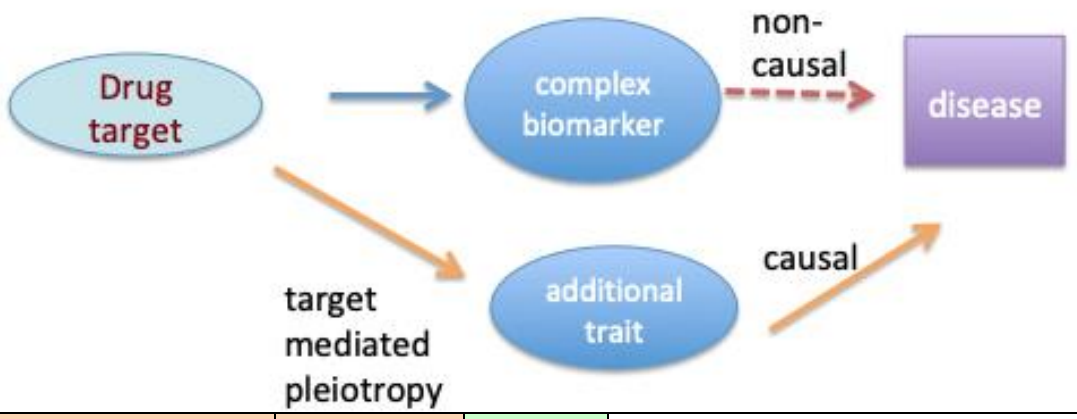
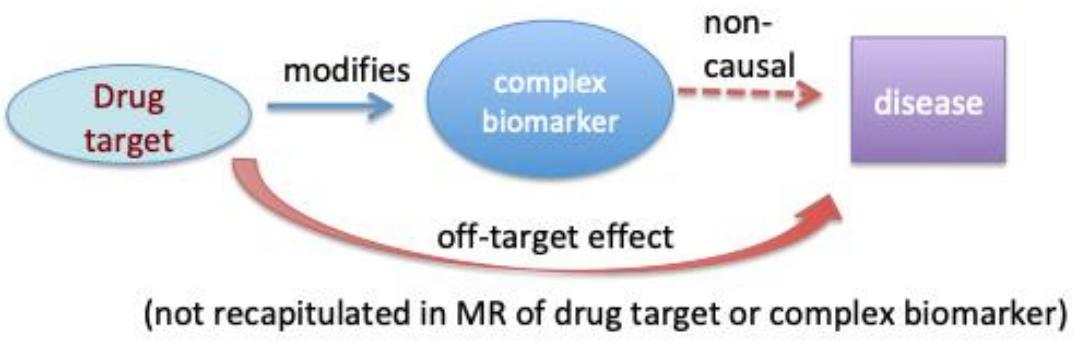
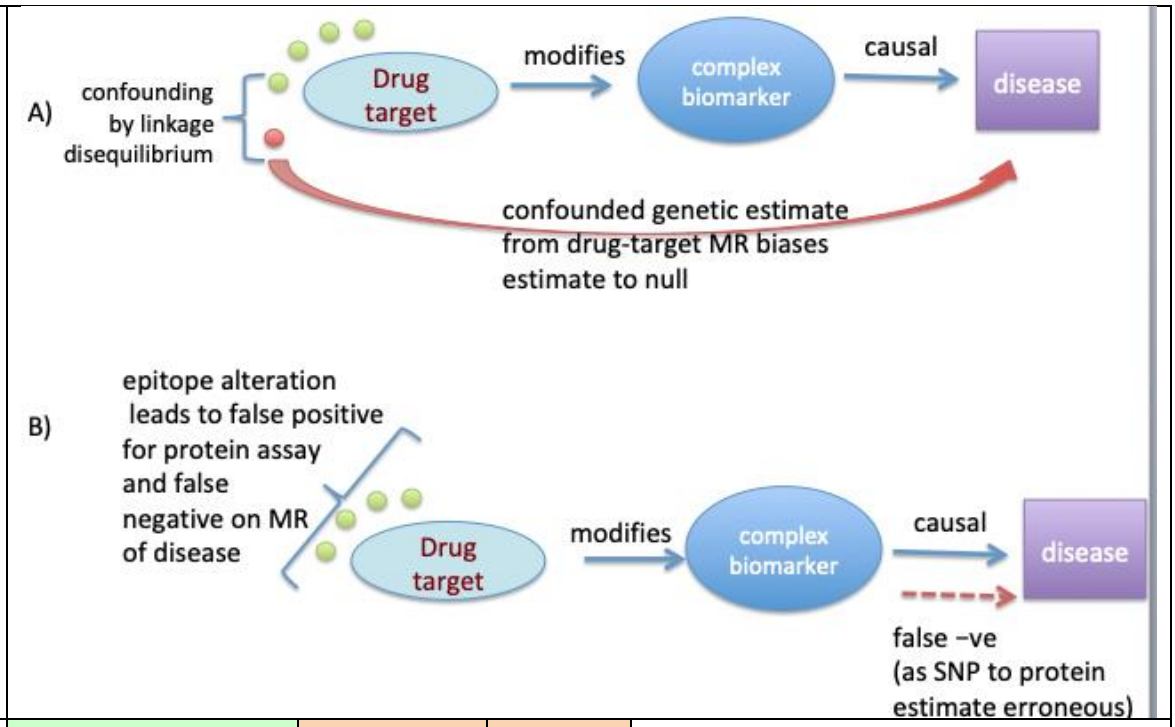
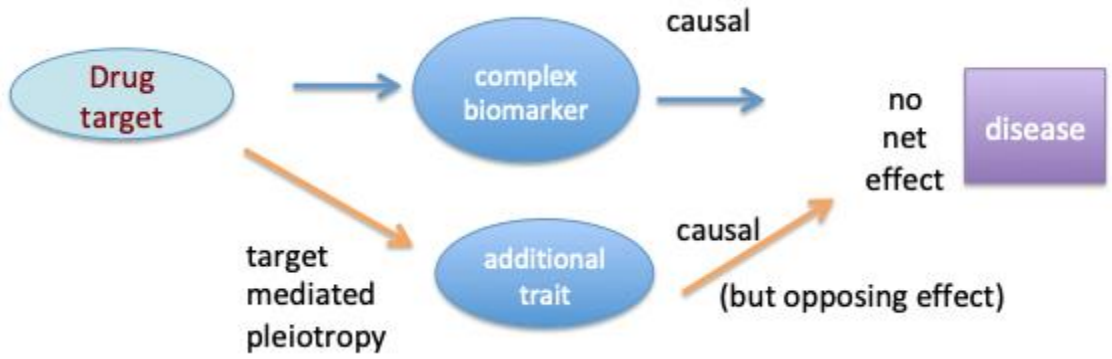
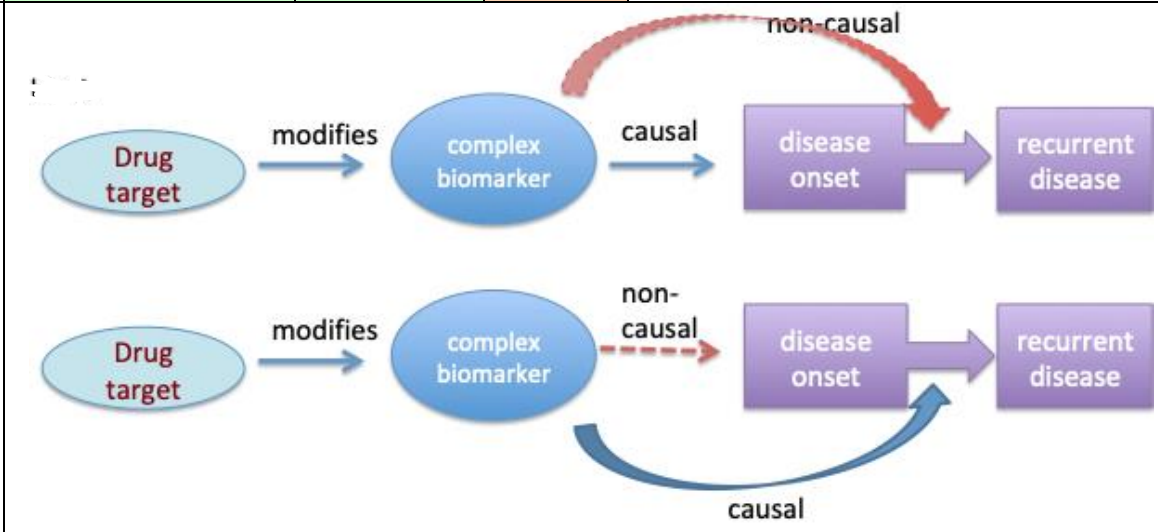
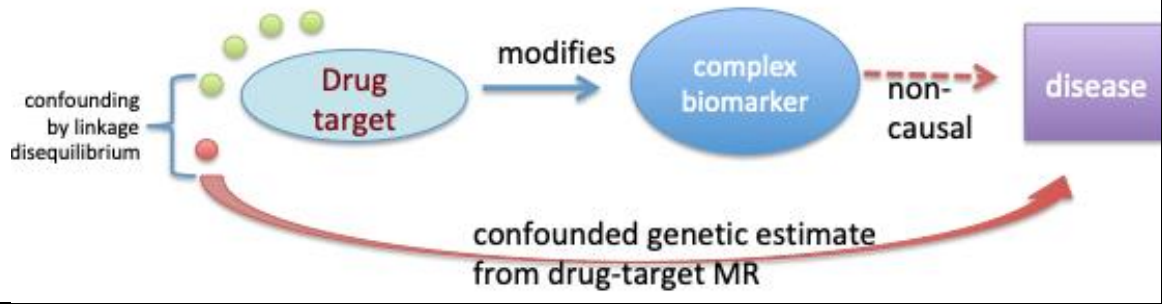
Table 2. Scenarios where findings from MR of phenotype, drug target and treatment trials may diverge

Scenario	MR of phenotype	MR of drug target	RCT	Potential Explanation(s)* and examples
1	Causal	Causal	Causal	Biomarker causes disease; and modifying biomarker through the drug target has same effect on disease. E.g. LDL-C and apolipoprotein B (phenotype) [40] and HMGCR [107], PCSK9, NPC1L1 (drug targets); statins [39,108,109] [110] PCSK9 mAbs and ezetimibe (drugs)
				
2	Non-causal	Non-causal	Non-causal	Biomarker doesn't cause disease, nor does the drug target. E.g. HDL-C [58], *CETP* variants that don't associate with LDL cholesterol [77], CETP therapeutic inhibitors that don't lower LDL-cholesterol (or apolipoprotein B) [76], and apo-A1 infusions [111]
				
3	Non-causal	Causal	Causal	Drug target shows target-mediated pleiotropy that leads to beneficial effect on disease. E.g. *CETP* variants that lower apolipoprotein B [23], and potent

				pharmacological CETP inhibitors [75]
				
4	Non-causal	Non-causal	Causal	Drug has off-target effects; E.g. CETP inhibitors and systolic blood pressure[74,79] Also: drug target tested in RCT causal for recurrent events but not incident events (in which MRs conducted) (e.g. biomarkers involved in plaque stability [112]).
				
5	Causal	Non-causal	Causal	(a) SNPs used for drug target MR confounded by correlation with other SNPs (through linkage disequilibrium) that have opposing effect on disease risk with the net effect to bias disease associations to the null; (b) Genetic variant alters an epitope binding site for assay, which has no qualitative or quantitative effect of the protein

				
6	Causal	Non-causal	Non-causal	Biomarker causes disease, but drug target has target-mediated pleiotropy that off-sets this effect to yield net null effect
				
7	Causal	Causal	Non-causal	Biomarker important for disease onset but not for disease progression with biomarker and drug target MRs conducted in general population but RCT conducted in patients with disease (and vice versa). Biomarker (and drug target modifying biomarker) only causal for disease during a specific time (e.g. adolescence) and clinical trial conducted during adulthood – e.g. vitamin D and multiple sclerosis [113].

				Development of neutralizing antibodies to biological therapies e.g. development of antibodies to PCSK9 mAbs [114]
				
8	Non-causal	Causal	Non-causal	Drug-target MR using cis-pQTL (protein quantitative loci) confounded by local LD
				

Footnote: * potential explanations assume that studies are adequately powered.

Box 1: Notable examples of Mendelian randomization studies of biomarkers, drug targets and randomized controlled trials

MR of biomarker	MR of drug target	RCT	Comments
LDL cholesterol – shows evidence of causation with CHD [19]	LDL-C lowering variants in *HMGCR*, *PCSK9*, *NPC1L1* [107] [115] associate with CHD	Multiple trials of drugs targeting LDL-C lowering show cardiovascular benefit [39,116]	The exemplar, where MR of a biomarker, drug targets and RCTs align
HDL cholesterol [17,19] MRs do not provide evidence of causation, once unbalanced horizontal pleiotropy is taken into account	*CETP*[23] variants associate with risk of CHD; *APOA1*[117] variants do not associate with risk of CHD	REVEAL trial of anacetrapib [75] demonstrated evidence of vascular benefit; RCTs of apoA-I infusion [111] have thus far not shown benefit	HDL-C and apoA-I are likely to be non-causal in CVD. CETP inhibitors are likely to lower risk of CHD if they lower apoB.
BMI increases risk of T2D and CHD in MR [14] with one potential mechanism being increased blood glucose	*GLP1R* variants that associate with lower blood glucose show evidence of a potential causal role of glucagon-like peptide-1 receptor (GLP1R) in T2D and CHD[118] *SGLT1* variants are associated with altered blood glucose and risk of vascular disease[119] but it has proven challenging to identify variants in	The Look-AHEAD trial showed directionally consistent evidence of lower vascular risk [121] but was potentially underpowered, supported by post-hoc analyses [122] Monoclonal antibody agonists to GLP-1R have shown efficacy for CHD [70] SGLT2 monoclonal antibody inhibitors have shown efficacy	An example were modifying a complex biomarker (BMI) with recognised harmful effects on multiple diseases at the population level is challenging, requiring pharmacological modification of a protein upstream. Notably such protein drug targets may display target-mediated pleiotropic effects that are

	SLC5A2 (which encodes SGLT2) to instrument therapeutic inhibition of SGLT2[120]	for multiple endpoints related to metabolic and vascular disease[123,124].	independent of BMI (see **Box 5**)
Phospholipase A2 (PLA_2) is a family of enzymes that hydrolyse fatty acid from membrane phospholipids [125]. Secretory phospholipase A2-IIA ($sPLA_2$-IIA) and lipoprotein-associated PLA2 (Lp-PLA_2) were both thought to play potential roles in atherogenesis. sPLa-IIA [42] and Lp-PLA [126,127] drug-target MRs showed lack of evidence of causal roles in CVD		Varespladib (a drug targeting $sPLA_2$-IIA) failed in VISTA16 [41]; Darapladib (which targeted Lp-PLA_2) failed in SOLID-TIMI 52 [128] and STABILITY [129] trials	Billions of US dollars were spent on developing therapies that were destined to fail for lack of target-mediated disease causation.
MR of CRP [51] failed to demonstrate a causal role in CHD	The interleukin 6 receptor (IL6R) is a drug target that alters CRP (see **Figure 3**), and for which genetic evidence supports a causal role of IL6R in CHD [20 54]	Selective IL-1 beta inhibitor (canakinumab) [52], which is upstream of IL6R demonstrates benefit in phase III cardiovascular outcome trials	An example of applying randomized evidence from biomarkers on a causal pathway to elucidate where to intervene, illustrated in **Figure 3**.
MR of bone mineral density (BMD) found that higher BMD increased risk of CHD and T2D [130]	BMD-associated variants in *SOST* (used as a genetic instrument for sclerostin inhibition) show higher risk of CVD [73]	Early evidence from RCTs of drugs that modify sclerostin suggest potential excess risk of CVD [73]	An example of human genetics providing evidence to support a potential target-mediated adverse effect of a therapy

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Box 2: How can genetic evidence can enhance the evidence base of causation to facilitate the conduct of randomized controlled trials?

Drug target Mendelian randomization studies can provide a source of valuable information that is of value to understanding whether the modification of a drug target is likely to lead to clinical benefit.

Feature	**Application of Mendelian randomization**
Trial design	When a trial might be unethical (e.g. insufficient clinical equipoise, potentially harmful intervention or when there are potential ethical issues of withholding treatment or placebo comparator unethical)
	When a previously-conduced large-scale trial was underpowered (e.g. adiposity and vascular disease in Look-AHEAD [121])
	To inform on whether a trial should be conducted in particular phenotypically-defined and/or genetically-defined subgroups
	To predict the outcome of a factorial trial design, e.g. by predicting drug by drug interactions (e.g. HMGCR and CETP [23])
Characteristics of the intervention	When no therapies exist (e.g. drugs not yet developed – for example prior to development of PCSK9 inhibitors)
	To deprioritise development of non-causal therapies (e.g. those targeting CRP [51], $sPLA_2$-IIA [42] or Lp-PLA_2 [126])
	To explore the nature of the causal relationship across the physiological distribution of the exposure - notably individual trials of a single dose of drug are unable to speak to causal effects across the physiological distribution of exposure on which they intervene
Mechanistic insights	To explain mechanisms of effect (e.g. **Figures 3-4, and Box 3**)
	To clarify pleiotropic effects (a) Off-target effects of a *drug class*: e.g. whether the drug has effects on an outcome beyond that which is considered to be the primary 'exposure' (e.g. HMGCR/statins and CHD – effect estimates are proportionate to LDL-C reductions[23] seen with other lipid-associated genetic variants and lipid lowering therapies, arguing against pleiotropic effects that lead to CVD reduction through other mechanisms, such as a reduction in inflammation) (b) Off-target effects of an *individual drug*: e.g. torcetrapib blood pressure effects vs other CETP inhibitors[74] (c) To explore the full repertoire of target-mediated effects (including those that are beneficial and potentially harmful) in

	order to inform clinical trial design to enable accurate synthesis of risk benefit analyses, and potential repurposing to new indications
Adaptive trial design	MR estimates with disease can be used to guide the selection of diseases used in a composite primary endpoint (e.g. removing elements from a composite outcome if MR shows that one element is not causally modified by the drug target). If this information becomes available during the conduct of a trial, the constituents of the composite primary endpoint might be modified with such knowledge through an adaptive design.
	Re-estimate power based on MR point estimate[92] (with appropriate scaling of effect estimate, if possible), prolong recruitment to increase number of participants and/or prolong duration of follow-up
	Use of MR to inform on subgroups to alter trial eligibility criteria to enrol participants that will experience: (a) greater benefit (may allow trial to recruit fewer numbers of participants and/or have a shorter duration to lower the cost of drug development) E.g. a recent re-appraisal of the CETP inhibitor evacetrapib that failed to demonstrate an overall effect on CAD was examined to explore whether an interaction existed on stratifying individuals by a CAD PGS. Perhaps unsurprisingly, this analysis showed no CAD PGS by evacetrapib interaction [131]. However, repeating the analysis by conducting a GWAS of apoB lowering by evacetrapib and constructing a *de novo* PGS based on SNPs demonstrated to modify the drug-induced apoB lowering might well display a qualitative interaction (between evacetrapib and the PGS) on risk of CAD (b) fewer adverse effects: although it may not be possible to separate out the mechanism-based adverse effects of a therapeutic (e.g. *CYP2C19*/clopidogrel[132]; *GUCY1A3*/aspirin[133]) if an adverse event arises due to a mechanistic pathway that is separate to the mechanistic pathway leading to benefit, it may be possible to identify genetic variants that modify the propensity of the pharmacological agent to modify the pathway leading to adverse events, and by doing so, disentangle benefit from harm.

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Box 3: Using Mendelian randomization to explore potential mediators of the relationships of complex exposures and disease risk.

One of the prevailing challenges for population health is that while we understand the causal relationships of multiple risk factors for disease, we are unable to modify them at a population-level in order to gain maximal benefit [61] (e.g. BMI and CVD). Such population-wide interventions are likely to be identified in the future but may take decades to develop. What can be done in the mean time? One as-of-yet untapped, but potentially lucrative, application of MR is in its ability to identify mediators on the causal pathway from complex exposures (such as adiposity[55], physical activity[134], education[135,136]) and disease risk (e.g. CHD, T2D). With the repertoire of metabolites[137] and proteins[138] that are being measured in sufficient numbers to characterize their genetic architecture through GWAS and construct reliable genetic instruments, MR is uniquely placed to elucidate what lies on the causal pathway from population-wide 'lifestyle' exposures (e.g. BMI) and disease. This can be conducted by performing MR of known causal risk factors (e.g. BMI) as the exposure on proteins and metabolites as outcomes, and, for those proteins and metabolites identified to be altered by genetically predicted differences in BMI, conduct *de novo* MRs of these proteins and metabolites onto disease. Through the intersection of this with drug development, it may be possible to identify druggable biomarkers that mediate (i.e. lie on the causal pathway(s)) between complex exposures and disease . Through the pharmacological modification of such intermediates, it may be possible to ameliorate the harmful effects of upstream complex exposures that are challenging to modify at the population level.

Box 4: How to conduct the optimal MR for a biomarker vs a drug target?

Several articles have described approaches to the conduct of MR analyses[37,139-141], obviating the need to provide an exhaustive prescription here.

Rather, we consider the following scenarios:

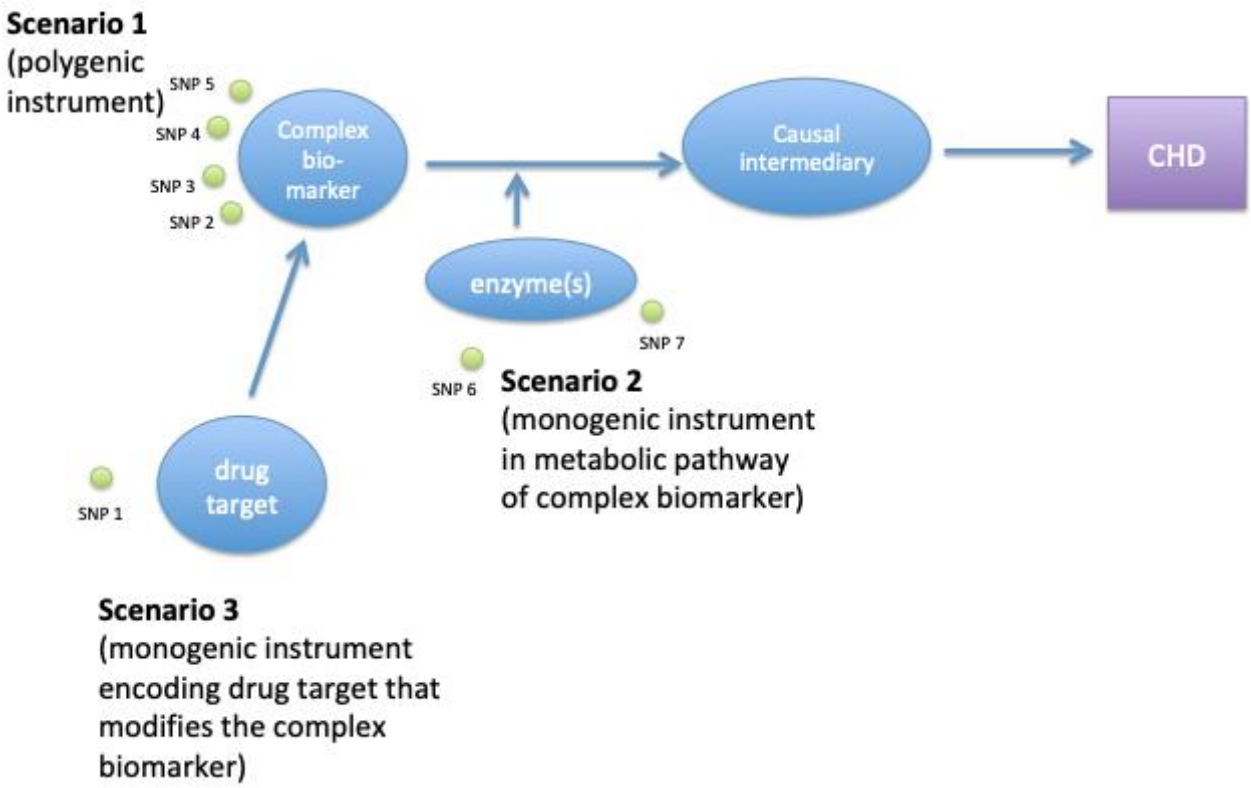
(1) *A polygenic biomarker*, for example, body mass index (BMI) or high-density lipoprotein cholesterol (HDL-C), the optimal approach is to use a polygenic instrument with as many SNPs as possible that reliably associate with the exposure of interest, while avoiding weak instrument bias and the introduction of horizontal pleiotropy. This is typically conducted through the use of SNPs reaching GWAS significance. The reason for the general preference to use as many SNPs that reliably associate with the exposure as possible is to capture the full repertoire of the genetic architecture of the trait. The trade-off is that this will invariably mean that many of the SNPs in the instrument will associate with the exposure through unknown mechanisms. However, such an approach to constructing genetic instruments permits sensitivity analyses to be conducted allowing a thorough appraisal of the potential for violation of the instrumental variable assumptions through e.g. horizontal pleiotropy.

(2) *A biomarker where the biological synthesis or metabolism is clearly defined* – e.g. the metabolism of alcohol by aldehyde dehydrogenase (encoded for by variants in *ALDH2*). In this case, while it may be feasible and informative to use a polygenic instrument for alcohol, it may be preferable to use one or more SNPs (a monogenic instrument) of known function that influence either the synthesis or metabolism of the biomarker[142]. The principal advantage here is specificity of the instrument. This comes at a strategic shortcoming: that the instrument

specificity relies on the biological plausibility of such specificity. A phenotypic scan of monogenic vs polygenic instrument and both instruments vs phenotype can help unravel such, but it may be challenging to interpret, owing to the potential issue of differential confounding (i.e. confounding differentially affecting the polygenic- vs monogenic -MR and observational analyses).

(3) Drug target– in this case there are three separate approaches that can be undertaken. (i) use only *cis* instruments; (ii) use only *trans* instruments (see Box 5 for definition); (iii) using both *cis* and *trans* instruments. The advantages of using *cis* SNPs include specificity, and the possibility of assessing such specificity through co-localisation. The disadvantages of using cis-only includes potential lack of power owing to an insufficient proportion of variance of the exposure explained, and potential inability to conduct sensitivity analyses that require multiple variants (e.g. MR-Egger and weighted median approaches, *inter alia*). Use of *trans* SNPs by definition includes SNPs that associate through either an upstream mechanism or gene regulatory functions, that may lead to biased estimates (see **Figure 6**), but which can potentially be (a) compared to the *cis* instrument for comparability and (b) analysed for pleiotropy using well-established MR approaches.

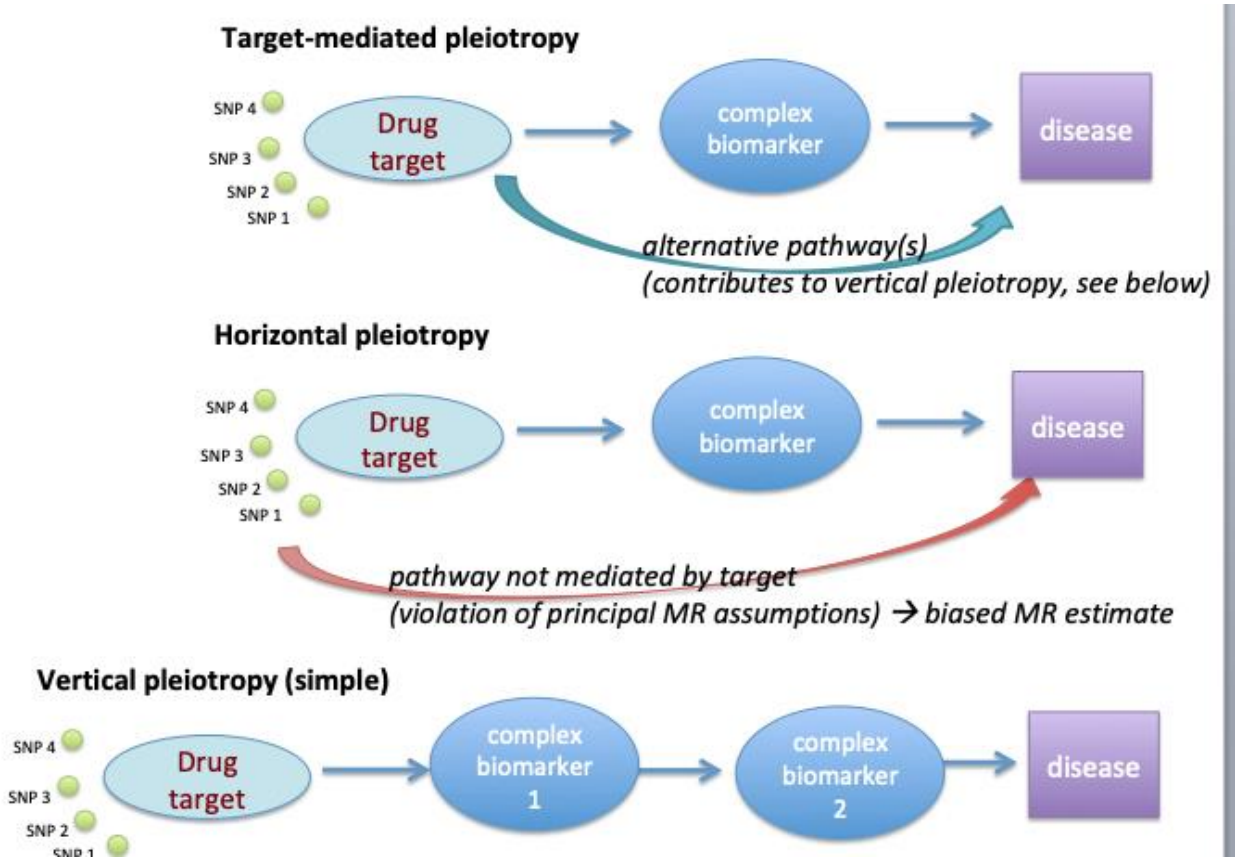
Figure to accompany Box 4

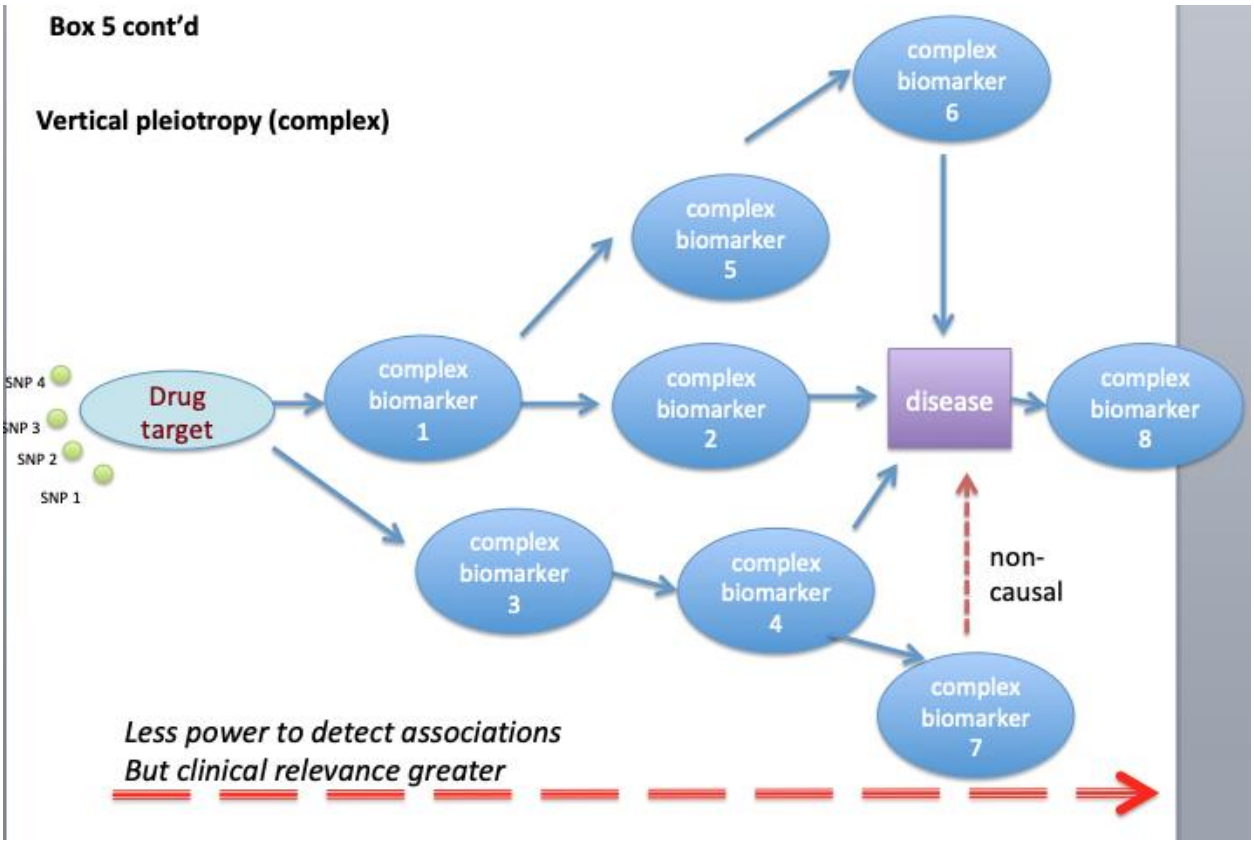


Box 5. Pleiotropy as applied to drug target Mendelian randomization

In this article we describe various forms of pleiotropy – namely that which is **target-mediated**, **horizontal**, and **vertical**. **Target mediated pleiotropy** is the situation where a drug target demonstrates effects that are discrete to those of the complex biomarker – this usually arises from pathways branching off upstream of the biomarker (see **Figure**). **Horizontal pleiotropy** is when the genetic instrument associates with the outcome through pathways other than those

which are mediated through the primary exposure (in this case a protein), and can violate the exclusion restriction criterion of instrumental variable analysis. **Vertical pleiotropy** is the principle on which Mendelian randomization rests – i.e. the association of the genetic instrument with traits (and disease) owing to them being downstream consequences of the protein drug target.





Box 6: *Cis* and *trans* genetic variants

Genetic variants that are within or close to gene that encodes for the protein of interest can affect proteins either qualitatively or quantitatively, with the latter leading to e.g. lower levels of a protein. These proximal genetic variants are known as *cis*-acting variants. By altering the exposure of interest (i.e. the protein) through the gene that directly encodes it, such cis-acting variants are likely to have effects specific to the protein of interest, and are less likely to be pleiotropic. In contrast, genetic variants that affect a protein that are not close to the gene that encodes the protein of interest are known as *trans*-acting variants. By virtue of being distal to the gene-encoding locus, trans-acting variants are more likely to have pleiotropic effects that affect multiple pathways (as illustrated in **Figure 6**). As a result, trans-acting variants can be more challenging to interpret in protein drug-target MR studies, as their effects can be

mediated by multiple pathways, some of which might be pleiotropic and lead to confounded interpretations from MR.

Box 7. Use of tissue-specific gene expression data for drug-target MR

Proteins are synthesized following the transcription of DNA to messenger RNA (mRNA), with mRNA being subsequently translated, through transfer RNA, into amino acids, which are polymerised into proteins. Characterisation of mRNA provides a means to quantify gene expression in tissues, and evaluate pre-translational effects of genes, especially those encoding drug targets, across different tissue types [143]. Increasing availability of genetic data linked to tissue-specific mRNA expression in human tissues (so-called expression quantitative trait loci, or eQTL) makes it possible to leverage this information to help guide instrument selection for drug-target MR. Data from gene expression in human tissues (so-called expression quantitative trait loci, or eQTL) are increasingly available making it possible to leverage this information to help guide instrument selection for drug-target MR. For example, one is the earliest LDL cholesterol signals detected by GWAS was at the locus on chromosome 1p13, likely caused by the *SORT1* gene [144]. Applying the principals of MR to evaluate the genetically predicted effects of *SORT1* expression on LDL cholesterol highlights the importance of tissue-specificity when selecting eQTL as instruments[145]. The genotype-tissue expression (GTEx) project has provided evidence that expression of *SORT1* in liver tissues affects LDL cholesterol levels (Beta=0.067, SE=0.005, P=7.03x10^{-38}) [143]. As LDL cholesterol is synthesised by the liver, using gene expression data derived from this organ is likely the most pertinent in capturing the functional mechanism responsible for this GWAS signal. In contrast, using eQTL derived from other tissues types which

are not relevant to LDL cholesterol synthesis incorrectly implicates *PSRC1* as the putative causal gene at this locus (e.g. skin tissue Beta=0.063, SE=0.011, P=8.38x10^{-09}).

A major limitation currently in using these tissue-specific molecular datasets is that the sample sizes are typically modest compared to GWAS standards (e.g. the latest version of GTEx ranges between n=21 and n=755 across tissues). This often results in having only a single independent cis-regulatory variant for MR analyses, meaning that findings need to be subsidised by evidence from genetic colocalization analyses (see refs[83-85]). Another challenge encountered is disentangling the effects of nearby genes which are often co-expressed and thus correlated, which may suggest that a non-causal gene as responsible for a GWAS signal (e.g. as described above for *PSRC1* and LDL cholesterol). Furthermore, publicly available data on proteins are predominantly derived using whole blood rather than disease-relevant human tissues, which will be preferential for drug target validation purposes once they are accessible at the same scale and coverage as eQTL datasets. Once this is the case, they are likely to be a valuable source of instruments which may be capable of detecting effects potentially overlooked by analysing data from the plasma proteome[146].

Box 8. Heterogeneity of treatment effects

An increasingly seen criticism of randomized controlled trials is that they are carried out in highly selected populations, and therefore their findings may not be generalizable to other patient groups [99,147]. MR can contribute here by using genetic proxies for treatments to examine whether the same predicted effects of either biomarker change or drug treatment are

seen in different subgroups of the population. For example, an MR study of body mass index (BMI) effects on type II diabetes yielded the same predicted relative risk reduction for a given difference in BMI among groups at different level of risk [148]. Similar analyses could be done using drug targets across risk groups. Such groups can include those taking other drugs, either though applying MR in those taking such agents, or by proxying both drugs using MR – as, for example, in a factorial study of MR predicted effects of PCSK9 inhibition and HMGCR inhibition on CHD and type II diabetes [107]. It is also possible to test for interactions of effects of a genetic proxy for drug treatment with genome wide genetic variation, to examine whether there is any evidence of overall heterogeneity of effect, with a study of *HMGCR* finding no strong predicted effect modification [149,150]. These analyses can be useful for prospectively designing clinical trials, in order to maximize effect estimates, and also as an approach to stratified medicines.

Box 9. Promoting equitable development of therapeutic targets through Mendelian randomization

As the conduct of Mendelian randomization studies becomes increasingly feasible, facilitated by availability of data and analytical platforms permitting rapid analyses at scale, an important question is whether our focus is on asking the right questions? For cardiovascular disease drug development, this should ensure that drugs are developed equitably, ensuring that effective treatments are developed and made available for minority under-represented groups.

Cardiovascular disease and risk factors for CVD disproportionately affects individuals of lower socioeconomic status, is unevenly balanced between women and men, and incidence is higher in individuals of certain ethnic and minority groups (e.g. Asians [151,152]). Women and minorities

have historically been under investigated and under-represented in clinical research[153]. It is plausible that the relationship between risk factors and disease may differ meaningfully between women and men, with potential consequences on providing appropriate treatment and preventative strategies [154]. In clinical trials, a difference in treatment effect is typically investigated by a priori defined subgroup analyses where the effect of an intervention on an outcome is stratified by sex, and analysis permits quantification of whether there is heterogeneity in treatment effects between women and men. In Mendelian randomization, a similar approach can be used[155], but requires additional features including representation of minority groups in GWAS. As of 2017, almost 90% of participants in GWAS were European ancestry [156]. If the genetic architecture of the exposure (be it a drug target or complex biomarker) and outcome differs between the strata under investigation (e.g. defined by sex), then ideally this should be reflected in MR analysis by using e.g. sex-stratified GWAS in order to select representative genetic instruments. This can pose challenges if genetic instruments are differentially available across strata and/or if biases exist (e.g. selection biases into the study that are differential by exposure and stratum [157]).

In a similar vein, GWAS in non-European ethnic groups can facilitate explorations of whether there are important differences of the causes of disease by ethnicity.