eGastroenterology

Drug-target Mendelian randomisation applied to metabolic dysfunction-associated steatotic liver disease: opportunities and challenges

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To cite: Luo S, Zheng M-H, Wong VW-S, *et al*. Drug-target Mendelian randomisation applied to metabolic dysfunction-associated steatotic liver disease: opportunities and challenges. *eGastroenterology* 2024;**2**:e100114. doi:10.1136/egastro-2024-100114

► Prepublication history for this paper is available online. To view these files, please visit the journal online (https://doi.org/10.1136/egastro-2024-100114).

Received 05 July 2024
Accepted 13 September 2024

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ABSTRACT

Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as the most prevalent cause of chronic liver disease worldwide affecting over one-third of the adult population. Despite the recent evolution of new nomenclature and diagnostic criteria for MASLD, progress in drug development for this condition remains limited. This review highlights the potential of drug-target Mendelian randomisation (MR), a study design that leverages human genetics and genomics, for the discovery, repositioning and safety assessment of drug targets in MASLD. We summarised key aspects of designing and appraising a drug-target MR study, discussing its inherent assumptions and considerations for instrument selection. Furthermore, we presented real-world examples from studies in MASLD which focused on opportunities and challenges in identifying novel drug targets, repositing existing drug targets, informing adjunctive treatments and addressing issues in paediatric MASLD.

INTRODUCTION

Liver disease accounts for approximately two million deaths annually[1] with non-alcoholic fatty liver disease (NAFLD) being one of the most prevalent conditions affecting 32.4% of the global population.[2] Recent advances in understanding the pathophysiology of metabolic dysfunction have led to the development of new nomenclature and diagnosis criteria for liver diseases.[3] The term steatotic liver disease encompasses various aetiologies of steatosis including metabolic dysfunction-associated steatotic liver disease (MASLD), alcohol-associated liver disease (ALD) and a combination of MASLD and ALD known as MetALD.[3] In this review, we used the new nomenclature MASLD, except when quoting previous studies based on older diagnostic criteria.

Despite the substantial global burden of MASLD, progress in drug development for this condition has been stagnating.[4] Real-world data, such as pharmacoepidemiological studies are susceptible to various sources of bias, including confounding by indication and immortal time bias.[5] Randomised controlled trials (RCTs), the gold standard for assessing treatment effects, can be costly, time-consuming and logistically challenging. These factors contribute at least partly to the relatively slow and high failure rate of investigational medications to reduce the burden of MASLD. As of 2024, the US Food and Drug Administration has approved Rezdiffra (resmetirom) for the first-ever treatment of patients with non-cirrhotic dysfunction-associated steatohepatitis, a specific form of MASLD.[6]

Recent evidence suggests that drug targets validated by human genetic evidence are 2.6 times more likely to succeed in clinical development,[7] thereby potentially enhancing the cost-effectiveness of the drug development pipeline.[8] One such genetic approach is Mendelian randomisation (MR), an instrumental variable analysis leveraging naturally randomised genetic variants to infer causal relationships within observational data. The independent segregation of alleles at conception creates a natural experimental analogous to randomised trials, theoretically rendering MR studies less susceptible to confounding and reverse causation.[9] Over the past decade, the applications of MR have significantly increased in fields such as cardiology,[10] oncology,[11] neurology,[12] COVID-19[13] and hepatology.[14] Recently, an emerging subfield of drug-target MR has evolved employing specific methodological approaches for assessing the therapeutic potential and adverse effects of drug targets.[15]

In this review, we concentrate on the application of human genetics and genomics for drug target discovery, repositioning and safety assessment in MASLD using MR. We

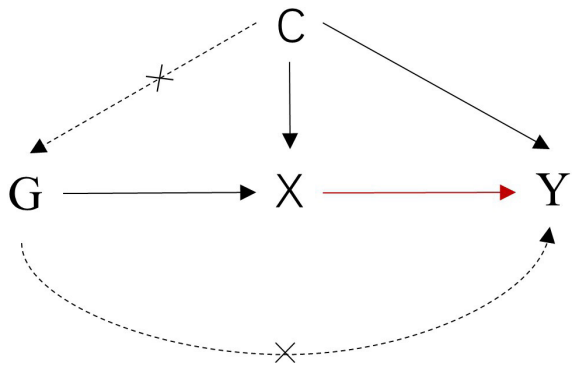

Figure 1 Directed acyclic graph of a Mendelian randomisation study. Nodes are presented in boldface and labelled paths represent the effects between nodes. G represents a genetic variant, X the exposure, Y the outcome and C (potential unmeasured) common causes of X and Y. Instrumental variable assumption. (1) Relevance: genetic variant G is associated with the exposure X; (2) independence: the association of genetic variant G and the outcome Y is not confounded; (3) exclusion restriction: genetic variant G does not affect the outcome Y except through its effect on the exposure. The causal effect of interest is the exposure on the outcome Y, depicted in solid red lines.

delve into key aspects of designing and evaluating a drug-target MR study including its inherent assumptions and considerations for instrument selection. Furthermore, we provide real-world examples demonstrating the integration of genome-wide and drug-target MR highlighting their application in identifying emerging drug targets, informing adjunctive treatments, repositing drug targets and addressing paediatric MASLD.

THE ASSUMPTIONS OF MR

All study designs have their own assumption and MR is no exception. A valid genetic instrument (instrumental variable) used in MR must satisfy three core assumptions. The instrument should be associated with the exposure (relevance); the association of the instrument in the outcome is not confounded (independence); the instrument should only affect the outcome through the exposure (exclusion restriction, also known as 'no horizontal pleiotropy'), as shown in figure 1.[9] In the context of drug-target MR, the genetically predicted or proxied exposure represents a measure of pharmacological perturbation of the relevance drug target.[16] It is important to note that instruments for drug target perturbation and drug usage or prescription are not equivalent or interchangeable. This distinction has been demonstrated by the controversy surrounding the investigation of medication effects via inappropriate MR.[17]

STATISTICALLY VERSUS BIOLOGICALLY INSTRUMENT SELECTION FOR MR

The identification and validation of appropriate genetic variants as instrumental variables for an exposure is fundamental to the design and interpretation of all MR investigations. There are two main strategies for instrument selection: A statistically driven approach and a biologically driven approach, figure 2. In a statistically driven approach, all variants robustly associated with the exposure (often using a p value below the genome-wide significance threshold of 5×10^{-8}) across the entire genome are included regardless of their biological function. This approach is also referred to as 'polygenic' or 'genome-wide' MR.[18] While the inclusion of more variants may increase statistical power, it also raises the likelihood of incorporating invalid, horizontally pleiotropic instruments, potentially introducing bias into the analysis. By contrast, a biologically driven approach typically selects variants from a single gene region with a known biological link to the exposure. This approach may likely yield a more valid inference when investigating proximal gene products such as proteins or gene expression levels as the exposures, based on established functional relevance of the instrument to the exposure. Prior to the emergence of large genome-wide association studies (GWAS), earlier MR studies also employed this approach in selecting instruments such as *FTO* for obesity[19] and *ALDH2* and *ADH1B* for alcohol consumption.[20 21]

INSTRUMENT SELECTION CONSIDERATION FOR DRUG-TARGET MR

Drug-target MR focuses on locating a gene known to encode a druggable protein, also known as *cis*-MR, and genetic variants within or near the gene of interest are used to characterise the effect(s) of the drug target on a single or multiple outcome(s).[16] Ideally, selecting causal variants known to affect the drug target while maximising precision (statistical power) is preferred. However, typically the nature and number of causal variant(s) is unknown and hence impeding the instrument selection process.[16] There is no gold standard strategy for instrument selection and the optimal approach often depends on the specific investigation and available data. Here, we outline several key model decisions.

The location of genetic variants relative to protein-encoding gene

Cis-acting genetic variants are within or near the protein-encoding gene of interest. The optimal distance between the variant and the gene has not been standardised and efforts to define the ideal range for determining *cis* versus *trans* functions are ongoing.[22] Some previous drug-target MR studies used stringent selection criteria for location, such as variants within 100 kilobase pairs upstream and downstream of a protein-encoding gene or transcription start site.[23] In contrast, others have used a broader flanking region (eg, 1 megabase (Mb) or even 5 Mb) to include more variants.[24 25] However, using a broader genetic flanking region may include variants located

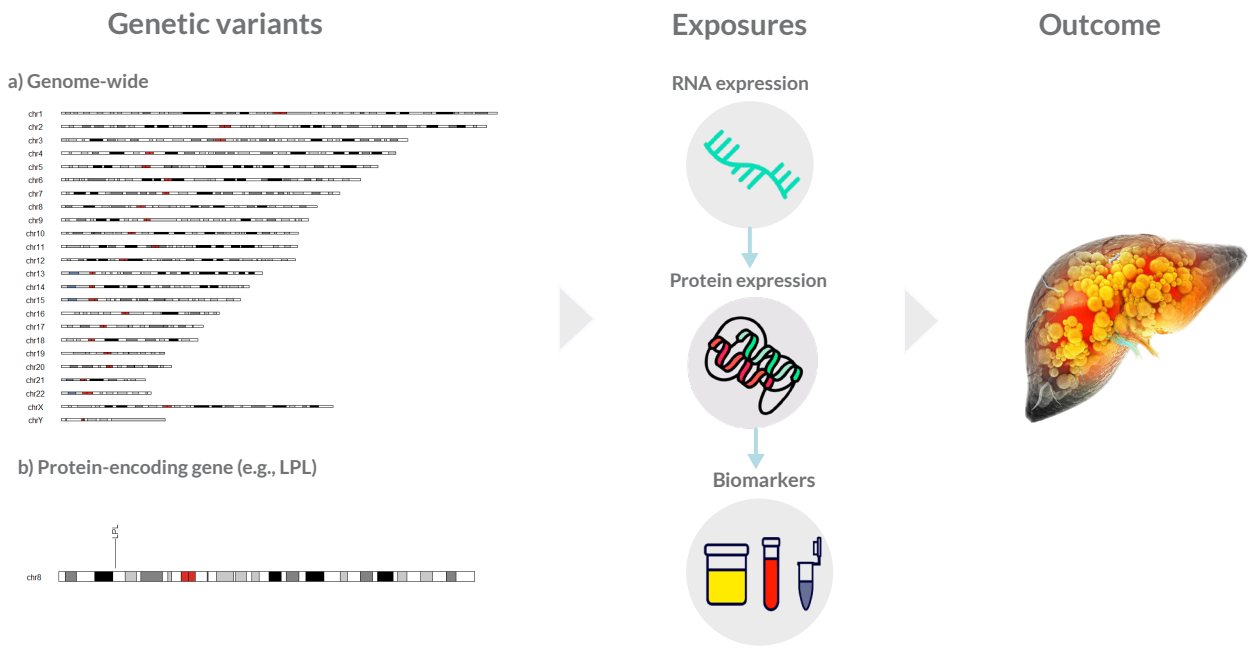

Figure 2 Schematic diagram of Mendelian randomisation study designs in metabolic dysfunction-associated steatotic liver disease. This figure illustrates two distinct approaches on genetic variants selection. The statistically driven approach, often termed as 'genome-wide' or 'polygenic' Mendelian randomisation, employs all genetic variants associated with the exposure across the entire genome. The biologically driven approach, typically referred to as 'drug-target' Mendelian randomisation, selectively uses genetic variants from a single gene region with a known biological link to the exposure. The selection of exposures could be based on various phenotypes that reflects a measure of pharmacological perturbation of drug target, including gene expression, protein expression at disease relevant tissue(s), downstream biomarkers and clinical outcomes. LPL, lipoprotein lipase.

at neighbouring genes (*trans*-acting) not encoding the drug target of interest and may erroneously model effects due to horizontal pleiotropy (ie, violation of exclusion restriction assumption).

Independent versus correlated variants within protein-encoding gene

Genetic instruments should be conditionally independent genetic predictors of the putative causal trait. In the context of *cis*-MR, a single genetic variant with the smallest univariate p value in the protein-encoding region is typically used as the instrument. The putative causal effect estimate can be obtained using the Wald ratio with a single instrument. However, this approach precludes the applications of robust methods for sensitivity analyses that require multiple instruments, such as the weighted median and MR-Egger, which are commonly employed in genome-wide MR analyses. In *cis*-MR, multiple *cis*-variants may be available if the GWAS is sufficiently large. However, using variants from the same gene region could violate assumptions underlying sensitivity analyses due to shared pleiotropy and non-independence.[26] Therefore, genetic colocalisation analysis is often used as a complementary analysis to *cis*-MR to assess potential biases arising from linkage disequilibrium (LD).[27] When multiple causal variants are presented within the protein-encoding gene, single-variant MR may not adequately capture all the genetic effects in the region, potentially leading to a loss of statistical power. Conversely, including all genetic variants from the same gene region may result in numerical instability due to multicollinearity among the variants.[28] To enhance statistical power, researchers often select multiple candidate variants that are in partial LD as instruments. Various techniques have been introduced for this purpose including stepwise-pruning, conditional analysis, principal component analysis, factor analysis and Bayesian variable section.[28]

Stepwise-pruning and conditional analysis both depend on a correlation threshold parameter. The correlated instruments inverse-variance weighted method (also known as the generalised least squares method) is used to account for the genetic covariance matrix.[28] There is no consensus on the optimal choice of LD correlation threshold. Empirically, employing larger thresholds ($r^2 \geq 0.8$) will result in a numerically unstable causal effect estimate due to the inversion of an ill-conditioned genetic correlation matrix.[16] Principal component analysis is widely used in GWAS to adjust for population stratification.[29] In *cis*-MR, its goal is to identify linear combinations of variants that are orthogonal to each other as an instrument and explain either 99% or 99.9% of the variance in the genetic data.[30] Where weak instrument bias is a concern, factor analysis and Bayesian variable selection (eg, joint analysis of marginal summary statistics) can provide more reliable estimates.[28 31]

The choice of exposure to weight the genetic associations

The availability of high-throughput proteomics platforms, such as the aptamer-based multiplex protein assay SomaScan 11K Assay V.5.0 (SomaLogic) and the antibody-based affinity reagents Olink Explore 3072, enables large-scale drug-target MR, also known as 'proteome-wide MR'. These platforms measure biological targets of many approved or developmental therapeutics, significantly improving drug development yield with available circulating proteins as well as other novel unknown opportunities for drug targeting.

Ideally, a drug-target MR will be most reliable when using protein abundance as the exposure where these variants are known as protein expression quantitative loci (pQTL) measured in disease-relevant tissues when available. For example, previous studies integrated human proteome data derived from brain tissue to identify and prioritise drug targets for neurological phenotypes (eg, Alzheimer disease).[32] Although tissue-specific protein data has not yet been generated at a large scale,[33] circulating protein levels are more readily accessible and offer a practical alternative in large biobanks, such as the UK Biobank Pharma Proteomics Project.[34] However, these data may not necessarily capture biological effects from tissue types that are more relevant to the disease being studied.[32]

When a circulating protein is unavailable or does not accurately represent the protein perturbation arising from drug action, researchers can use other phenotypes upstream or downstream of the druggable protein to search for relevant genetic variants and corresponding weights for MR analyses. The weights refer to the effect sizes of these genetic variants on the relevant phenotypes. For upstream phenotypes, this can be achieved by using blood gene expression quantitative loci (eQTL) from eQTLGen consortium[35] and/or tissue-specific eQTL from the Genotype-Tissue Expression consortium.[36] Prior study showed that genetic effects on circulating protein abundance are often but not exclusively driven by regulation of transcription.[37] However, the tissue-specific heterogeneity in eQTL/pQTL, if present, likely reflects actual biological differences between tissues. In drug development, ascertaining the tissue specificity is essential for ensuring that the drug exerts appropriate pharmacokinetic properties while minimising the risk of potential adverse effects in unrelated tissues.[15]

Where a drug exerts specific action on a protein and subsequently influences the downstream trait, drug-target MR using weights from the genetic associations with known downstream traits (biomarker or disease outcome) may provide a valid test for the effect of protein on disease. An example in cardiovascular medicine was lipid-modifying medication which involves the use of low-density lipoprotein cholesterol (LDL-C) to locate known drug targets encoding loci HMGCR (statins), NPC1L1 (ezetimibe), PCSK9 (PCSK9 inhibitors) for inferring the effects of pharmacological perturbations on coronary artery disease[38] and subsequently investigation on MASLD.[39] Similar examples include using glycated haemoglobin (HbA_{1c}) reduction to identify functional variants related to antidiabetic drugs action[40] and systolic blood pressure reduction for antihypertensive drugs to inform drug efficacy and potential side effects.[41] Alternatively, genetic variants may be weighted by their association with a binary disease outcome which is an intermediate phenotype in the pathway between the exposure and the outcome of interest. For example, recent studies have used variants associated with type 2 diabetes and HbA_{1c} reduction to identify functional variants related to glucagon-like peptide-1 receptor agonists (GLP1-RA).[42 43]

The principle of instrument selection should be to maximise statistical power while safeguarding against incorrect inferences. If selected genetic variants in the flanking region do not mimic the effects of pharmacological perturbation of the drug, this may raise concerns over the validity of the drug-target MR study. Ideally, selected instruments should be validated using positive control outcome(s) such as a clinically confirmed indication of the drug. Employing various instrument selection methods as sensitivity analyses can enhance the reliability of the study.

INTEGRATION OF GENOME-WIDE AND DRUG-TARGET MR

Combining genome-wide and drug-target MR analyses, where feasible, can address complementary research questions. The genome-wide MR establishes a causal relationship between a modifiable exposure which is typically a downstream phenotype of the drug (eg, LDL-C), and the outcome. In contrast, the drug-target MR investigates whether perturbation of this exposure through a specific drug target or mechanism (eg, LDL-C reduction via HMGCR inhibition) could mitigate the outcome risk. A prime example in cardiovascular medicine is the causal role of LDL-C in coronary artery disease with lowering plasma LDL-C levels via therapeutic targets (eg, HMGCR, PCSK9 and NPC1L1) consistently reducing the risk of coronary artery disease.[38]

Of note, it is plausible that targeting the same phenotype via different mechanisms can yield varying effects on outcomes. For example, accumulating evidence from genome-wide MR studies consistently suggests a positive association between plasma triglycerides and NAFLD.[39 44–46] However, recent drug-target MR studies have found that among various therapeutic targets (PPARA, ANGPTL3, ANGPTL4, APOC3 and LPL) aim at lowering plasma triglycerides, only LPL (lipoprotein lipase) reduces the risk of NAFLD.[39 47] LPL, the main enzyme regulating the hydrolysis of triglycerides-rich lipoproteins,[48] is biologically relevant to excessive accumulation of hepatic triglycerides, a characteristic pathological feature of MASLD. Thus, targeting LPL activation may represent a unique therapeutic strategy to mitigate MASLD risk. Moreover, insights into mechanistic nuances within broader drug indication categories (eg, triglycerides-modifying agents) can enhance our

understanding of the underlying biological mechanisms through metabolic profiling.[38 49] A deeper understanding of this mechanism could aid in refining drug development strategies, ultimately leading to more targeted and effective interventions for the outcome.

TARGETING THE LPL PATHWAY THROUGH ANGPTL3 TO LOWER MASLD RISK: INSIGHTS FROM RARE LOSS-OF-FUNCTION AND COMMON VARIANTS

Angiopoietin-like protein 3 (ANGPTL3), a hepatokine secreted by the liver, acts as an endogenous inhibitor of LPL, resulting in the intravascular clearance of plasma triglycerides.[50] Individuals with heterozygous predicted loss-of-function (pLoF) variants at *ANGPTL3* locus have significantly lowered triglyceride and a decreased risk of coronary artery disease.[51] Several therapeutic strategies targeting ANGPTL3 are under investigation, including pharmaceutical inhibition, hepatically-targeted antisense oligonucleotide inhibition (Vupanorsen),[52] neutralisation of circulating ANGPTL3 protein by monoclonal antibodies (Evinacumab)[53] and CRISPR gene editing to introduce LoF mutations in *ANGPTL3* locus.[54]

Drug-target MR is able to mimic the effects of different modes of action for a therapeutic target and inform the design of clinical trials. In the case of ANGPTL3, recent studies have employed liver *cis*-eQTL,[49] *cis*-pQTL and protein-truncating variants (PTVs)[47 55] to mimic the effects of RNA-based therapies, antibodies and gene-editing strategies, respectively.[56] Genetic inhibition of ANGPTL3 via these approaches has all demonstrated significant reductions in lipoprotein-lipid levels (eg, triglycerides and LDL-C) but no effects on coronary artery disease, NAFLD or hepatic fat fraction.[56] However, a recent meta-analysis of LoF variant genetic association studies found that lifelong genetic inactivation of ANGPTL3 confers protection against coronary artery disease.[47]

The pLoF variants, including PTVs, can provide valuable insights into the function of specific genes. Studying the disease profile of individuals with pLoF variants has correctly predicted the efficacy and safety of therapeutic targets corresponding to these genes, as seen in the cases of PCSK9 and ANGPTL3.[57] This can be achieved from next generation sequencing techniques, such as whole-exome sequencing and whole-genome sequencing, with data from large-scale biobanks.[58 59] However, pLoF variants are often rare in the general population which may limit the reliability of drug-target MR in providing quantitative estimates of the causal effects of therapeutic interventions.

INFORMING ADJUNCTIVE TREATMENT USING FACTORIAL MR

The multifaceted pathophysiological nature of MASLD, as reflected by its new nomenclature, suggests that future clinical trials for MASLD should be oriented towards combination interventions or therapies.[60] Using the MR approach in a factorial design can help investigate interactions between two (or potentially more) distinct exposures as well as explore interactions between pharmacological interventions on an outcome similar to a factorial RCT.[61] In a factorial MR study, participants are categorised into different levels of each exposure based on their genetic risk score which is the sum of risk alleles corresponding to the exposure of interest, with or without weighting by their genetic associations with the exposure. In the simplest 2×2 factorial MR design, the study population can be divided into four groups using dichotomised genetic risk scores at their median, ensuring balanced numbers of participants across each group. These subgroups are analogous to 2×2 factorial RCTs corresponding to no intervention, intervention A only, intervention B only and both interventions A and B, figure 3.[61]

For example, Cater *et al* explored the joint effects of body mass index (BMI) and alcohol consumption with liver injury biomarkers and incident liver disease using 2×2 factorial MR design[62] with reference to the study by Ference *et al*.[63] The study suggested that combined interventions, targeting both BMI and alcohol consumption, could potentially lead to a greater reduction in population levels of liver injury than interventions that aimed at reducing either BMI or alcohol use individually.[62] This approach has also been employed to compare the joint effect of drugs belonging to the same class (eg, LDL-C lowering targets HMGCR, PCSK9, NPC1L1 and ACLY)[24] and different classes (eg, intervention on interleukin-6 receptor (IL6R) signalling and LDL-C lowering therapies) on coronary artery disease.[64]

A previous methodological review has indicated that, despite the large sample size in previous applications of factorial MR, the statistical power to detect a statistical interaction using a dichotomised genetic risk score at the median is generally inefficient.[61] More recent applications using continuous genetic risk scores have shown improved efficiency compared with using dichotomised scores.[64 65] Efficiency can be optimised by maximising the difference in the mean levels of risk factors across sufficiently large groups to detect statistical interactions. This can be achieved either by identifying a natural break in the risk factor distribution or by establishing a threshold that divides the population into equal-sized groups as much as possible.[61]

DRUG TARGET REPOSITIONING FOR MASLD

Drug repositioning, or repurposing, is a cost-effective and time-effective strategy that involves discovering new therapeutic indications for existing drugs that have already undergone safety and efficacy testing for their initial indication.[66] The drug-target MR approach has successfully identified repositioning opportunities and prioritised clinical trials of drug targets for early management of COVID-19.[67] To successfully reposition a drug, it is essential to understand its known molecular mechanisms of action and to identify plausible genetic instruments that

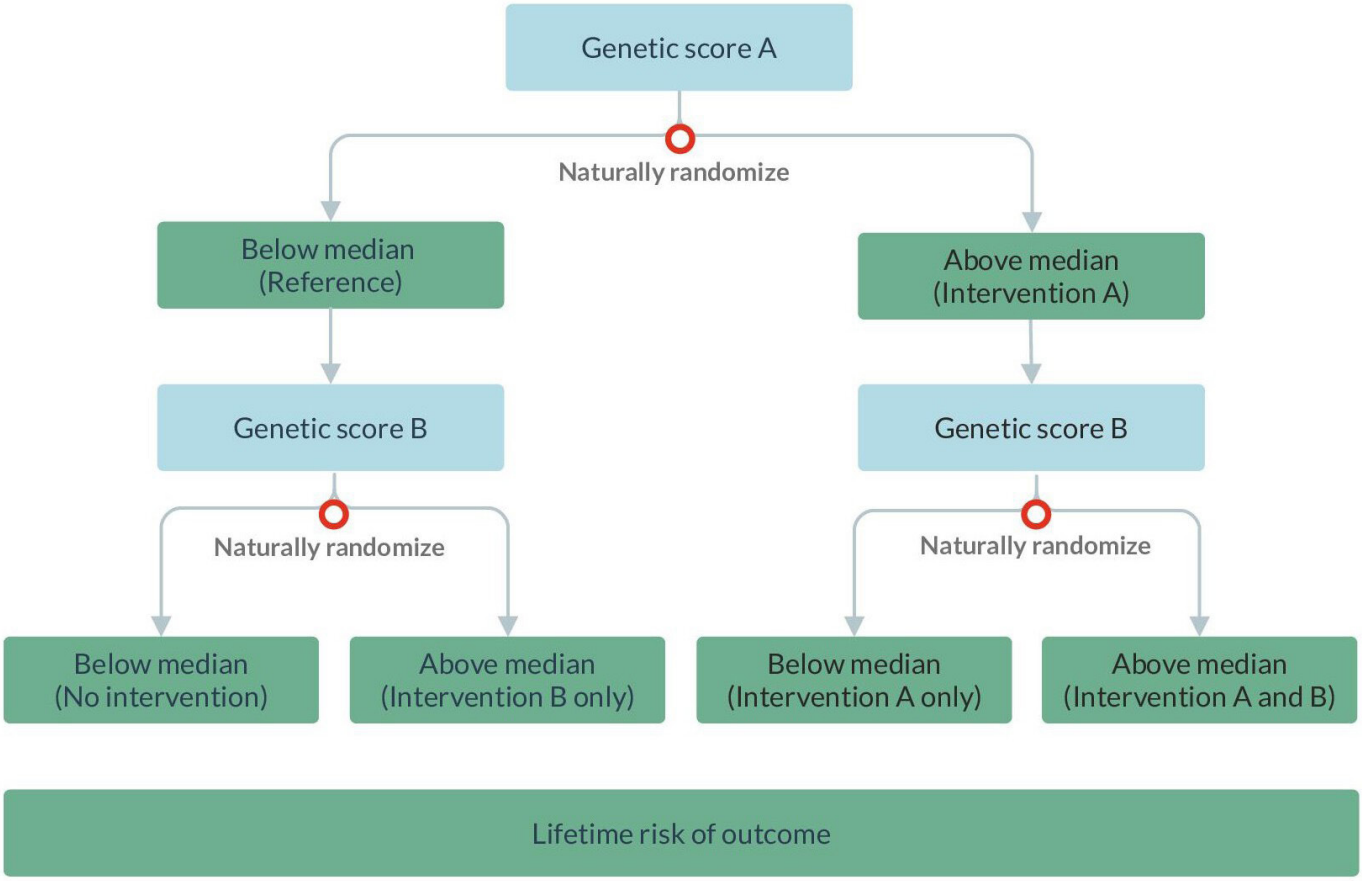

Figure 3 Schematic diagram illustrating a 2×2 factorial Mendelian randomisation design with dichotomised genetic risk scores that acts proxies for interventions on exposures A and exposure B.

can mimic these effects. For example, the genetic mimicry IL-6 receptor inhibition recapitulates the established downstream inflammatory signalling pathways such as increased soluble IL-6 receptor and circulating IL-6 levels and along with decreased levels of C-reactive protein and fibrinogen. This mechanism has been suggested to confer protective effects against COVID-19[68 69] as subsequently corroborated by RCTs.[70]

Similarly, drug-target repositioning holds promise for addressing the epidemic of MASLD and preventing its complications including cirrhosis and hepatocellular carcinoma. Current pharmacological interventions under evaluation include those targeting energy efficiency and disposal such as GLP1-RA and sodium-glucose cotransporter 2 (SGLT2) inhibitors[71–73] as well as those mitigating lipotoxic liver injury and associated inflammation and fibrosis such as metformin and aspirin.[74] However, challenges may arise when plausible genetic instruments are unavailable for certain drug targets, when the drug's mechanisms of action are unclear or when a drug exerts its effects through multiple pathways.

SGLT2 inhibitors are effective antidiabetic drugs but the extensive pleiotropic effects of SGLT2 inhibitors extend beyond their known glycaemic control via urinary sodium excretion.[75] A previous study using variants within the solute carrier family 5 member 2 (*SLC5A2*) gene which encodes SGLT2 did not identify any common variants associated with both HbA_{1c} and urinary sodium excretion (positive control outcomes).[76] Another study using a rare LoF variant *SLC5A2* rs61742739 (Asn654Ser) associated with familial renal glucosuria suggested cardioprotective effects are not explained by glycaemic control.[77] This is consistent with findings from large cardiovascular outcome trials which indicates the cardiometabolic protection is unlikely related to improvement in glycaemic control.[75] Thus, previous drug-target MR studies that selected genetic instruments based on glycaemic traits may not be appropriate[78] especially these glycaemic traits may not be equivalent to each other.[79]

Aspirin, a non-steroidal anti-inflammatory drug, is being repositioned in clinical trials as a treatment for MASLD.[80] Like metformin and certain antihypertensive agents such as calcium channel blockers, aspirin has a complex pharmacological profile likely targeting multiple proteins.[81] Accurately assessing the overall effect of such a drug requires considering the proportions of its effects on each target, provided this information is available from pharmacological studies. This task becomes increasingly complex when the drug's targets and mechanisms of action are still under investigation as seen with metformin.[82] In these circumstances, deciphering

the drug's target-specific effects could provide valuable insights for future target-specific drug trials.

Lastly, MR may not necessarily instrument a drug that simultaneously influences multiple parallel biological pathways. For example, metformin's weight loss effect, which inhibits hepatic gluconeogenesis via an AMP-activated protein kinase-dependent mechanism, may function independently of its ability to elevate circulating growth differentiate factor 15 which suppresses appetite.[83] In such cases, to model the total effect of the drug, molecular mediators may be instrumented individually[40] or in combination using a factorial MR approach. In another example, recent genetic evidence supports the findings from clinical trials that GLP1-RA exerts body weight reduction and glycaemic control through distinct signalling mechanisms.[27 84] A didactic example of how phenotypic heterogeneity at *GLP1R* locus is used in multivariable *cis*-MR design to investigate the mechanism and site-of-action of the causal effect of pharmacological intervention on a disease outcome.[85]

TACKLING PAEDIATRIC MASLD: DRUG EFFICACY AND SAFETY USING MR

The global epidemic of overweight and obesity has led to a growing prevalence of paediatric MASLD with far-reaching implications for childhood, adolescence and adulthood.[86] However, few data are available on the safety and efficacy of medicines in children and adolescents resulting in frequent off-label or unlicensed administration of medicines in everyday paediatric practice.[87] Obstacles hindering the development of paediatric indications for drugs primarily intended for the adults include a lack of suitable infrastructure for conducting paediatric clinical trials and difficulties in trial design including ethical concerns.[87] To date, off-patent drugs such as antioxidants (vitamin E) and insulin sensitisers (metformin) are being evaluated for their efficacy in treating children and adolescents with NAFLD.[88 89] However, these studies are limited by small sample sizes and short duration and hence may have limited statistical power to detect genuine efficacy and side effects.

Although there are generally more RCTs in adults, similarities and differences exist in the causes, natural history and prognosis of fatty liver diseases in children compared with adults.[90] This is also reflected by the new diagnostic criteria for MASLD in children and adolescents,[91] though some argue that diagnostic criteria for metabolic dysfunction-fatty liver disease (MAFLD) may better capture the impact of metabolic derangements on fibrosis.[92] While extrapolation from adult data is an important feature in the development of drugs intended for children, it is crucial not to assume that children will have similar disease progression or response to interventions found to be effective in adults including side effects.[93]

To address the knowledge gap regarding the efficacy and safety of medicines, drug-target MR studies have been implemented to ascertain the efficacy and possible side effects of medications in vulnerable populations such as adolescents[94] and pregnant women.[95] Such drug-target MR leveraging reliable surrogate and safety endpoints could be a more cost-effective study design to inform the efficacy and safety of therapeutic agents in reversing the course of MASLD/MAFLD in children and adolescents. By providing evidence on safety particularly regarding growth and sexual development, this approach could provide additional prior information on efficacy and safety for children and adolescents without exposing these vulnerable populations to unnecessary risk.

VARIATION IN PHENOTYPING OF MASLD

The validity of an MR study is directly related to the quality of GWAS data, in particular the classification of the disease and trait measurement. Liver biopsy is the reference standard for diagnosing and staging hepatic diseases. However, its invasive nature limits its usage especially in paediatric population. Among non-invasive methods, MRI-derived proton density fat fraction stands out for its superior accuracy in detecting and quantifying liver steatosis.[96] Nevertheless, its high cost and limited availability can restrict its widespread application especially among large-scale epidemiological studies. Liver enzymes, such as alanine aminotransferase and aspartate aminotransferase, are routinely assessed in primary care to screen for liver fibrosis. However, these biomarkers are non-specific and do not always correlate with liver disease severity.[96] Alternatively, the use of International Classification of Diseases codes in electronic health records diagnosis provides a feasible approach for population-based diagnosis but may lead to misclassification and is insufficient for assessing disease severity.

MASLD comprises a spectrum of progressive liver conditions ranging from isolated hepatic steatosis to metabolic dysfunction-associated steatohepatitis. Variants that contribute solely to the progression of steatohepatitis, fibrosis or cirrhosis without promoting the initial occurrence of steatosis may remain unidentified. To enhance the utility of drug-target MR, conducting GWAS on steatotic liver disease and its subcategories[3] diagnosed histologically or by imaging could provide more informative insights into treatment responses and disease progression.[97 98]

CONCLUSION

MR holds significant promise for advancing drug target identification and repositioning in MASLD, yet it is important to acknowledge its assumptions and limitations. The growing accessibility of large-scale multi-omics data provides unprecedented opportunities for progression in this field. Large-scale GWAS and/or next-generation sequencing of MASLD as well as employing state-of-the-art methodologies would be crucial for the conduct

of credible drug-target MR to facilitate drug development in the prevention and treatment of MASLD.

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Funding This work is supported by Seed Fund for Basic Science (2302101604), the University of Hong Kong.

Competing interests SLAY has received honoraria from SomaLogic for giving presentation on scientific work related to proteomics. MHZ has received honoraria for lectures from AstraZeneca, Hisky Medical Technologies and Novo Nordisk, consulting fees from Boehringer Ingelheim, serves as a consultant for Eieling Technology. The other authors declared no conflict of interest. VWSW is an Editorial Board Member for *eGastroenterology*.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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