Title: Phenome-wide Mendelian randomization mapping the influence of the plasma proteome on complex diseases

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Abstract:

The human proteome is a major source of therapeutic targets. Recent genetic association analyses of the plasma proteome enable systematic evaluation of the causal consequences of variation in plasma protein levels. Here, we estimated the effects of 1002 proteins on 225 phenotypes using two-sample Mendelian randomization (MR) and colocalization. Of 413 associations supported by evidence from MR, 130 (31.5%) were not supported by results of colocalization analyses, suggesting that genetic confounding due to linkage disequilibrium (LD) is widespread in naive phenome-wide association studies of proteins. Combining MR and colocalization evidence in cis-only analyses, we identified 111 putatively causal effects between 65 proteins and 52 disease-related phenotypes (www.epigraphdb.org/pqtl/). Evaluation of data from historic drug development programmes showed that target-indication pairs with MR and colocalization support were more likely to be approved, evidencing the value of this approach in identifying and prioritising potential therapeutic targets.

Despite increasing investment in research and development (R&D) in the pharmaceutical industry[1], the rate of success for novel drugs continues to fall[2]. Lower success rates make new therapeutics more expensive, reducing availability of effective medicines and increasing healthcare costs. Indeed, only one in ten targets taken into clinical trials reaches approval [2], with many showing lack of efficacy (~50%) or adverse safety profiles (~25%) in late stage clinical trials after many years of development[3,4]. For some diseases, such as Alzheimer's disease, the failure rates are even higher[5].

Thus, early approaches to prioritize target-indication pairs that are more likely to be successful are much needed. It has previously been shown that target-indication pairs for which genetic associations link the target gene to related phenotypes are more likely to reach approval[6]. Consequently, systematically evaluating the genetic evidence in support of potential target-indication pairs is a potential strategy to prioritise development programmes. While systematic genetic studies have evaluated the putative causal role of both methylome and transcriptome on diseases[7,8], studies of the direct relevance of the proteome are in their infancy [9,10].

Plasma proteins play key roles in a range of biological processes and represent a major source of druggable targets[11,12]. Recently published genome-wide association studies (GWAS) of plasma proteins have identified 3606 conditionally independent single nucleotide polymorphisms (SNPs) associated with 2656 proteins ('protein quantitative trait loci', pQTL) [9, 13,14,15,16]. These genetic associations offer the opportunity to systematically test the causal effects of a large number of potential drug targets on the human disease phenome through Mendelian randomization (MR)[17]. In essence, MR exploits the random allocation of genetic variants at conception and their associations with disease risk factors to uncover causal relationships between human phenotypes, and has been described in detail previously[18,19].

For MR analyses of proteome, unlike more complex exposures, an intuitive way to categorise protein-associated variants is into cis-acting pQTLs located in the vicinity of the encoding gene (defined as ≤ 500kb from the leading pQTL of the test protein in this study) and trans-acting pQTLs located outside this window. The cis-acting pQTLs are considered to have a higher biological prior and have been widely employed in relation to some phenome-wide scans of drug targets such as *CETP*[20] and *IL6R*[21]. Trans-acting pQTLs may operate via indirect mechanisms and are therefore more likely to be pleiotropic[22], although may support causal inference where they are likely to be non-pleiotropic.

Here, we pool and cross-validate pQTLs from five recently published GWAS and use them as instruments to systematically evaluate the causal role of 968 plasma proteins on the human phenome, including 153 diseases and 72 risk factors available in the MR-Base database [23]. Results of all analyses are available in an open online database (www.epigraphdb.org/pqtl/), with a graphical interface to enable rapid and systematic queries.

Results

Characterising genetic instruments for proteins

Figure 1 summarises the genetic instrument selection and validation process. Briefly, we curated 3606 pQTLs associated with 2656 proteins from five GWAS[9,13,14,15,16]. After removing proteins and SNPs using criteria such as LD-pruning listed in **Online Method:** *instrument selection*, we retained 2113 pQTLs for 1699 proteins as instruments for the MR analysis (**Supplementary Table 1**). Among these instruments, we conducted further validation by categorising them into three tiers based on their likely utility for MR analysis (**Online Methods**: *Instrument validation*): 1064 instruments of 955 proteins with the highest relative level of reliability (tier 1); 62 instruments which exhibited SNP effect heterogeneity across studies (**Supplementary Figure 1** and **2**), indicating uncertainty in the reliability of one or all instruments for a given protein (tier 2; **Supplementary Table 2** and **3**); and 987 non-specific instruments which were associated with more than five proteins (tier 3). For the 263 tier 1 instruments associated with between two and five proteins, 68 of them influenced multiple proteins in the sample biological pathway and thus are likely to reflect vertical pleiotropy and remain valid instruments (**Supplementary Note**: *Distinguishing vertical and horizontal pleiotropic instruments using biological pathway data*)[22].

Amongst the 1126 tier 1 and 2 instruments, 783 (69.5%) were cis-acting (within 500kb of the leading pQTL) and 343 were trans-acting. Of 1002 proteins with a valid instrument, 765 had only a single cis or trans instrument. 66 were influenced by both cis and trans SNPs (**Supplementary Table 4**) and 153 had multiple conditionally distinct cis instruments (381 cis instruments showed in **Supplementary Table 5**).

Estimated effects of plasma proteins on human phenotypes

We undertook two-sample MR to systematically evaluate evidence for the causal effects of 1002 plasma proteins (with tier 1 and tier 2 instruments) on 153 diseases and 72 disease related risk factors (**Supplementary Table 6**, **Online Methods**: *Phenotype selection*). Overall, we observed 413 protein-trait associations with MR evidence ($P < 3.5 \times 10^{-7}$ at a Bonferroni-corrected threshold) using either cis or trans instruments (or both for proteins with multiple instruments).

Genetically filtering out predicted associations between proteins and phenotypes may indicate 4 explanations: causality; reverse causality; confounding by LD between the leading SNPs for proteins and phenotypes, or horizontal pleiotropy (**Supplementary Figure 3**). Given these alternative explanations, we conducted a set of sensitivity analyses to establish whether the MR association reflects a causal effect of protein on phenotype: tests of reverse causality using bi-directional MR[24] and MR Steiger filtering[25,26]; heterogeneity analyses for proteins with multiple instruments[27], and colocalization analyses[28] to investigate whether the genetic associations with both protein and phenotype shared the same causal variant (**Figure 1**). To avoid unreliable inference from colocalization analysis due to the potential presence of multiple neighbouring association signals, we also developed and performed pair-wise conditional and colocalization analysis (PWCoCo) of all conditionally independent instruments against all conditionally independent association signals for the outcome phenotypes (**Online methods**: *Pairwise conditional and colocalization analysis*; **Figure 2**). For this study, MR and colocalization were the two methods filtering reliable associations. After the colocalization analysis, 283 of the 413 protein-phenotype associations had profiles supportive of causality.

Estimating protein effects on human phenotypes using cis pQTLs

In the MR analyses using cis-pQTLs, we identified 111 putatively causal effects of 65 proteins on 52 phenotypes, with strong evidence of MR ($P< 3.5x10^{-7}$) and colocalization (posterior probability>80%; after applying PWCoCo) between the protein- and phenotype-associated signals (**Figure 3**, **Supplementary Table 7**). A further 69 potential associations had evidence from MR but did not have strong evidence of colocalization (posterior probability<80%; **Supplementary Table 8**), highlighting the potential for confounding by LD and the importance of colocalization analyses in MR of proteins. Evidence of potentially causal effects supported by colocalization was identified across a range of disease categories including anthropometric phenotypes and cardiovascular and autoimmune diseases (**Supplementary Note:** Disease areas of protein-trait associations) and our findings replicated some previous reported associations (**Supplementary Note:** MR results replicated previous findings).

Of 437 proteins with tier 1 or tier 2 cis instruments from Sun *et al.*[9] and Folkersen *et al.*[14], 153 (35%) had multiple conditionally independent SNPs in the cis region identified by GCTA-COJO[29] (**Supplementary Table 5**). We applied an MR model which takes into account the LD structure between conditionally independent SNPs in these cis regions[30]. In this analysis, we identified 10 additional associations, which had not reached our Bonferroni corrected P-value threshold in the single variant cis analysis. Generally, the MR estimates from the multi-cis MR analyses were consistent with the single-cis instrumented analyses (**Supplementary Table 9**).

In regions with multiple cis instruments, 16 of the 111 top cis MR associations only showed evidence of colocalization after conducting PWCoCo analysis for both the proteins and the human phenotypes, where none was observed between marginal results (**Supplementary Table 7**). For example, interleukin 23 receptor (IL23R) had two conditionally independent cis instruments: rs11581607 and rs3762318[9]. Conventional MR analysis combining both instruments showed a strong association of IL23R with Crohn's disease (OR=3.22, 95%CI= 2.93 to 3.53, $P=6.93x10^{-131}$; **Supplementary Table 9B**). There were 4 conditionally independent signals (conditional P value$<1x10^{-7}$) predicted for Crohn's disease in the same region (data from de Lange *et al*[31]). In the marginal colocalization analyses, we observed no evidence of colocalization (**Figure 4** and **Supplementary Figure 4**, colocalization probability=0). After performing PWCoCo with each distinct signal in an iterative fashion, we observed compelling evidence of colocalization between IL23R and one of the Crohn's disease signals for the top IL23R signal (rs11581607) (colocalization probability=99.3%), but limited evidence for the second conditionally independent IL23R hit (rs7528804) (colocalization probability = 62.9%). Additionally, for haptoglobin, which showed MR evidence for LDL-cholesterol (LDL-C), there were two independent cis instruments. There was little evidence of colocalization between the two using marginal associations (colocalization probability=0.0%). However, upon performing PWCoCo, we observed strong evidence of colocalization for both instruments (colocalization probabilities = 99%; **Supplementary Table 10; Supplementary Figure 5**). Both examples demonstrate the complexity of the associations in regions with multiple independent signals and the importance of applying appropriate colocalization methods in these regions. Of the 413 associations with MR evidence (using cis and trans instruments), 283 (68.5%) also showed strong evidence of colocalization using either a traditional colocalization approach (260 associations) or after applying PWCoCo (23 associations),

suggesting that one third of the MR findings could be driven by genetic confounding by LD between pQTLs and other causal SNPs.

Due to potential epitope-binding artefacts driven by protein-altering variants[32], we also flag putatively causal links where the lead instrument is a protein-altering variant or is in high LD (r2>0.8) with one (**Supplementary Table 7** and **8** filtered by column "VEP_pQTL_Ldproxy" including missense, stop-lost/gained, start-lost/gained and splice-altering variants).

Using trans-pQTLs as additional instrument sources

Trans pQTLs are more likely to influence targets though pleiotropic pathways. Among the 1316 trans instruments we identified from 5 studies, 73.5% were associated with more than 5 proteins, compared with 1.8 % of cis instruments (**Supplementary Table 1**). However, in a MR context, including non-pleiotropic trans-pQTLs may increase the reliability of the protein-phenotype associations since (1) they will increase variance explained of the tested protein and increase power of the MR analysis; (2) the causal estimate will not be reliant on a single locus, where multiple instruments exist; and (3) further sensitivity analyses, such as heterogeneity test of MR estimates across multiple instruments, can be conducted. Therefore, we extended our MR analyses to include 343 non-pleiotropic trans instruments (**Supplementary Figure 6**).

To utilize trans instruments, we first combined cis and trans instruments for 66 proteins that had both cis and trans instruments (noted as cis + trans analysis). However, none reached our pre-defined Bonferroni-corrected threshold, and only two protein-phenotype associations showed even suggestive evidence ($P<1x10^{-5}$) (**Supplementary Table 11**). Further, after including trans instruments, 17 of the cis-only signals were attenuated (is it in a table?). Secondly, we performed trans-only MR analyses of 293 proteins, and identified 158 associations with 44 phenotypes that also had strong evidence (posterior probability>0.8) of colocalization (**Supplementary Table 12**). A further 54 trans-only MR associations did not have strong evidence of colocalization (**Supplementary Table 13**).

Some of the trans analyses with MR and colocalization evidence suggest causal pathways that are confirmed by evidence from rare pathogenic variants or existing therapies. For example, although we had no cis instrument for Protein C (Inactivator Of Coagulation Factors Va And VIIIa) (PROC) (**Supplementary Figure 7A**), we found evidence for a causal association between PROC levels and deep venous thrombosis ($P=1.27x10^{-10}$; colocalization probability>0.9) using a trans pQTL, rs867186 (**Supplementary Figure 7B**), which is a missense variant in *PROCR*[33], the gene encoding the endothelial protein C receptor (EPCR). Patients with mutations in *PROC* have protein C deficiency, a condition characterised by recurrent venous thrombosis for which replacement protein C is an effective therapy.

From 47 proteins with multiple trans instruments, we identified four additional MR associations, but none showed strong evidence of colocalization (**Supplementary Table 13**) and little evidence of heterogeneity (**Supplementary Table 14**).

Estimating protein effects on human phenotypes using pQTLs with heterogeneous effects across studies

Among the 2113 selected instruments, we checked whether the 1062 instruments with

association information in at least two studies showed consistent effect size across studies (**Supplementary Table 15**). For these SNPs, we found that 62 showed evidence of difference in effect size across studies (tier 2 instruments), which we performed MR analyses using the most significant SNP across studies and report the findings with caution. Some proteins that are targets of approved drugs were found to have potential causal effects in this analysis, such as interleukin-6 receptor (IL6R) on rheumatoid arthritis (RA)[34], and coronary heart disease (CHD)[21] (**Supplementary Table 16**). Tocilizumab, a monoclonal antibody against IL6R, is used to treat RA, while canakinumab, a monoclonal antibody against interleukin-1 beta (an upstream inducer of interleukin-6), has been shown to reduce cardiovascular events specifically among patients who showed reductions in interleukin-6[35].

As another test of heterogeneity across studies, where the same protein was measured in two or more studies, we performed colocalization analysis of each pQTL (in one study) against the same pQTL (in another study) for the two studies in which we had access to full summary results (Sun *et al.*[9] and Folkersen *et al.*[14]). Of the 41 proteins measured in both studies, 76 pQTLs could be tested using conventional colocalization and PWCoCo (**Supplementary Table 15**). We found weak evidence of colocalization for 51 pQTLs (posterior probability<0.8), which suggested either two different signals were present within the test region or the protein has a pQTL in one study but not in the other. In either case, as one of the two distinct signals may be genuine, we performed MR analysis of these 25 pQTLs using instruments from each study separately. 8 associations had MR evidence but only one showed colocalization evidence (IL27 levels on human height; **Supplementary Table 17**).

Sensitivity analyses to evaluate reverse causality

For potential associations between proteins and phenotypes identified in the previous analyses, we undertook two sensitivity analyses to highlight results due to reverse causation: bi-directional MR[24] and Steiger filtering[25] (**Online Methods**: *Distinguishing causal effects from reverse causality*). In general, we found little evidence of reverse causality for genetic predisposition to diseases on protein level changes (more details in **Supplementary Note:** Bi-directional MR and Steiger filtering results; **Supplementary Data 1**).

Drug target prioritisation and repositioning using phenome-wide MR

Given that human proteins represent the major source of therapeutic targets, we sought to mine our results for targets of molecules already approved as treatments or in ongoing clinical development. We first compared MR findings for 1002 proteins against 225 phenotypes with historic data on progression of target-indication pairs in Citeline's PharmaProjects (downloaded on the 9th of May 2018). Of 783 target-indication pairs with an instrument for the protein and association results for a phenotype similar to the indication for which the drug had been trialled, 9.2% (73 pairs) had successful (approved) drugs, 69.1% had failed drugs (including 195 failed drugs in the clinical stage and 354 drugs which failed in the preclinical stage) and 20.3% were for drugs still in development (161 pairs). The 268 pairs for successful (73) or failed (195) drugs were included in further analyses (**Supplementary Table 18**). We observed eight target-indication pairs of successful drugs with MR and colocalization evidence of a potentially causal relationship between protein and disease (**Supplementary Table 19**). After removing duplicate genetic evidence for related indications for the same therapy (**Online Methods**: *Drug target validation and repositioning*)**,** six successful drugs remained from 214 pairs (**Supplementary Table 20**). In addition to the PROC and IL6R

examples discussed earlier, we found Proprotein convertase subtilisin/kexin type 9 (PCSK9) (target for evolocumab) for hypercholesterolemia and hyperlipidaemia, Angiotensinogen (AGT) for hypertension, IL12B for psoriatic arthritis and psoriasis and TNF Receptor Superfamily Member 11a (TNFRSF11A) for osteoporosis. At each of these examples, the direction of effect between circulating protein and disease risk was consistent with the therapeutic mechanism, except IL6R and PROC at first sight. However, for IL6R and PROC, the alleles associated with higher soluble protein levels have been shown to also lead to lower intracellular pathway activation[36,37], indicating consistency of direction with the therapeutic approach. These examples highlight the importance of careful examination of the biological mechanisms underlying plasma pQTLs to enable translation. Further removing associations potentially driven by protein-altering variants, as well as drugs which were in large part motivated by genetic evidence (e.g. PCSK9 fits both exclusion criteria), comparisons of the remaining 191 pairs indicated that protein-phenotype associations with MR and colocalization evidence remained more likely to become successful target-indication pairs (**Table 1**). Although we acknowledge the limited sample size of the test set, this raises enthusiasm for the utility of pQTL MR analyses with colocalization as a method for target prioritization.

Previous efforts have highlighted the opportunities and challenges of using genetics for drug repositioning[38]. We identified 3 approved drugs for which we found pQTL MR and colocalization evidence for 5 phenotypes other than the primary indication and 23 drug targets under development for 33 alternative phenotypes (**Supplementary Table 21**). An example of urokinase-type plasminogen activator (PLAU) levels associated with lower inflammatory bowel disease (IBD) risk is in **Supplementary Note**: *Case study for drug repurposing* and **Supplementary Figure 8**.

We also evaluated drugs in current clinical trials and identified 8 additional protein-phenotype associations with MR and colocalization evidence (**Supplementary Table 22**), for which we observe MR evidence implicating an increased likelihood of success.

Finally, we compared the 1002 instrumentable proteins (i.e. those that passed our instrument selection procedure) against the druggable genome[39]. 682 of the 1002 (68.1%) instrumentable proteins overlapped with the druggable genome (**Supplementary Table 23** and **Online Methods**: *Enrichment of proteome-wide MR with the druggable genome*). A further enrichment analysis was conducted to assess the overlap between putative causal protein-phenotype associations and the druggable genome (**Supplementary Table 24**). Of the 295 top findings (120 proteins on 70 phenotypes) with both MR and colocalization evidence, 250 of them (87.7%) overlapped with the druggable genome (**Figure 5**). This enrichment analysis will become more valuable with the continuous evolution of the druggable genome[38].

Discussion

MR analysis of molecular phenotypes against disease phenotypes provides a promising opportunity to validate and prioritise novel or existing drug targets through prediction of efficacy and potential on-target beneficial or adverse effects[40]. Our phenome-wide MR study of the plasma proteome employed five pQTL studies to robustly identify and validate genetic instruments for thousands of proteins. We used these instruments to evaluate the potential effects of modifying protein levels on hundreds of complex phenotypes available in MR-Bas[23] in a hypothesis-free approach[17]. We confirmed that protein-phenotype associations with both MR and colocalization evidence predicted a higher likelihood of a particular target-indication pair being successful and highlight 283 potentially causal associations. Collectively, we underline the important role of pQTL MR analyses as an evidence source to support drug discovery and development and highlight a number of key analytical approaches to support such inference.

In particular, we note the distinct opportunities and methodological requirements for MR of molecular phenotypes, such as transcriptomics and proteomics, compared to other complex exposures. For example, the number of instruments is often limited for proteins, restricting the opportunity to apply recently developed pleiotropy robust approaches[41,27]. New methods such as MR-robust adjusted profile scoring (MR-RAPS)[42] allow inclusion of many weak instruments in the MR analysis and have been applied to a recent proteome-wide MR study[10]. However, we note some examples where inclusion of multiple weaker instruments can reduce power and yield different results to those based on cis instruments alone[40,43], and we note very limited additional gain from inclusion of trans instruments. A major advantage of proximal molecular exposures is the ability to include cis instruments (or interpretable trans instruments) with high biological plausibility, limiting the likelihood of horizontal pleiotropy[22,44]. Further, we note the limited gain from inclusion of trans instruments in our analysis. However, undue focus on single SNP MR approaches brings susceptibility to other pitfalls, such as the inability to examine heterogeneity of effect and to evaluate and remove potential epitope artefacts.

To provide robust MR estimates for proteins, we note the important role of a number of sensitivity analyses following the initial MR in order to distinguish causal effects of proteins from those driven by horizontal pleiotropy, genetic confounding through LD[45] and/or reverse causation[25.] Of note, only two-thirds of our putative causal associations had strong evidence of colocalization, suggesting that a substantial proportion of the initial findings were likely to be driven by genetic confounding through LD between pQTLs and other disease-causal SNPs. To avoid misleading results, we suggest that for regions with multiple molecular trait QTLs, it is important to consider methods such as PWCoCo, which can avoid the assumptions of traditional colocalization approaches of just a single association signal per region[46]. In the current study, application of PWCoCo identified evidence of colocalization for 23 additional protein-phenotype associations hidden to marginal colocalization[46]. We note that recent recommendations support the use of colocalization as a follow up analysis to reduce false positives[47].

An important limitation of this work is that protein levels are known to differ between cell types[48]. In this study, we have estimated the role of protein measured in plasma on a range of complex human phenotypes but are unable to assess the relevance of protein levels in

other tissues. Whilst eQTL studies highlight a large proportion of eQTLs being shared across tissues[37], there are many which show cell type and state specificity[49], highlighting the potential value of applying the current approach to data from proteomics analyses in other cell types and tissues. We also hypothesize that in instances with multiple conditionally distinct pQTLs, but where we observe colocalization of only certain conditionally distinct pQTL-phenotype pairs, that this may reflect underlying cell- and state-specific heterogeneity in bulk plasma pQTLs, among which only certain cell-types or states are causal[50]. Although pQTL studies have not yet been performed as systematically across tissues or states as eQTL studies, it remains encouraging that our analyses using plasma proteins identify associations across a range of disease categories, including for psychiatric diseases for which we may expect key proteins to function primarily in the brain.

Evaluating the potential of MR to inform drug target prioritisation, we demonstrated that the presence of pQTL MR and colocalization evidence for a target-indication pair predicts a higher likelihood of approval. One of the limitations of our approach is the lack of comprehensive coverage of genetic data for all phenotypes for which drugs are in development, as well as our inability to instrument the entire proteome through pQTLs. As such, ongoing expansions in the scale, diversity and availability of GWAS will be important in providing more precise estimates of the value of MR and colocalization in drug target prioritization and in enabling its broader application.

Another potential limitation of our work is the presence of epitope-binding artefacts driven by coding variants that may yield artefactual cis-pQTLs[32]. In particular, such instances may lead to false negative conclusions where, in the presence of a silent missense variant causing an artefactual pQTL but with no actual effect on protein function or levels, we do not correctly instrument the target protein. In instances where the missense variant appears to be driving the association with the phenotype, we suggest that causal inference may remain valid but inference on direction of association is challenged. Finally, the limited coverage of the proteome afforded by current technologies, leaves the possibility of undetected pleiotropy of instruments. While cis-pQTLs are less likely to be prone to horizontal pleiotropy than trans-pQTLs, it is well known from studies of gene expression that cis variants can influence levels of multiple neighbouring genes and hence the same is likely to be true for proteins. Future larger GWAS of the plasma proteome are likely to uncover many more variant-protein associations, increasing the apparent pleiotropy of many pQTLs.

In conclusion, this study identified 283 putatively causal effects between the plasma proteome and the human phenome using the principles of MR and colocalization. These observations support, but do not prove, causality, as potential horizontal pleiotropy remains an alternative explanation. Our study provides both an analytical framework and an open resource to prioritise potential new targets and a valuable resource for evaluation of both efficacy and repurposing opportunities by phenome-wide evaluation of on-target associations.

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Author contribution

JZ, VH and DB performed the Mendelian randomization analysis; JZ and DB performed the colocalization analysis; JZ performed the conditional analysis; VH, YL, BE and TRG developed the database and web browser; JZ and VW performed the drug target prioritisation and enrichment analysis. JZ and RS conducted the druggable genome analysis; JZ and PE conducted the pathway and protein-protein interaction analysis; AG, TGR, BE, HM, JY, CL, SL and JR conducted supporting analyses; JS, BBS, JD, HR, JCM provided key data and supported the MR analysis; JL, KE, LM, MVH, MH, DW, MRN reviewed the paper and provided key comments. JZ, VH, DB, VW, PH, AB, GDS, GH, RAS and TRG wrote the manuscript. JZ, TRG and RAS conceived and designed the study and oversaw all analyses.

Competing Interests Statement

AG, LM, MH, DW, MN, RS and RAS are employees and shareholders in GlaxoSmithKline. HR, JL and KE are employees and shareholders in Biogen. VH is employed on a grant funded by GlaxoSmithKline. DB is employed on a grant funded by Biogen. TRG, GH and GDS receive funding from GlaxoSmithKline and Biogen for the work described here. AB has received grants from Merck, Novartis, Biogen, Pfizer and AstraZeneca.

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Figure Legend

Figure 1. Study design of this phenome-wide MR study of the plasma proteome. The study included instrument selection and validation, outcome selection, 4 types of MR analyses, colocalization, sensitivity analyses and drug target validation.

Figure 2. A demonstration of pair-wise conditional and colocalization (PWCoCo) analysis. Assume there are two conditional independent association pQTL signals (SNP 1 and SNP 2) and two conditional independent outcome signals (SNP 1 and SNP3) in the tested region. A naïve colocalization analysis using marginal association statistics will return weak evidence of colocalization (showed in regional plots A and D). By conducting the analyses conditioning on SNP 2 (plot B) and 1 (plot C) for the pQTLs and conditioning on SNP 1 (plot E) and 3 (plot F) for the outcome phenotype, each of the 9 pair-wise combinations of pQTL and outcome association statistics (represented as lines with different colours in the middle of this figure) will be tested using colocalization. In this case, the combination of plot B and plot E shows evidence of colocalization but the remaining 8 do not.

Figure 3. Miami plot for the cis-only analysis, with circles representing the MR results for proteins on human phenotypes. The labels refer to top MR findings with colocalization evidence, with each protein represented by one label. The colour refers to top MR findings with $P<3.09x10^{-7}$, where red refers to immune mediated phenotypes, blue refers to cardiovascular phenotypes, green refers to lung related phenotypes, purple refers to bone phenotypes, orange refers to cancers, yellow refers to glycemic phenotypes, brown refers to psychiatric phenotypes, pink refers to other phenotypes and **grey** refers to phenotypes that showed less evidence of colocalization. The X-axis is the chromosome and position of each MR finding in the cis region. The Y-axis is the -log10 P value of the MR findings, MR findings with positive effects (increased level of proteins associated with increasing the phenotype level) are represented by filled circles on the top of the Miami plot, while MR findings with negative effects (decreased level of proteins associated with increasing the phenotype level) are on the bottom of the Miami plot.

Figure 4. Regional association plots of IL23R plasma protein level and Crohn's disease in the IL23R region. A. and B. the regional plots of IL23R protein level and Crohn's disease without conditional analysis, Plot B listed the sets of conditionally independent signals for Crohn's disease in this region: rs7517847, rs7528924, rs183020189, rs7528804 (a proxy for the second IL23R hit rs3762318, r2=0.42 in the 1000 Genome Europeans) and rs11209026 (a proxy for the top IL23R hit rs11581607, r2=1 in the 1000 Genome European), conditional P value < 1x10-7; C. the regional plot of IL23R with the joint SNP effects conditioned on the second hit (rs3762318) for IL23R; D. the regional plot of Crohn's disease with the joint SNP effects adjusted for other independent signals except the top IL23R signal rs11581607; E. the regional plot of IL23R with the joint SNP effects conditioned on the top hit (rs11581607) for IL23R; F. the regional plot of Crohn's disease with the joint SNP effects adjusted for other independent signals except the second IL23R signal rs3762318. The heatmap of the colocalization evidence for IL23R association on Crohn's disease (CD) in the IL23R region was presented in **Supplementary Figure 4**.

Figure 5. Enrichment of phenome-wide MR of the plasma proteome with the druggable genome. In this figure, we only showed proteins with convincing MR and colocalization

evidence with at least one of the 70 phenotypes. The X-axis shows the categories of 70 human phenotypes, where the phenotypes have been grouped into 8 categories: 8 autoimmune diseases (red), 3 bone phenotypes (purple), 8 cancers (orange), 12 cardiovascular phenotypes (blue), 4 glycemic phenotypes (yellow), 2 lung phenotypes (green), 4 psychiatric phenotypes (brown) and 29 other phenotypes (pink). The Y-axis presents the tiers of the druggable genome (as defined by Finan et al) of 120 proteins under analysis, where the proteins have been classified into 4 groups based on their druggability: tier 1 contained 23 proteins which are efficacy targets of approved small molecules and biotherapeutic drugs, tier 2 contained 11 proteins closely related to approved drug targets or with associated drug-like compounds, tier 3 contained 58 secreted or extracellular proteins or proteins distantly related to approved drug targets, and 28 proteins have unknown druggable status (Unclassified). The cells with colours are protein-phenotype associations with strong MR and colocalization evidence. Cells in green are associations overlapping with the tier 1 druggable genome, where cells in yellow, red or purple were associations with tier 2, tier 3 or unclassified. More detailed information shown in **Supplementary Table 24**.

Table 1. Enrichment analysis comparing target-indication pairs with or without MR and colocalization evidence

Target-indication pair approved after clinical trials	Mendelian randomization and colocalization evidence: YES	Mendelian randomization and colocalization evidence: NO
YES	4	40
NO	0	147

Note: The protein-phenotype association pairs were grouped into four categories: 1) pairs with both MR/colocalization indications of causality and drug trial success; 2) pairs with MR and colocalization evidence but no drug trial evidence; 3) pairs with no strong MR or colocalization evidence but with drug trial evidence; and 4) pairs with no strong MR, colocalization or drug trial evidence. The cut-off for MR evidence was $p < 3.5 \times 10^{-7}$; the cut off for colocalization evidence was posterior probability > 80%. The drug trial evidence was obtained from PharmaProjects database. The MR and colocalization analysis results involved in this analysis including both Tier 1 and Tier 2 instruments in both cis and trans region. More results comparing MR and trial evidence for cis-only and tier 1 instruments can be found in **Supplementary Table 20**.