Genetic Risk Factors and Mendelian Randomization in Cardiovascular Disease

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Abstract

Cardiovascular disease encompasses several diverse pathological states that place a heavy burden on individual and population health. The aetiological basis of many cardiovascular disorders is not fully understood. Growing knowledge of the genetic architecture underlying coronary heart disease, stroke, cardiac arrhythmias and peripheral vascular disease has confirmed some suspected causal pathways in these conditions but also uncovered many previously unknown mechanisms. Here, we consider the contribution of genetics to the understanding of cardiovascular disease risk. We evaluate the utility and relevance of findings from genome-wide association studies, and explore the role that Mendelian randomisation has to play in exploiting these. Mendelian randomisation permits robust causal inference in an area of research where this has been hampered by bias and confounding in observational studies. In doing so, it provides evidence for causal processes in cardiovascular disease that could represent novel targets for much-needed new drugs for disease prevention and treatment.

Introduction

Cardiovascular disease (CVD) is frequently cited as an illustrative example of a 'complex disease', and encompasses several pathological states and events, including hypertension, hyperlipidaemia, myocardial infarction (MI) and stroke. These share many features of aetiology, pathogenesis, risk factors and clinical presentation, but large variation exists between and within conventional disease categories. Differences in CVD manifestation across a population are ascribed to many behavioural, environmental and genetic determinants and the interactions between these. Ascertainment of the true contribution of each, their interrelationships, and the relevance of these to clinical practice and to individual and population health is a considerable scientific challenge. The genetic determinants of CVD risk have attracted increasing attention in recent years because of their unique capacity to inform on causation, prediction and novel therapies.

Two key scientific aims prevail in CVD research: 1) improvement in prediction of future disease events, and 2) understanding of the pathogenic process underlying disease, and by extension, identification of potential therapeutic and preventive targets. Existing methods for prediction of CVD events are informed by prospective observational studies, within which factors measured earlier in life are found to predict subsequent disease onset and progression[1–3]. Risk prediction models have clinical utility but are by no means definitive, leaving wide scope for improving the accuracy and reliability of prediction. Risk factors included in prediction models must inform estimation of future risk, but need not themselves be causal determinants of CVD. As observational studies continue to identify novel CVD risk factors[4], their relevance for prediction, aetiological investigation, or both, must be carefully considered. Some important risk factors have, however, been found to have both predictive and causal roles. Among these hyperlipidaemia[5], hypertension[6], systemic and local inflammation[7,8], and hyperglycaemia[9] are arguably the most influential. While these factors together contribute substantially to CVD pathogenesis, many of the causal pathways leading to CVD are not recognised or understood. Existing pharmacological therapeutic and preventive strategies considerably reduce CVD risk, and generally target well-characterised causal pathways, such as blood lipids, blood pressure and hyperglycaemia. However, even with optimal treatment with current drugs, a sizeable proportion of individual and population risk persists[10], leaving a need for novel treatments, and identification of additional pathogenic processes may elucidate targets for new, or existing drugs. Genetic research offers opportunities for investigating prediction, aetiology and novel therapeutics, which we review below with a focus on specifically cardiac aspects of CVD.

Identifying genetic risk factors

Much of the foundation of cardiovascular genetics has grown from investigation and understanding of the familial hyperlipidaemias. Familial hypercholesterolaemia (FH) is an autosomal dominant disease with a frequency of between 1/500 and 1/300 members of the general population. It is caused by rare variants in the genes encoding the low-density lipoprotein cholesterol (LDL-C) receptor (*LDLR* chromosome 19p13.2), apolipoprotein B (*APOB*, chromosome 2p24), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*, chromosome 1p32.2)[11]. Patients with FH have very high plasma LDL-C concentrations, accelerated atherogenesis and early onset of CVD events, in particular coronary heart disease (CHD). Concerted efforts in family screening and technological developments such as next-generation sequencing of whole exomes continue to identify new FH-causing genes[12–14], many of which are potential candidates for the development of novel therapies. Investigation of FH genetics has made important contributions to understanding of CVD pathogenesis, however these rare, highly penetrant mutations are responsible for only a small fraction of the global burden of CVD and leave many aetiological questions unanswered.

Identification of common genetic variants associated with CVD risk and related biomarkers, and their exploitation for translation has been the focus of genetic research in this field since the advent of genome-wide association (GWA) studies[15]. GWA studies seek to identify genetic variants across the genome that are associated with differences in CVD risk or CVD-related biomarkers, and have generated a broad and complex body of evidence for the genetic contribution to CVD risk[16]. In general, these variants are common and have small phenotypic effects. For example, odds ratios for MI associated with a GWA study-identified genetic variant are typically in the order of 1.10 to 1.20. GWA studies consequently require very large samples of tens of thousands of individuals to achieve sufficient statistical power, with data from several samples often combined using meta-analysis[17] (**Table 1**).

Coronary artery disease and its related biomarkers

Several GWA studies of CHD have identified common variants at a number of genomic loci in samples now including many thousands of cases and controls[17–19] (**Table 1**). Some identified loci are involved in known pathogenic pathways, including those related to blood lipids (*LDLR*, *PCSK9*, *APOE*). Also identified have been some less predictable loci - *ABO* (chromosome 9q34.2), involved in determining ABO blood type; *IL6R* (chromosome 1q21) - encoding the proinflammatory interleukin-6 receptor[17]; and a variant in an intergenic region of chromosome 9 (9p21.3) of which the function and biological relevance is now under close scrutiny but remains uncertain[20,21]. In addition to risk of disease events, GWA studies have examined genetic determinants of conventional CVD risk factors, including blood pressure[22], lipids[23], body composition[24], and smoking behaviour[25]. These studies have added to our understanding of CVD pathogenesis, and have begun to highlight potential drug targets for disease prevention. Notably, variants in *PCSK9* were first identified as causes of FH and were subsequently identified by general population GWA studies of LDL-C concentration and CHD risk. PCSK9 is the target of novel lipid-lowering drugs developed following the GWAS findings that are currently undergoing evaluation in phase III trials (ClinicalTrials.gov NCT01764633, NCT01975376).

Arrhythmias

GWA studies of heart rhythm disorders have identified a number of dysrhythmia-associated loci. As might be expected, many of the reported variants are in genes encoding ion channels. For example, variants in the potassium channel-encoding genes *HCN4* and *KCNN3* have been associated with risk of atrial fibrillation

(AF)[26,27]. In addition to studies of risk of specific arrhythmia diagnoses, GWA studies of quantitative ECG traits have also yielded important results. Variants in the gene encoding voltage-gated sodium channels (*SCN5A* and *SCN10A*) have been found to associate with QRS complex duration[28–30], and PR interval[28,31–33]. Reported by a number of GWA studies for its association with QT segment duration are variants at the *NOS1AP* locus encoding nitric oxide synthase 1 adaptor protein[32–34], for which a role in calcium channel-mediated myocardial repolarisation has been proposed[35]. These findings, while confirming previously suspected aetiological pathways may suggest promising targets for novel anti-arrhythmic drugs.

Heart failure

'Heart failure' encompasses a diverse group of phenotypes with different, though often overlapping aetiologies. This diversity has presented a challenge to those investigating heart failure genetics as detailed phenotyping is required to ensure accurate case ascertainment, and relatively few studies are reported. In a collaborative GWA study taking incident heart failure as a single disease entity in nearly 24,000 individuals, variants were reported near the *USP3* locus in people of European ancestry and *LRIG3* in those of African ancestry[36]. Of note, no other variants in that analysis met the genome-wide threshold for statistical significance ($p<1x10^{-7}$), possibly as a result of between- and within-study heterogeneity. Investigation of heart failure-related phenotypes has also yielded limited success, with only a handful of loci identified for their associations with dilated cardiomyopathy (*BAG3*, *ZBTB17*)[37] and left ventricular mass (*RAI14*, *CD36*)[38], though these were reported in relatively small samples. One GWA study of circulating N-terminal pro-brain natriuretic peptide (NT-proBNP), a biomarker of heart failure, reported associations at three loci, including the chloride channel, *CLCN6*[39]. Heart failure is an area where larger samples of finely phenotyped individuals may be needed to advance our understanding of the underlying genetics of its multiple constituent phenotypes, although the potential for greater understanding of the disease and new therapeutic strategies is great.

Valvular heart disease

Heart valve disease has been investigated by relatively few studies, but provides an important illustration of the investigative pathway from GWA study to Mendelian randomisation (MR) analysis. A 2013 GWA study of aortic and mitral valve calcification reported associations of a variant at the *LPA* locus with both circulating lipoprotein(a) (Lp(a)) concentrations and aortic valve calcification[40]. The authors also reported an MR analysis demonstrating that genetically raised Lp(a) concentrations were associated with aortic valve calcification, and supporting a causal role for Lp(a) in this complex phenotype. Similar findings were subsequently elsewhere[41,42], and the variants in *LPA* have also been reported by GWA studies of CHD[18,43]. Lp(a) appears, therefore, to be a causal determinant of more than one area of CVD, and offers a potentially important therapeutic opportunity.

Stroke

Findings from GWA studies of stroke have been less fruitful than for CHD, with a relative paucity of disease-associated variants. Notably, several loci showing genome-wide associations with ischaemic stroke are also associated with CHD. Among these are the chromosome 9p21 locus and *ABO* (chr9q34.2), both of which have been reported by a number of CHD GWA studies, and which show directionally concordant effects on both disease phenotypes[44]. Stroke presents a challenge similar to that of heart failure, encompassing a range of very different clinical phenotypes, broadly grouped as ischaemic stroke (including embolic, atherosclerotic,

large and small vessel subtypes), and intracranial haemorrhage. The differences in the phenotypes and the relative difficulty in precisely determining their aetiology has made investigation of stroke in GWA studies more challenging. A number of important loci with large effects on stroke have, however, been identified[45].

Abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) is a common disease of later life, which, if untreated, can rupture with high resultant mortality[46]. The genetic architecture AAA has been investigated using GWA studies, although these emerged some years after the first studies of CHD. The chromosome 9p21.3 region, strongly associated with CHD[47], has been shown also to contribute to AAA development with an associated excess risk of approximately 30% per allele[48], a finding that has been replicated in several subsequent studies[49]. Larger, later GWA studies have reported additional AAA-associated variants at the *DAB21P* locus (chr9q33)[50], *LRP1* (chr12q13.3)[51] and *LDLR* (chr 19p13.2)[52], and a large candidate gene study using variants in *IL6R* (chr1q21) confirmed a role in AAA for signalling at the interleukin-6 (IL-6) receptor[53]. Current conventional management of AAA comprises watchful waiting for low risk aneurysms, and endovascular interventions and open surgical repair for those at higher risk of rupture; there are no definitive pharmacotherapeutic options. As the genetic determinants of AAA has become clearer, potential novel therapeutic targets have emerged. Of particular note are the IL-6 receptor, for which a routinely prescribed inhibitor drug exists, and cholesterol pathways, which can be modified using a range of lipid-lowering agents. Findings from genetic studies may help to prioritise new targets for drugs that may help reduce risk of rupture in patients who do not meet prevailing criteria for interventional management.

Emerging approaches to CVD genetic discovery

Methods used to discover novel CVD-related genetic variants are beginning to shift away from the 'classical' GWA study, in a move driven principally by technological advances. The rapidly declining price of whole genome or exome sequencing[54] offers new means for ultrafine dissection of the genetic architecture of disease in large numbers of individuals. As large-scale sequencing projects progress (e.g. UK 100,000 Genomes project www.genomicsengland.co.uk/the-100000-genomes-project), these data may reveal novel, rarer genetic determinants of CVD risk. Furthermore, the range of available phenotypes continues to expand. Proteomic, metabolomic and lipidomic technology provides high-resolution CVD biomarkers of which the genetic foundations can be investigated. The profile of individuals included in genetic studies is also transforming. Early GWA studies were largely restricted to individuals of European ancestry. In the USA, cohorts including people of African and Hispanic ancestry are increasingly reporting genetic findings, and investigators in China, Japan and Korea are also publishing at an growing rate. All these developments provide opportunities to broaden and deepen our understanding of CVD through genetics.

Below, we explore how recent findings are being exploited in translational cardiovascular research.

Genetics and risk prediction

Could genetics add value to conventional risk prediction strategies?

CVD risk prediction tools currently in routine clinical use are based on findings relating to conventional risk factors such as age, sex, smoking status and blood lipids from prospective observational studies[1,2]. While these perform with reasonable accuracy, none has perfect specificity or sensitivity. Pursuit of improved prediction is ongoing, and use of genetic data has been proposed as a means of achieving this[55].

Attempts have been made to demonstrate the added value of incorporating genetic information into prediction models. These have largely involved a composite model of conventional and genetic risk factors, with the hypothesis that the genetic data will account for a sufficient proportion of unexplained risk to improve predictive utility. Integrating personal genomic information into conventional clinical assessment was shown to have merit for improving prediction in a single individual[56], but the yield of population-based studies using multi-locus predictive gene scores has been modest and inconsistent[57–62]. The reasons for this failure are not yet clear. One possibility is that the majority of CVD-associated variants identified by GWA studies influence disease risk through effects on variables already included in conventional prediction models, such as blood lipids. Another is that common genetic variants generally have such modest phenotypic effects that these are unable to contribute meaningfully to clinical practice where larger effects are sought. One strategy for overcoming this is to combine information from a number of CHD-associated SNPs in a genetic risk score[63] (discussed below). Despite early optimism for a role for genetics in CVD prediction, this has not yet become a reality in routine practice. Commercial offerings of direct-to-consumer personalised genetic prediction burgeoned in the years immediately following the completion of early GWA studies, but have now fallen from favour with both consumers and regulators[64]. The rapidly falling price of whole genome and exome sequencing may, however, facilitate greater success in this field and allow more successful prediction.

Mendelian randomisation in CVD

Introduction to Mendelian randomisation

MR uses genetic data to dissect the roles of CVD-associated risk factors, and particularly to separate those merely marking the presence or advent of disease from those with a causal contribution[65,66]. MR exploits unique properties of genotype to enable robust causal inference, often in settings where this would otherwise be impossible. The traditional benchmark test of causality is the randomised controlled trial (RCT), which measures the effects of an exposure-modifying intervention on disease risk or related biomarkers. The RCT framework permits causal inference by virtue of three key features of its design: 1)random allocation to intervention or control groups, which avoids confounding; 2) blinding, which avoids bias; and, 3) and the act of intervention with prospective follow-up, which avoids reverse causation. Such trials have helped to confirm or refute the causal role of proposed risk factors in a number of complex disease, e.g. low-density lipoprotein cholesterol (LDL-C)[67] and blood pressure[68] as causal mediators of coronary heart disease risk, and to finding no evidence for a causal role for antioxidant supplements in cardiovascular disease prevention[69].

MR offers a natural parallel to the RCT (**Figure 1a**), with similar features allowing causal inference. Random allocation of alleles at conception which, according to Mendel's second law of independent assortment are independent from each other, mirrors the randomisation in the RCT[70,71]. Potential confounders are distributed equally between different genotype groups for a given variant, such that any observed phenotypic associations of the variant can be deemed direct consequences of that mutation and free of confounding. Furthermore, genotype exerts a longitudinal action throughout life from conception, and there is a unidirectional flow of biological influence from DNA sequence, through transcription and protein synthesis to more complex phenotypes. These features together help to overcome the phenomenon of reverse causation in which a supposed causal factor of disease is in reality a consequence of the disease process itself. Finally, individuals are largely unaware of their genotype at a given locus, which replicates blinding used in RCTs. In an MR study, common genetic variants (usually single nucleotide polymorphisms, SNPs) associated with the biomarker or exposure of interest are used as the 'intervention'. The biomarker or exposure may be an endogenous factor such as LDL-C or body mass index (BMI), or an exogenous or environmental exposure such as alcohol consumption. Genetic variants associated with such a risk factor are used as its proxies, or instruments (**Figure 1b**). Associations of the genetic instrument with disease risk or presence of another outcome phenotype can be interpreted as supporting a causal role for the risk factor in those outcomes.

Investigating causation in CVD using Mendelian randomisation

MR has been used extensively to address important aetiological uncertainties in CVD, and MR studies of CVD risk factors provide clear illustrations of the utility of the technique. Genetic determinants of LDL-C concentration have been shown to causally associated with higher CHD risk in proof-of-concept MR studies confirming existing evidence from randomised trials of lipid-lowering medication [72,73]. Furthermore, the clear observational relationship of higher lipoprotein (a) concentrations with CHD risk was shown by MR studies to be causal [74,75]. More controversially, MR has been used to dissect the relationships of circulating HDL-C and triglycerides with CHD risk. Findings have been difficult to interpret because of the challenges in selecting genetic instruments that associate specifically with the lipid fraction of interest. A variant in the *APOA5* gene (chr11q23) associated with both higher triglyceride levels and CHD risk, implicated triglyceride-mediated pathways in the aetiology of CHD[76]. When a genetic risk score (GRS) composed of several triglyceride-associated variants was used as the MR instrument, the apparent association between higher triglycerides and CHD risk was attenuated after adjustment for lipid-related covariates[77,78]. With novel HDL-C-raising drugs in advanced stages of development[79], greatest interest has fallen on the causal role of HDL-C. MR studies have used single HDL-C-associated variants in a range of different genes but report no association of any of these with CHD risk [80–82], a finding replicated with an HDL-C GRS [77,82]. Together, these findings appear to argue against a causal role for HDL-C in CHD, however the many determinants of HDL-C level and function complicate their interpretation and leave open the possibility of both a causal role and a beneficial effect of HDL-C-raising therapies.

The role of inflammation in CVD has been addressed using MR, with high profile examples involving C-reactive protein (CRP) and IL-6. The strong epidemiological association between higher plasma CRP concentrations and CHD risk[7], and the long-held pathophysiological hypothesis of atherosclerosis as an inflammatory disease[83] suggested that CRP may itself cause CHD. Variants in the gene encoding CRP (*CRP* 1q23.2) associate strongly with CRP concentrations, but are not associated with CHD risk[84], providing strong evidence that CRP merely marks the presence of atherosclerotic disease rather than contributing to its development. Interpretation here is simpler than for the lipid studies described above, since the protein CRP is the sole product of the *CRP* gene and has no other direct determinants. Causal roles for other inflammation markers have, however, been demonstrated. Like CRP, higher concentrations of IL-6 are associated with CHD risk[8], and importantly, the IL-6 receptor (IL-6R) is the target of an existing drug used to treat rheumatoid arthritis[85]. Variants in the *IL6R* gene (1q21), encoding the IL-6R, influence IL-6 signalling and have been shown to associate with CHD risk[86,87]. These findings demonstrated for the first time a causal role for an inflammatory mediator in CHD, and highlighted the IL-6R as a promising drug target for CHD prevention.

MR has been used to investigate the relevance of more complex risk factors in CVD. Studies using the GRS approach have reported inconsistent estimates of the causal role of BMI in CHD[88,89], despite clear associations with higher risk of type 2 diabetes (T2D). Observational studies suggest a non-linear relationship between alcohol consumption (a behavioural risk factor) and CHD risk, with an apparent benefit associated with light-to-moderate consumption[90]. Genetic variants that associate strongly with alcohol consumption in populations of European ancestry are, however, associated with *higher* CHD risk, with a relationship that remained constant across all categories of alcohol consumption[91]. These genetic findings imply that the observed non-linear relationship of alcohol with CHD risk is a consequence of confounding and any level of alcohol consumption leads to higher CHD risk.

Validating CVD drug targets using Mendelian randomisation

The properties of MR that allow causal inference as described above can be exploited for the validation of drug targets. Since the MR model is viewed as a natural 'RCT', variants in a gene encoding and influencing the level

or function of a protein drug target can be used as naturally occurring 'interventions' with which to investigate the consequences of modulating that target pharmacologically. Since the majority of known and proposed drug targets are proteins[92] and SNPs in protein-coding regions occur frequently across the genome, this approach is broadly practicable. In the example of the IL-6 receptor described above, close concordance was noted between the drug tocilizumab that inhibits IL-6 receptor signalling and variants in its encoding gene in their effects on biomarkers of inflammation and risk of CHD[86] and abdominal aortic aneurysm[53]. From this was drawn the inference that the IL-6 receptor represents a potentially valuable drug target for prevention of these two important diseases. The MR approach has also been used to predict the outcome of ongoing RCTs. Secretory phospholipase A2 ($sPLA_2$)-IIA has been identified as a potential target for CHD prevention. An inhibitor of the enzyme, varespladib, was pursued until the phase III RCT stage[93], which was abandoned prematurely on grounds of insufficient efficacy for CHD prevention. An MR study using variants in the gene encoding $sPLA_2$-IIA (*PLA2G2A*, chr 1p35) demonstrated a similar lack of effect on CHD[94], which was corroborated by the subsequently published trial findings[95].

In addition to investigating main therapeutic effects, MR has been used to explore adverse drug effects. The cholesteryl ester transfer protein (CETP) inhibitor torcetrapib was designed to raise circulating HDL-C concentrations in the hope that this would reduce CHD risk[96]. A large phase III RCT of torcetrapib was, however, halted early because of excess cardiovascular mortality in the torcetrapib-treated arm, which was thought to have resulted from an increase in blood pressure in those patients[97]. With other CETP inhibitors at that time in advanced development, concern was high that the hypertensive effect of torcetrapib was a class-wide, on-target effect of the drug likely to be shared by the other agents. In a subsequent MR study, variants in the *CETP* gene (chr 16q2) were strongly associated with HDL-C but not with differences in blood pressure, suggesting that the effects observed with torcetrapib were *off*-target, molecule-specific effects unlikely to be shared by the other CETP inhibitor drugs[98]. As a second example, the modestly increased risk of T2D with statin treatment observed in RCTs[99,100] raised the question of whether this was an on- or off-target effect of the drugs; that is, whether the dysglycaemic effect was a direct consequence of inhibition of HMG-CoA, the intended target of statins. Again, using the MR approach, variants in the gene encoding HMG-CoA reductase (*HMGCR*, chr 5q13.3) associated with lower LDL-C were used as proxies for statin treatment. Both statin treatment and the genetic variants were associated with *higher* T2D risk and higher bodyweight, and the genetic variants with higher plasma glucose and insulin, and waist and hip circumferences[101]. These strong points of directional concordance strongly suggested that the higher T2D risk caused by statin therapy is at least in part a *direct consequence* of HMG-CoA reductase inhibition. Importantly, however, the benefits of statin treatment for CVD prevention heavily outweigh the small increase in T2D risk.

Limitations of Mendelian randomisation

Although the potential utility of MR is great, the technique is not without limitation. Every MR study requires a genetic instrument for the exposure of interest, and in some instances such an instrument may be unavailable. For example, genetic determinants of some environmental or behavioural exposures may not yet have been identified, or a GWAS from which MR instruments could be derived might not have been conducted for an endogenous biomarker. In such cases, an MR study may need to be reconsidered or postponed. Where a genetic instrument is available it may, for a range of reasons, be unsuitable. Firstly, linkage disequilibrium (LD) between variants in the genome may result in the observed effects of a variant at one locus being confounded by the linked effects of a different, possibly unidentified locus. In such a case, the genetic instrument is said to be confounded by LD, and the assumptions of the MR model may be violated. Differences in LD structure between ancestral populations can cause such confounding in one population but not another. Thus, MR instruments cannot be assumed to be transferable between ancestral groups without careful evaluation of the loci to which they are related through LD. Several online, open access tools using data from large cross-

ancestral sequencing projects are now available for such evaluation, and include the SNP Annotation and Proxy Search (SNAP) engine (http://www.broadinstitute.org/mpg/snap/). Finally, the detrimental effects of a variant allocated at conception may be moderated by the effects of other variants along the lifecourse of an individual in order to reduce the harm caused by the index variant. This process is known as canalisation and can lead to attenuation of the phenotypic effects of certain polymorphisms. Such phenotypic effects are therefore more difficult to detect and may hamper the design or analysis of an MR study.

Conclusions and the future of genetic research in CVD

Above, we have reviewed recent progress in the contribution of genetics to CVD research and clinical practice. With the field swiftly advancing, new developments are likely to emerge in the near future. These include pharmacogenetics, personalised genetic testing at the point of care, large-scale sequencing projects and broader exploitation of emerging metabolomic, proteomic and transcriptomic technologies for further enhancement of our knowledge of the genetic basis for CVD.

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Declarations

This article does not contain any studies with human or animal subjects performed by any of the authors.

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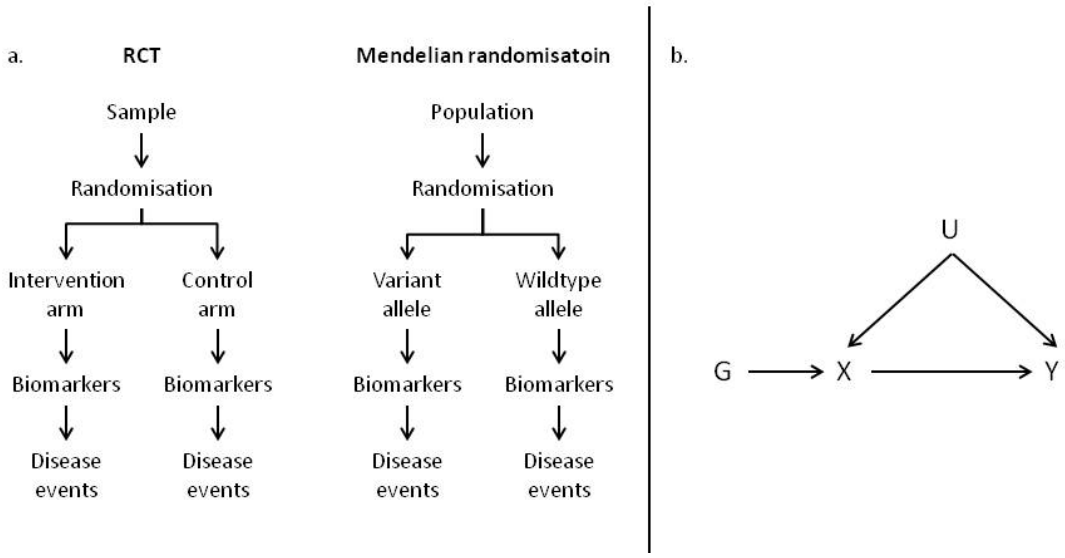
Figure 1



a. Mendelian randomisation as a natural parallel of the randomised controlled trial (Adapted from Hingorani AD and Humphries SE 2005[71].

b. The Mendelian randomisation model. In order to infer a causal role of an exposure, *X*, in a disease, *Y*, three assumptions must hold. First, the genetic instrument, *G*, must associate with *X*. Second, *G* must be independent of confounders, *U*, of the *X*-*Y* relationship. Third, *G* must be associated with *Y* only through its effect on *X*. If these three relationships can be demonstrated, the exposure, *X*, can be said to be causally related to the disease, *Y*.

Abbreviations: RCT - randomised controlled trial

Table 1. *Notable genetic loci identified by meta-analyses of large GWA studies of CHD risk in European populations (>63,000 cases and >110,000 controls)*[17]

Gene	Genomic location	Reported variant(s)	OR per-allele	P-value	Remarks
SORT1	1p31.3	rs602633	1.13	1.47×10^{-25}	Role of *SORT1* in lipid metabolism and CHD risk was unrecognised until reported by GWA studies[102]
PCSK9	1p32.2	rs11206510	1.04	1.79×10^{-5}	Rare *PCSK9* variants known to cause familial hypercholesterolaemia (FH). Drug inhibitors of PCSK9 have shown promise in randomised trials for LDL-C lowering, and data are awaited from phase III trials on their effects on CHD risk.
SLC22A3-LPAL2-LPA	6q26	rs3798220	1.28	4.90×10^{-5}	MR studies using variants in the gene encoding lipoprotein(a) been shown Lp(a) to have a causal role in aspects of CVD[41,74,74].
LDLR	19p13.2	rs1122608	1.06	3.72×10^{-6}	*LDLR* mutations have important causal role in FH.
COL4A1-COL4A2	13q34	rs4773144	1.07	1.43×10^{-11}	Encoding type IV collagen, *COL4A1* variants have also been associated with arterial stiffness in a GWA study[103]
IL6R	1q21.3	rs4845625	1.06	3.64×10^{-10}	MR studies have demonstrated associations of variants in this gene encoding the interleukin-6 receptor with CHD risk, and proposed it as a novel drug target[86,104].
APOB	2p24.1	rs515135	1.07	2.56×10^{-10}	*APOB* mutations have important causal role in FH.
LPL	8p21.3	rs164	1.11	2.88×10^{-9}	Variants in *LPL* (encoding lipoprotein lipase) have been associated in GWA studies with a range of lipid phenotypes, including HDL-C, triglycerides[23]

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