A Bayesian joint pQTL study sheds light on the genetic architecture of obesity

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Abstract

The genetic contribution to obesity has been widely studied, yet the functional mechanisms underlying metabolic states remain elusive. This has prompted analysis of endophenotypes via quantitative trait locus studies, which assess how genetic variants affect intermediate gene (eQTL) or protein (pQTL) expression phenotypes. However, most such studies rely on univariate screening, which entails a strong multiplicity burden and does not leverage shared regulatory patterns. We present the first multivariate pQTL analysis with our highly scalable Bayesian framework LOCUS, on plasma protein levels from a dual mass-spectrometry and SomaLogic assay, and show that it is more powerful than a standard univariate procedure on this data. We identify 136 pQTL associations in the Ottawa obesity cohort, of which $> 80\%$ replicate in the independent DiOGenes cohort and have significant functional enrichments; 15% of the hits would be missed by univariate analysis. By exploiting clinical data, we reveal the implication of proteins under genetic control in low-grade inflammation, insulin resistance, and dyslipidemia, opening new perspectives for diagnosing and treating metabolic disorders. All results are freely accessible online from our searchable database.

Keywords: Bayesian multivariate modelling; Metabolic Syndrome; Proteomic quantitative trait locus analysis; Stratified obesity cohorts; Two-stage integrative study; Variational inference.

Introduction

Genome-wide association studies (GWAS) have identified hundreds of loci associated with obesity susceptibility,[1] yet their functional impact on metabolism remains poorly understood. The analysis of endophenotypes via molecular quantitative trait locus (QTL) studies may provide deeper insight into the biology underlying clinical traits. While gene expression QTL (eQTL) studies are now routinely performed, protein expression QTL (pQTL) studies have emerged only recently.[2–6] These studies allow the exploration of the functional bases of obesity, as certain proteins act as proxies for metabolic

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endpoints.[3,7,8] However two major hurdles hamper pQTL analyses. First, owing to the number of tests that they entail, conventional univariate approaches lack statistical power for uncovering weak associations, such as *trans* and pleiotropic effects,[9,10] while better-suited multivariate methods fail to scale to the dimensions of QTL studies. Second, the clinical data complementing QTL data are often very limited, restricting subsequent investigation to external information from unrelated populations, health status or study designs, and rendering some degree of speculation unavoidable.

In this paper, we demonstrate that both concerns can be addressed using statistical approaches and data tailored to the problem under consideration: we present an integrative genome-wide pQTL study of two obesity clinical cohorts performed with our Bayesian joint QTL method LOCUS,[11] which simultaneously accounts for all the genetic variants and proteomic outcomes, thereby leveraging the similarity across proteins controlled by shared functional mechanisms. We employ a two-stage design, using data from the Ottawa clinical practice cohort[12] ($n = 1,644$) for discovery and replicating our findings in the independent DiOGenes cohort[13] ($n = 789$). Each dataset involves protein plasma levels quantified by mass-spectrometry and aptamer-based assays, which permits thorough cross- and intra-platform validation.

Our work aims to illustrate the utility and feasibility of multivariate Bayesian QTL analyses. Notably, we show the gain in power achieved by LOCUS on our data by confronting the validated hits with those of a classical two-stage univariate design. We also assess the statistical performance of both approaches in simulations emulating real data conditions.

Pertinent interpretation of pQTL effects for complex diseases hinges on a careful examination of metabolic and clinical parameters from the same subjects or, at a minimum, from a population presenting similar clinical characteristics. We demonstrate the biomedical potential of several replicated pQTL hits, using comprehensive clinical data from the two pQTL obesity cohorts. Our results reveal novel protein biomarkers under genetic control, in the context of obesity co-morbidities; they are available from our online browser `https://locus-pqtl.epfl.ch`.

Material and Methods

Ethics. The *Ottawa* and *DiOGenes* studies were approved by the local human research ethic committees. Participants provided informed written consent, and all procedures were conducted in accordance with the Declaration of Helsinki.

Study samples. The *Ottawa* study was a medically supervised program set up by the Weight Management Clinic of Ottawa.[12] Subjects under medication known to affect weight, glucose homeostasis or thyroid indices were excluded from all analyses.

The *DiOGenes* study was a multi-center pan-European program.[13] Eight partner states participated to the study: Bulgaria, the Czech Republic, Denmark, Germany, Greece, the Netherlands, Spain and the United Kingdom. Participants were overweight/obese (BMI between 27 and 45 kg/m^2), non-diabetic and otherwise healthy.

For both studies, subjects who were not under fasting conditions at plasma sample collection were excluded from the proteomic analyses. The main clinical characteristics of the cohorts are given in Supplementary Table S1.

Proteomic data. Plasma protein expression data were obtained using two types of technologies: mass-spectrometry (MS) and a multiplexed aptamer-based assay developed by SomaLogic.[14] Samples were randomized, ensuring that the plate numbers were not associated with age, gender, ethnicity,

weight-related measures, glycemic indices, measures of chemical biochemistry, and, for the DiOGenes samples, collection centers.

The MS proteomic quantification used plasma samples spiked with protein standard lactoglobulin (LACB). Samples were immuno-depleted, reduced, digested, isobarically 6-plex labeled and purified. They were analyzed in duplicates on two separate but identical systems using linear ion trap with Orbitrap Elite analyzer and Ultimate 3000 RSLCnano System (Thermo Scientific). Protein identification was done with the UniProtKB/Swiss-Prot database, using Mascot 2.4.0 (Matrix Sciences) and Scaffold 4.2.1 (Proteome Software). Both peptide and protein false discovery rates (FDR) were set to 1%, with a criterion of two unique peptides. The relative quantitative protein values corresponded to the $\log_2$-transformation of the protein ratio fold changes with respect to their measurements in the biological plasma reference sample. The sample preparation and all other manipulations relative to the MS measurements are detailed further in previous work.[15–17]

The SomaLogic protein measurements were characterized using the SOMAscan assay,[14] which relies on fluorescent labelling of poly-nucleotide aptamers targeting specific protein epitopes. Protein measurements were obtained in relative fluorescence unit and were then $\log_2$-transformed.

We discarded MS-based proteins if their measurements were missing for more than 5% of the samples, leaving 210 proteins in the Ottawa cohort and 136 in the DiOGenes cohort; we restricted all downstream analyses to the 133 proteins available for both cohorts. The SomaLogic measurements had no missing values. Totals of $1,100$ and $1,129$ proteins were assayed in the Ottawa and DiOGenes cohorts. All our analyses focused on the $1,096$ proteins quantified for both cohorts. The overlap between the MS and SomaLogic panels was of 72 proteins only.

We excluded samples with extreme expression values in more than 5% of the proteins, i.e., values beyond the outer fences of the empirical distribution ($q_1 - 3 \times \text{IQR}$, $q_3 + 3 \times \text{IQR}$, where q_1, q_3 are the lower and upper quartiles, and IQR is the interquartile range). After this quality control procedure, 577 and 428 Ottawa samples remained in the MS and SomaLogic datasets, respectively, and 481 and 563 DiOGenes samples remained in the MS and SomaLogic datasets, respectively.

Genotyping. Genotypes were generated using HumanCoreExome-12 v1.1 Illumina SNP arrays (Illumina, Inc., San Diego, CA), according to their manufacturer's instructions and were called with the GenomeStudio Software provided by Illumina. Preprocessing steps, including imputation and quality control, have been previously documented.[18] We discarded SNPs with call rate $< 95\%$, violating Hardy–Weinberg equilibrium (FDR $< 20\%$), and we discarded subjects with low call rate ($< 95\%$), abnormally high autosomal heterozygosity (FDR $< 1\%$), an XXY karyotype, or gender inconsistencies between genotype data and clinical records. For subjects with identity-by-state IBS$> 95\%$, we kept only the one with the highest call rate. The subjects from both cohorts were of European ancestry and the two cohorts had similar genetic structure. We used principal component analyses separately on each cohort to exclude subjects that were extremely heterogeneous genetically. We performed genotype imputation using SHAPEIT and IMPUTE2, based on the European reference panel from the $1,000$ Genome project (March 2012 release, phase 1 version 3). We then discarded SNPs with INFO score < 0.8, which left 4.9M imputed SNPs in both datasets. We applied a light LD pruning with PLINK using pairwise r^2 threshold 0.95 and used a minor allele frequency threshold of 5% after having restricted the genotype data to the subjects with available proteomic data.

The above steps were performed separately for the Ottawa and the DiOGenes cohorts, so in order to define a common set of SNPs for discovery and replication, we restricted each dataset to the SNPs available for both cohorts. After all genetic quality controls, and in both cohorts, $p = 275,485$ tag SNPs remained for the SomaLogic analysis and $p = 275,297$ tag SNPs remained for the MS analysis.

As SNPs were imputed, the 0.95-r^2 pruning led to a drastic cut of "redundant" markers: without this pruning step, the number of SNPs was $\approx$ 4M. Such a reduction is not surprising considering the nature of the underlying SNP arrays (essentially based on tag SNPs) and indicates that little information was discarded. In the Ottawa cohort $n = 376$ subjects had both genotype and MS proteomic data, and $n = 394$ subjects had both genotype and MS proteomic data. In the DiOGenes cohort, these numbers were $n = 400$ and 548.

Clinical data. Both cohorts had records on age, gender, anthropometric traits (weight and BMI), glycemic variables (fasting glucose, fasting insulin, HOMA-IR), and total lipid levels obtained from blood biochemistry (total cholesterol, triglycerides, HDL). We derived LDL values using the Friedewald formula,[19] and obtained gender-specific *visceral adiposity index* (VAI) values using the formula of Amato et al.[20] In each cohort and for each clinical variable, we removed a few samples with extreme measurements, similarly as for the proteomic data quality control.

LOCUS: fast Bayesian inference for multivariate QTL analysis. LOCUS[11] is a variational inference approach for joint mapping analysis at the scale required by current molecular QTL studies (Figure 1A). It implements a hierarchical sparse regression model that involves a collection of high-dimensional regressions,

$$\boldsymbol{y}_t = \boldsymbol{X}\boldsymbol{\beta}_t + \boldsymbol{\varepsilon}_t, \qquad \boldsymbol{\varepsilon}_t \sim \mathcal{N}_n\left(\boldsymbol{0}, \tau_t^{-1}\boldsymbol{I}_n\right), \qquad t = 1, \ldots, q, \tag{1}$$

where $\boldsymbol{y} = (\boldsymbol{y}_1, \ldots, \boldsymbol{y}_q)$ is an $n \times q$ matrix of q centered outcomes (e.g., genomic, proteomic, or metabolomic levels), and $\boldsymbol{X}$ is an $n \times p$ matrix of p centered candidate predictor SNPs, for each of n samples. Each outcome, $\boldsymbol{y}_t$, is related linearly to all p candidate SNPs, and has a specific residual precision, τ_t, to which we assign a Gamma prior, $\tau_t \sim \text{Gamma}(\eta_t, \kappa_t)$. As $p, q \gg n$, sparsity of the $p \times 1$ regression parameters $\boldsymbol{\beta}_t$ is enforced by placing a spike-and-slab prior on each of their components, namely, for $s = 1, \ldots, p$,

$$\beta_{st} \mid \gamma_{st}, \sigma^2, \tau_t \sim \gamma_{st}\,\mathcal{N}\left(0, \sigma^2\,\tau_t^{-1}\right) + (1 - \gamma_{st})\,\delta_0, \qquad \gamma_{st} \mid \omega_s \sim \text{Bernoulli}\left(\omega_s\right), \tag{2}$$

where δ_0 is the Dirac distribution. Hence, to each regression parameter β_{st} corresponds a binary latent parameter γ_{st}, which acts as a "predictor-outcome association indicator": the predictor $\boldsymbol{X}_s$ is associated with the outcome $\boldsymbol{y}_t$ if and only if $\gamma_{st} = 1$. The parameter σ represents the typical size of nonzero effects and is modulated by the residual scale, $\tau_t^{-1/2}$, of the outcome concerned by the effect; it is inferred from the data using a Gamma prior specification, $\sigma^{-2} \sim \text{Gamma}\left(\lambda, \nu\right)$. Finally, we let the probability parameter ω_s have a Beta distribution,

$$\omega_s \sim \text{Beta}\left(a_s, b_s\right), \tag{3}$$

where a_s and b_s are set so as to enforce sparsity as described in Ruffieux et al.[11] Since it is involved in the Bernoulli prior specification of all $\gamma_{s1}, \ldots, \gamma_{sq}$, the parameter ω_s controls the proportion of outcomes associated with the predictor $\boldsymbol{X}_s$, and hence directly represents the propensity of predictors to be pleiotropic "hotspots". Both ω_s and σ^2 allow the leveraging of shared association patterns across all molecular variables, which enhances the estimation of weak *trans* and pleiotropic QTL effects. A graphical representation of the model is provided in Figure 1B. LOCUS estimates interpretable posterior probabilities of association for all SNP-outcome pairs (Figure 1C), from which Bayesian false discovery rates are easily calculated.

Inference on high-dimensional Bayesian models is both computationally and statistically difficult. Previous joint QTL approaches[21,22] are based on sampling procedures, such as Markov Chain Monte

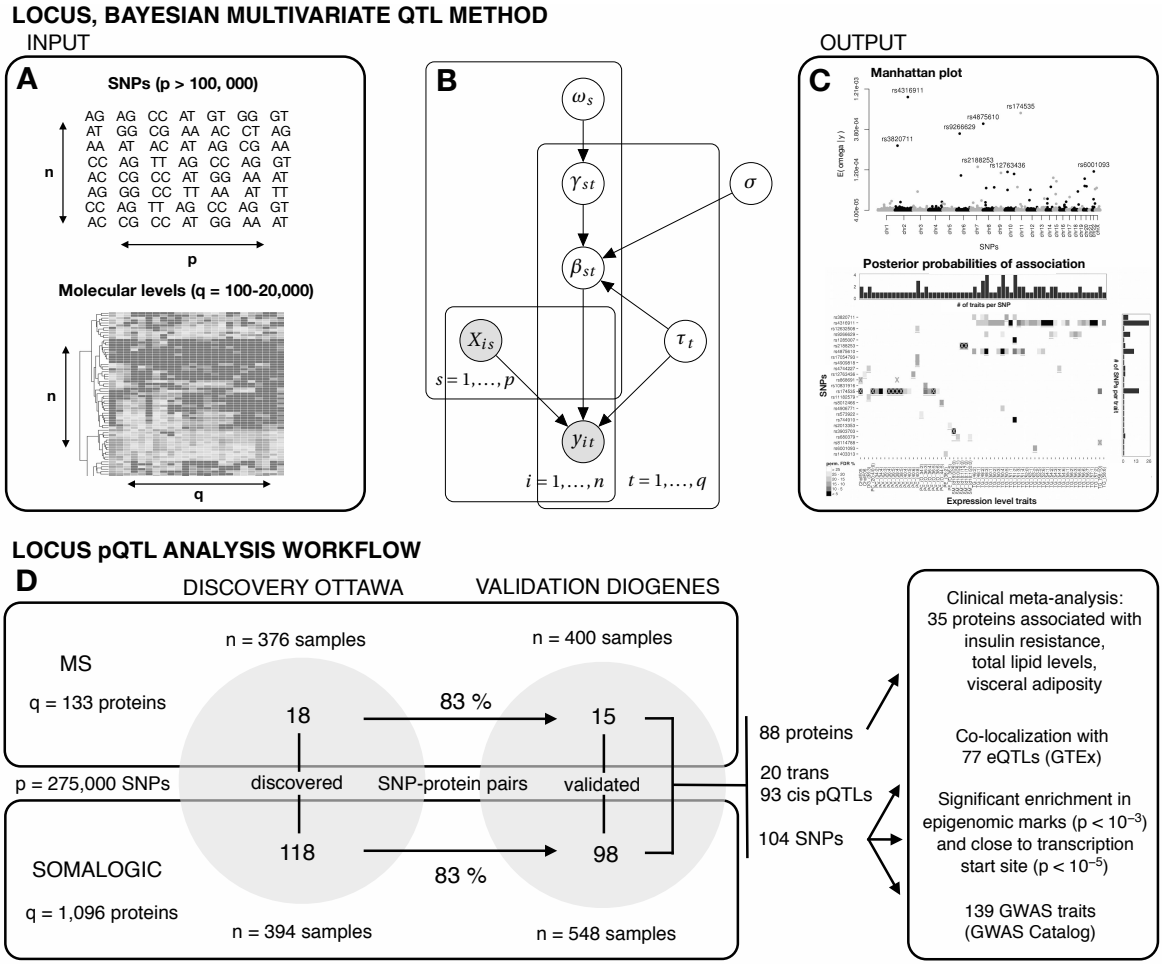

Figure 1: LOCUS model overview and study workflow. (A) Inputs to LOCUS are an $n \times p$ design matrix $\boldsymbol{X}$ of p SNPs, and an $n \times q$ outcome matrix $\boldsymbol{y}$ of q molecular traits, e.g., gene, protein, lipid or methylation levels, for n individuals. The model accounts for all the SNPs and molecular traits jointly. (B) Graphical model representation of LOCUS. The effect size between a SNP s and a trait t is modelled by β_{st}, and γ_{st} is a latent variable taking value unity if they are associated, and zero otherwise. The parameter ω_s controls the pleiotropic level of each SNP, i.e., the number of traits with which it is associated. The parameter σ represents the typical size of effects, and the parameter τ_t is a precision parameter that relates to the residual variability of each trait t. (C) Outputs of LOCUS are posterior probabilities of associations, $\mathrm{pr}(\gamma_{st} = 1 \mid \boldsymbol{y})$, for each SNP and each trait ($p \times q$ panel), and posterior means for the pleiotropy propensity of each SNP, $\mathrm{E}(\omega_s \mid \boldsymbol{y})$ (Manhattan plot). (d) Workflow of the pQTL study. The MS and SomaLogic pQTL data are analyzed in parallel. LOCUS is applied on the Ottawa data for discovery, and 83% of the 18 and 118 pQTL associations discovered with the MS and SomaLogic data replicate in the independent study DiOGenes. The relevance of the validated pQTLs in the obese population is assessed via analyses of clinical parameters from the Ottawa and DiOGenes cohorts. Further support is obtained by evaluating colocalization with eQTLs, epigenomic marks and GWAS risk loci.

Carlo (MCMC) algorithms, and require prohibitive computational times on data with more than few hundreds of SNPs or outcomes. LOCUS uses a fast deterministic variational inference algorithm, which scales to the typical sizes of QTL problems. Previous work [11] compared LOCUS with existing QTL methods, whether stochastic or deterministic, univariate or multivariate. Here, we augmented our algorithm with a simulated annealing procedure [23] to enhance exploration of multimodal parameter spaces, as induced by strong LD structures.

The applicability of a fully multivariate method to large molecular QTL data also hinges on the effective computational implementation of its algorithmic procedure. The annealed variational updates of LOCUS are analytical and performed by batches of variables. The software is written in R with

C++ subroutines and is publicly available at `https://github.com/hruffieux/locus`. Our MS and SomaLogic analyses completed in a few hours for 275K tag SNPs representing information from about 5M common markers, yet larger SNP panels can be considered as our method scales linearly in terms of memory and CPU usage. For instance, analyses of 2M SNPs and 1000 proteins run in less than 40 hours (see profiling in the Appendix A).

Simulation study design. We evaluated the performance of LOCUS expected on our data by conducting two simulation studies. We compared its statistical power to detect pQTL associations with that of the linear mixed model approach GEMMA,[24] which estimates the associations between each SNP and each outcome in a univariate fashion. We used the R package `echoseq` to generate synthetic data that emulate real data.

For the first simulation, we ran LOCUS and GEMMA on the SNPs of all $n = 376$ Ottawa subjects, and on simulated expression outcomes with residual dependence replicating that of the $q = 133$ MS proteomic levels. We used the SNPs from chromosome one ($p = 20,900$), and generated associations between 20 SNPs and 25 proteins chosen randomly, leaving the remaining variables unassociated. Some proteins were under pleiotropic control; we drew the degree of pleiotropy of the 20 SNPs from a positively-skewed Beta distribution, so only a few SNPs were hotspots, i.e., were associated with many proteins. We generated associations under an additive dose-effect scheme and drew the proportions of outcome variance explained by a given SNP from a Beta$(2, 5)$ distribution to give more weight to smaller effect sizes. We then rescaled these proportions so that the variance of each protein attributable to genetic variation was below 35%. These choices led to an inverse relationship between minor allele frequencies and effect sizes, which is to be expected under natural selection. We generated 50 replicates, re-drawing the protein expression levels and effect sizes for each.

For the second simulation, we re-assessed the performance of LOCUS for a grid of data generation scenarios. We considered a wide range of sparsity levels (numbers of proteins under genetic control) and effect sizes (proportions of outcome variance explained by the genetic variants). Given the large number of configurations (130), and in order to limit the computational burden, we used the first $p = 2,000$ SNPs, and ran LOCUS and GEMMA on 20 replicates for each configuration.

Proteomic quantitative trait locus analyses. We performed pQTL analyses separately for each platform, i.e., one analysis for the MS proteomic dataset, and another for the SomaLogic proteomic dataset. Each analysis comprised two stages: a discovery stage using the Ottawa cohort and a replication stage based on the DiOGenes cohort.

For discovery, we used LOCUS on both the MS and the SomaLogic datasets, with an annealing schedule of 50 geometrically-spaced temperatures and initial temperature of 20; pilot experiments indicated that estimation was not sensitive to these choices. We used a convergence tolerance of 10^{-3} on the absolute changes in the objective function as the stopping criterion. The algorithm can handle missing data in the outcome matrix, so no imputation was necessary for the MS proteomic data.

We adjusted all analyses for age, gender, and BMI at baseline. No important stratification was observed in the genotype data; the first ten principal components together explained little of the total variance ($< 4\%$), so we did not include them as covariates. We derived FDR values from the posterior probabilities of association obtained between each SNP and each protein, and reported pQTL associations using an FDR threshold of 5%.

We performed a validation study of the pQTLs discovered using the DiOGenes cohort with GEMMA,[25] with centered relatedness matrix (default) and p-values from (two-sided) Wald tests.

We then obtained adjusted p-values using Benjamini–Hochberg FDR, and validated our hits using again an FDR threshold of 5%.

Comparison with a standard two-stage univariate design. To assess the extent to which LOCUS two-stage pQTL analysis discovers more hits than the univariate procedures routinely applied for e- or pQTL analyses, we re-performed the entire study using GEMMA.[24] We followed standard practices and ran the method separately for the MS and SomaLogic analyses on the SNPs without LD pruning, i.e., on roughly 4 million SNPs. We then corrected for multiple testing using a conservative yet conventional Bonferroni threshold of 0.05 (based on the numbers of tested SNPs and proteins) and also discussed the results obtained with a more permissive Bonferroni threshold of 0.2.

To account for proxy hits arising from the SNP LD structure and provide grounds for comparison between GEMMA and LOCUS, we defined hits at the level of loci as follows: the hits identified by GEMMA and/or LOCUS as associated with a same protein (quantified by the same proteomic technology) were considered to be in a same locus if there was no more than 1Mb between two consecutive hits. The additional hits found by GEMMA at Bonferroni level 0.2 were assigned to the closest existing loci (mapping to the same protein), provided that the distance was less than 1Mb; new loci were defined for the remaining hits.

pQTL annotation. We used the Ensembl database (GRCh37, release 94) to retrieve the list of genes within 2Mb of each *sentinel* SNP (i.e., involved in the pQTL associations identified by LOCUS), and also listed the SNPs in LD ($r^2 > 0.8$), limiting the search to 500Kb upstream and downstream of the sentinel SNP position. We called *cis pQTLs*, all sentinel SNPs located within ±1Mb of the gene encoding for the controlled protein, and *trans pQTLs*, all other pQTLs.

We evaluated the overlap between our pQTL associations and previously reported pQTL signals with the PhenoScanner database,[26] using the default p-value threshold $p < 10^{-5}$ and an LD proxy search ($r^2 > 0.8$, in populations with European ancestry). As queries using the R-package `phenoscanner` are limited to 500 returned tuples, we downloaded a local copy of the database (retrieved on 26/03/2019). Moreover, since protein names in the database do not follow the official UNIPROT protein names, we retrieved the annotation files from all individual studies and remapped the protein names onto the official UNIPROT identifier, thereby enabling the comparison with our pQTL hits using dbSNP rsIDs and UNIPROT IDs.

Epigenomic annotation. We retrieved epigenomic annotations of $1,000$ Genomes Project (release 20110521) from Pickrell.[27] The data covered 450 annotation features, each binary-coded according to the presence or absence of overlap with the SNPs. The features corresponded to DNase-I hypersensitivity, chromatin state, SNP consequences (coding, non-coding, 5'UTR, 3'UTR, etc), synonymous and nonsynonymous status and histone modification marks. We obtained distances to the closest transcription start site from the UCSC genome browser. Ninety-seven of our 104 validated sentinel SNPs had annotation data; to evaluate their functional enrichment, we resampled SNP sets of size 97 from our initial SNP panel, and, for each set, we computed the cumulated number of annotations. We did the same for the distances to transcription start sites. We repeated this 10^5 times to derive empirical p-values.

Colocalization with known eQTLs and with GWAS risk loci. We evaluated the overlap of our pQTLs with the eQTL variants reported by the GTEx Consortium[28] (release 7) at q-value < 0.05. We considered all 49 tissues listed by GTEx but eQTL SNPs for several tissues were counted only

once. We made both general queries and queries asking whether a pQTL uncovered by LOCUS was an eQTL for the gene coding for the controlled protein.

We retrieved known associations between the validated sentinel pQTLs and diseases or clinical traits, based on the GWAS catalog[29] (v1.0 release e92), and also using an LD proxy search ($r^2 > 0.8$).

We evaluated enrichment for eQTL and risk loci using one-sided Fisher exact tests based on the 104 validated sentinel pQTLs.

Associations with clinical variables. We tested associations between the proteins under genetic control and clinical parameters separately in each cohort. For the DiOGenes data, we used linear mixed-effect models, adjusting for age, gender as fixed effects, and center as a random effect. For the Ottawa data, we used linear models, adjusting for age and gender. Except when testing associations with anthropomorphic traits, all analyses were also adjusted for BMI. For the clinical variables available in the two cohorts (total cholesterol, HDL, LDL, fasting glucose, fasting insulin, HOMA-IR, triglycerides and VAI), we performed meta-analyses using the R package `metafor`. We used random-effects models to account for inter-study variability, which may in part result from geographical differences, and employed two-sided Wald tests for fixed effects, and Cochran Q-tests for measuring residual heterogeneity; we did not interpret the results if between-study heterogeneity estimates were high ($I^2 > 80\%$), and evaluated the directional consistency of the effects between Ottawa and DiOGenes. We adjusted for multiplicity using Benjamini–Hochberg correction across all tests, i.e., involving the 88 tested proteins and the two proteomic technologies, and reported associations using a 5% FDR threshold.

We assessed whether the proteins under genetic control were enriched in associations with the clinical variables. We randomly selected 10^5 sets of 88 proteins from the panel used for the pQTL analyses and derived an empirical p-value by counting, for each set, the number of proteins with at least one clinical association at FDR 5%.

Data availability. The MS proteomic data have been deposited on the ProteomeXchange Consortium via the PRIDE partner repository, `http://www.proteomexchange.org`, with the dataset identifiers PXD005216 for DiOGenes and PXD009350 for Ottawa. The SomaLogic proteomic data are available from the Open Science Framework, at `https://osf.io/v8mes/?view_only=13e4ccd127024ee7b4c819385325925c` and `https://osf.io/s4v8t/?view_only=90637f2941e14ec986e5888491fbdbbb`, respectively for Ottawa and DiOGenes. All pQTL and clinical association results are available as supplementary tables and can be browsed from our online database: `https://locus-pqtl.epfl.ch`. Other data that support the findings of this study are available from the corresponding authors upon reasonable request.

Code availability. All statistical analyses were performed using the R environment (version 3.3.2). LOCUS and ECHOSEQ are freely available from GitHub.

Results

Two-stage pQTL analyses. We analyzed the pQTL data from the Ottawa and the DiOGenes cohorts in a two-stage study (Figure 1D); the DiOGenes cohort recruited overweight/obese, non-diabetic subjects, while the Ottawa study was led in a specialized obesity practice where subjects had severe obesity, dyslipidemia and insulin resistance disorders (Supplementary Table S1).

We used LOCUS for multivariate analyses of both proteomic datasets from the Ottawa cohort, quantified by mass spectrometry (MS) and the multiplexed aptamer-based technology SomaLogic,[14] respectively. At FDR 5%, LOCUS identified 18 pQTL associations from the MS analysis, corresponding to 14 unique proteins and 18 SNPs, and 118 pQTLs from the SomaLogic analysis, corresponding to 99 proteins and 111 SNPs; see Supplementary Table S2. We then undertook to replicate all uncovered pQTLs in the independent DiOGenes cohort, using MS and SomaLogic data. We validated 15 of the 18 discovered MS pQTLs, and 98 of the 118 discovered SomaLogic pQTLs at FDR 5% (Supplementary Table S3), yielding a replication rate of 83% in both cases. While the two platforms had inherent differences, 72 proteins were quantified by both, enabling cross-platform comparison. Eight of the MS pQTLs could be assessed with SomaLogic (i.e., had protein levels available), and 7 of them replicated at FDR 5%. Likewise, of the 20 SomaLogic associations having MS measurements, 14 were confirmed, demonstrating appreciable cross-technology replication.

We evaluated replication rates separately for *cis* and *trans* effects. With the MS data, all 15 *cis* Ottawa pQTLs replicated in DiOGenes, while the 3 *trans* pQTLs did not. With the SomaLogic data, 78 of 81 *cis* and 20 of 37 *trans* pQTLs could be validated. The overall replication rates reached 97% for the *cis* pQTLs and 50% for the *trans* pQTLs; the *trans*-pQTL rate is in line with other pQTL studies.[2,4,5] Finally, 35 of our validated pQTLs are, to our knowledge, new, i.e., they do not overlap with pQTLs previously identified in the general population,[2,4–6,30] and this number drops to 20 using proxy search $r^2 > 0.8$; four of these 20 hits have isoforms involved in known pQTLs (Supplementary Table S4).

Comparison with a standard univariate pQTL analysis. The high replication rates and the novel discoveries uncovered by LOCUS are largely attributable to its flexible hierarchical sparse regression model which exploits shared association patterns across all SNPs and proteomic levels (Figures 1A–C), as extensively shown in previous numerical experiments.[11] Here, we provide additional evidence for our specific study and data by comparing LOCUS with the univariate method GEMMA in two ways. First, we evaluate variable selection performance in two simulation studies and, second, we confront the hits of LOCUS real data analysis to those found by re-analysing the Ottawa and DiOGenes datasets with GEMMA.

The first simulation study uses the SNPs from chromosome one of the Ottawa cohort and 133 outcomes mimicking the MS proteomic levels, enforcing that the SNPs explain together at most 35% of each protein variance (Material and Methods). The ROC curves of Figure 2A show a net gain in power for selections with LOCUS compared to GEMMA. The average standardized partial areas under the curve (pAUC) with 95% confidence intervals are 0.926 ± 0.005 for LOCUS and 0.840 ± 0.005 for GEMMA. The second simulation study generalizes this observation to a grid of data generation scenarios with different effect sizes and number of proteins under genetic control: Figure 2B indicates that the average standardized pAUC is greater for LOCUS than for GEMMA in most cases, and suggests a similar performance for very low proportions of protein variance explained by the SNPs.

By design, univariate screening approaches do not exploit association patterns common to multiple outcomes or markers; they analyze the outcomes individually, and do not account for LD, which often results in a number of redundant proxy discoveries at loci with strong LD (Figure 2C). At a given FDR, this hampers the detection of weak but genuine signals, because of the large multiple testing burden. Post-processing strategies accounting for local LD structures exist[31,32] and alleviate the problem to some extent. Instead of applying such corrections, our multivariate approach anticipates and addresses the question by inducing sparsity directly at the modelling stage, also jointly exploiting shared information across the outcomes to enhance the detection of weak effects. Owing to

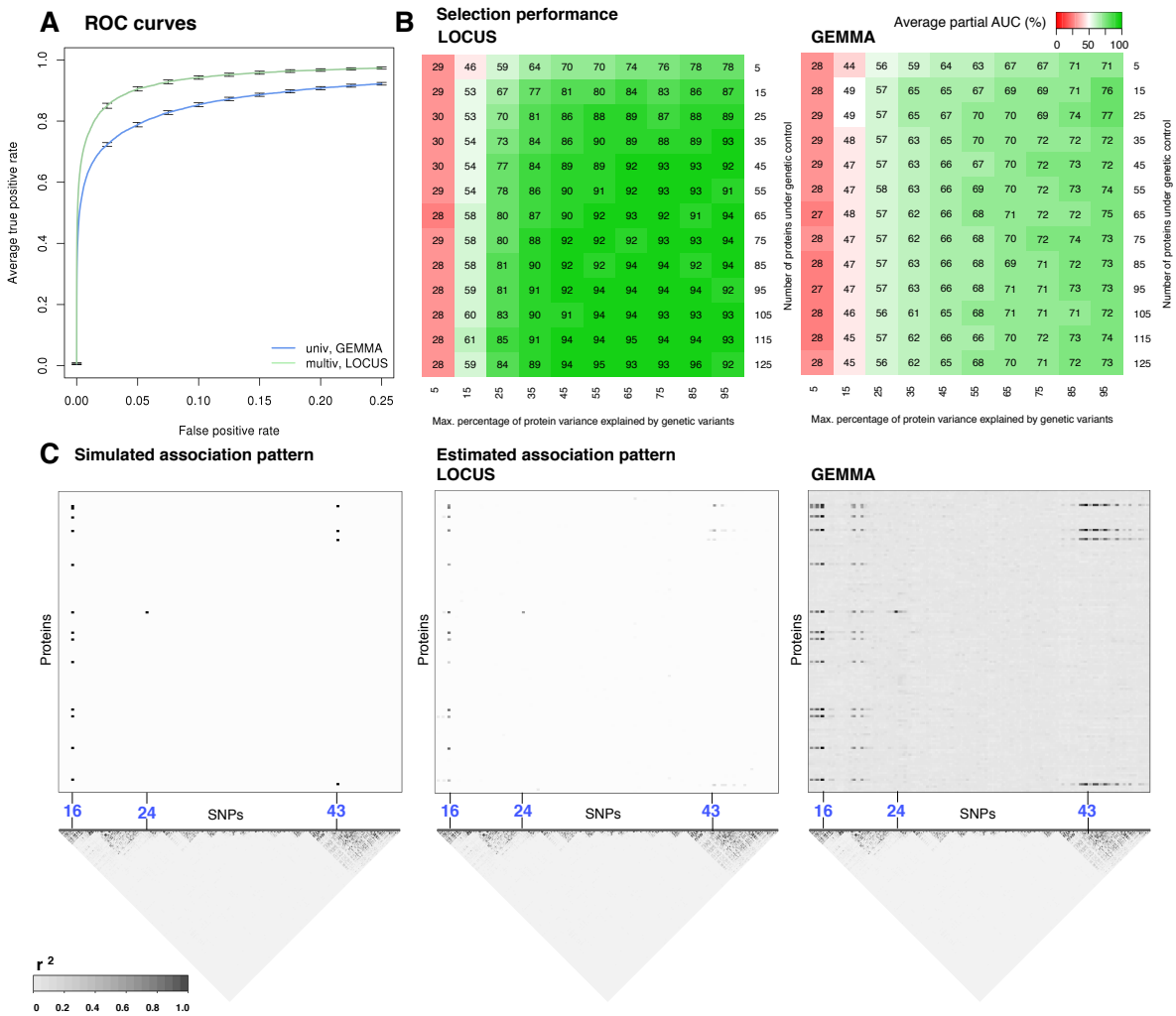

Figure 2: Selection performance of LOCUS and GEMMA on simulated data. (A) Truncated average ROC curves with 95% confidence intervals, obtained from 50 replications, for identification of SNP-trait associations. (B) Average standardized partial AUC of LOCUS and GEMMA for a grid of effect sizes (x-axis) and signal sparsity (y-axis), using 20 replications for each scenario. (C) Simulated pattern, and patterns recovered by LOCUS and GEMMA, averaged over the 50 replications. The plots display a window of 350 SNPs (x-axis) containing the first three SNPs having simulated associations (blue labels), along with their linkage disequilibrium (LD) pattern. GEMMA cannot discriminate the true signal from proxy hits in regions of high LD and highlights a series of SNPs as possible candidates, while LOCUS directly pinpoints the relevant SNPs.

its simulated annealing procedure that improves exploration at loci with strong LD, LOCUS better discriminates truly associated SNPs from their correlated neighbours.

These simulations tailored to the real data at hand prefigure the gain of power achieved by LOCUS over standard methods for our pQTL study, as we next discuss. We compared LOCUS and GEMMA under the typical two-stage GWA scenario employed in pQTL studies.[2,5,6] Namely, we contrasted the results of LOCUS with those of GEMMA applied to the Ottawa and DiOGenes data, but this time considering all 4 million SNPs available before LD pruning and employing a standard genome-wide Bonferroni correction of $\alpha = 0.05$. We acknowledge that the 0.05-Bonferroni correction may be overly conservative and also discuss the results using a permissive 0.2-Bonferroni threshold (Material and Methods).

The number of locus-protein hits identified and replicated with at least one of the two methods was 100, using Bonferroni correction of 0.05 for GEMMA (full list in Supplementary Table S5).

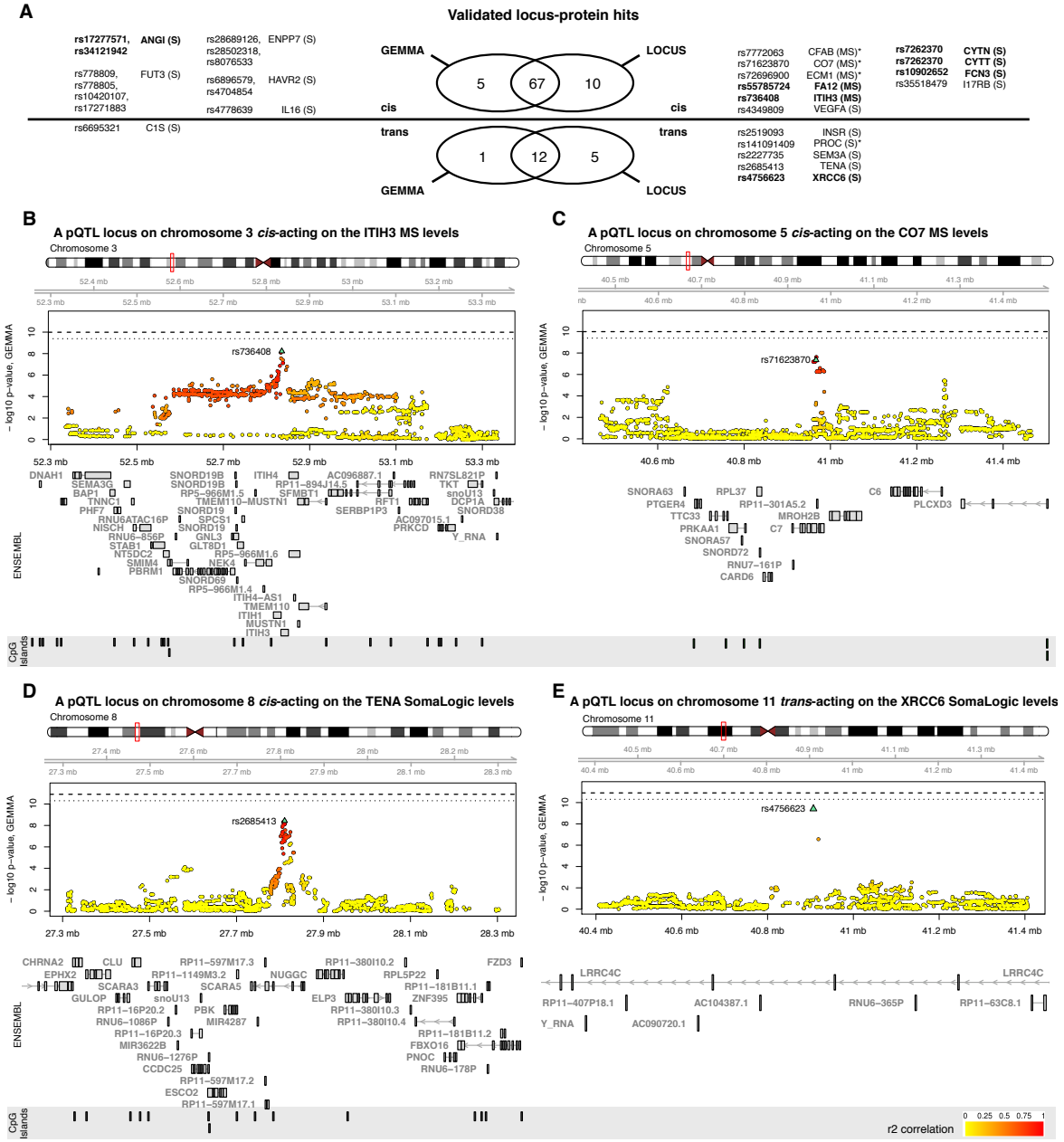

Figure 3: Comparison of LOCUS and GEMMA signal recovery on real data. (A) Venn diagram showing the locus-protein hits identified by the GEMMA and LOCUS two-stage analyses. The hits uncovered by GEMMA but not by LOCUS (left) and the hits uncovered by LOCUS but not by GEMMA (right) are listed; the former were not tagged so not detectable by the LOCUS analyses. When multiple SNPs correspond to the same locus-protein hit, the SNP(s) with the top association(s) in the Ottawa discovery cohort is/are shown. The novel hits (using a $r^2 > 0.8$-proxy search) are marked in bold and the hits with dual replication in the alternative proteomic platform are marked with a star (4 over the 4 quantified with both platforms). (B–E) Regional association plots for four loci, identified by the MS (B–C) and SomaLogic (D–E) pQTL analyses. In each case, the top panel displays the nominal $-\log_{10} p$-values obtained when re-analyzing the Ottawa data with GEMMA;[24] the dashed horizontal line corresponds to a Bonferroni level of $\alpha = 0.05$ and the dotted horizontal line corresponds to a Bonferroni level of $\alpha = 0.2$. The SNP identified by LOCUS is marked with a green triangle, and its correlation in r^2 with the surrounding SNPs is indicated by the yellow to red colors. The middle panel shows the transcript positions and the bottom panel shows the CpG island positions.

Among these 100 hits, GEMMA missed 15 hits validated by LOCUS (9 with the permissive Bonferroni correction of 0.2). As many as 5 of these 15 hits were *trans* associations and 6/15 hits were not previously described in the literature using a $r^2 > 0.5$-proxy search (Supplementary Table S5 and

Figure 3A); these two observations highlight again the ability of LOCUS to identify weak/*trans* effects that may go unnoticed by univariate analysis, in line with the above simulation studies. Moreover, all 4 hits whose protein was quantified by both proteomic technologies had a successful dual replication using the alternative technology.

Figure 3B–E displays four examples of hits missed by GEMMA and validated by the LOCUS two-stage MS (CO7 and ITIH3 loci) and SomaLogic (TENA and XRCC6 loci) analyses. Each panel shows how some univariate signal is present, of different nature depending on the type of linkage disequilibrium in the loci, but is too weak to be detected after multiplicity correction. In contrast, the multiplicity-adjusted LOCUS analyses could effectively single out and validate individual hits among the strongest GEMMA signals.

Finally, the LOCUS analysis missed 6 locus-protein hits identified by the GEMMA analysis, but, importantly, these hits were not detectable by LOCUS. Indeed, all 115 SNPs selected by GEMMA have been removed by the $r^2 > 0.95$ pruning employed to define the tag SNP panel used for the LOCUS study.

The next two sections focus on characterizing the hits validated using the LOCUS two-stage analyses in terms of functional enrichment and colocalization patterns.

Colocalization with eQTLs and evidence for regulatory impact. We assessed the overlap of the 113 validated pQTLs with known eQTLs (Supplementary Table S6). Seventy-seven of the 104 sentinel SNPs involved in our pQTL associations had one or more eQTL associations in at least one tissue. These SNPs have been implicated in 83 eQTL associations, representing a significant enrichment ($p < 2.2 \times 10^{-16}$). Forty-nine of these 77 SNPs were eQTL variants for the gene coding for the protein with which they were associated in our datasets. Our pQTLs were also enriched in epigenome annotation marks ($p = 9.20 \times 10^{-4}$) and significantly closer to transcription start sites compared to randomly chosen SNP sets ($p = 9.99 \times 10^{-6}$). These observations suggest potential functional consequences for our pQTL hits.

Colocalization with GWAS risk loci. A total of 217 previously reported genome-wide associations overlapped our validated pQTL loci, corresponding to 139 unique traits mapping to 68 distinct regions (based on LD $r^2 > 0.8$). Nineteen SNPs were directly involved in these associations (Supplementary Table S7) representing a significant enrichment ($p < 2.2 \times 10^{-16}$).

Some of these results generate useful hypotheses to be explored in future research. For instance, a HGFL *cis* pQTL, rs1800668, is in strong LD ($r^2 > 0.95$) with rs9858542 and rs3197999, which are known to associate with Crohn's disease.[33,34] Our pQTL finding may be of clinical relevance given the prevalence of Crohn's disease in overweight and obese subjects;[35] the region would merit follow-up in inflammatory bowel disease cohorts.

Another example concerns an association between rs3865444 and the Siglec-3 protein, whose coding gene, *CD33*, has been reported as a risk factor for Alzheimer's disease.[36] As subjects obese in midlife are more at risk of developing late-life Alzheimer's,[37] this pQTL may help to better understand the genetic bases of Alzheimer's disease and dementia; its potential as a prognosis biomarker should be studied in Alzheimer's cohorts, ideally using weight records.

Proteins as endophenotypes to study the genetics of obesity. Annotation from public databases suggested that most pQTLs had implications in inflammation, insulin resistance, lipid metabolism or cardiovascular diseases. We performed a more systematic evaluation of their clinical relevance in a meta-analysis of the DiOGenes and Ottawa clinical and proteomic data, and found

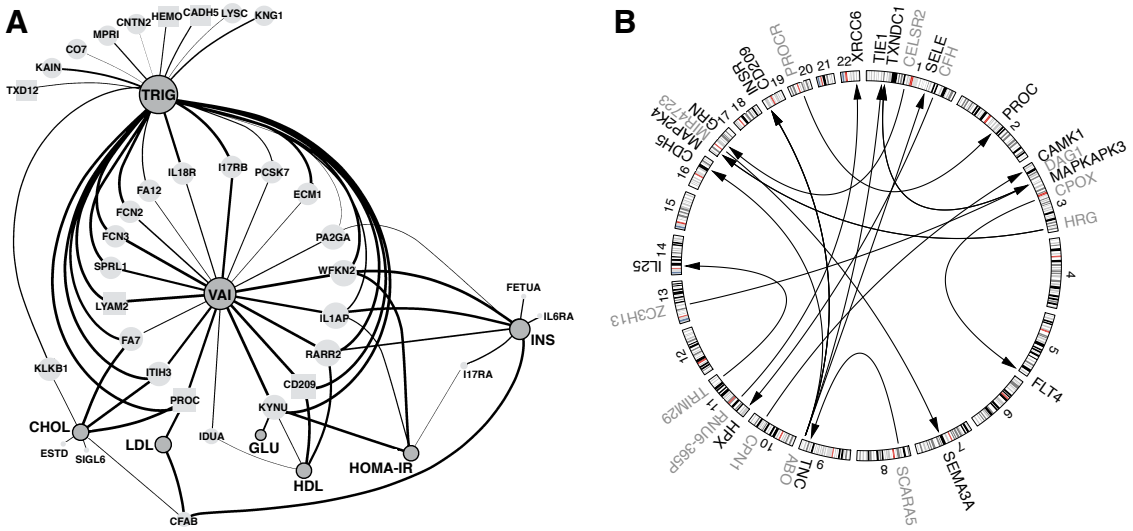

Figure 4: Associations of proteins under genetic control with clinical parameters, and *trans*-pQTLs associations. (A) Network displaying the associations (FDR < 5%) between protein levels and clinical variables obtained by meta-analysis, adjusting for age, gender and BMI. The nodes representing clinical parameters are in dark grey with black borders (fasting glucose, HDL, HOMA-IR, insulin resistance, LDL, total cholesterol, triglycerides, visceral adiposity index), and those representing proteins are in light grey with the type of genetic control, *cis* or *trans*, depicted with circles or squares, respectively. The edge thickness is proportional to the significance of association, and the node size is proportional to its connectivity. (B) Circular plot showing the *trans*-pQTL associations uncovered by LOCUS (FDR < 5% for discovery and validation). Each arrow starts from the pQTL SNP with label indicating its closest gene (grey) and points to the gene (black) coding for the controlled protein.

that 35 of the 88 proteins under genetic control had associations with dyslipidemia, insulin resistance or visceral fat-related measurements at FDR 5%; these associations should be attributable metabolic factors independently of overall adiposity, as we controlled for BMI as a potential confounder. They are displayed as a network in Figure 4A and are listed in Table 1. We observed consistent directions of effects in the two cohorts (see Forest plots of Figure 5, and Supplementary Table S8 for full details). Remarkably, we found that the 88 genetically-driven proteins are significantly more associated with the clinical variables than randomly chosen protein sets ($p = 0.014$); this enrichment suggests that the primary pQTL analyses can help uncover potential proteomic biomarkers for the Metabolic Syndrome and other obesity-related complications.

As shown in the network of Figure 4A, the triglyceride measurements and visceral adiposity index (VAI) have the highest degree of connectivity and are connected with measures of insulin resistance and other lipid traits via proteins such as FA7, IL1AP, KYNU, PROC, RARR2 and WFKN2. CFAB, FETUA, PA2GA have lower connectivity, yet are relevant in the context of obesity.[38–40] *Trans*-regulated proteins (Figure 4A–B) were also implicated in clinical associations: CADH5, CD209 and LYAM2, all controlled by the pleiotropic *ABO* locus; HEMO (Hemopexin), a liver glycoprotein controlled by the *CFH* locus, itself coding for another liver glycoprotein; PROC controlled by its own receptor *PROCR*; and TXD12 (thioredoxin domain containing 12), controlled by the *DAG1/BSN* locus.

In the subsequent sections, we expand on the possible functional and biomedical relevance of three representative examples of pQTL associations in the context of obesity complications. Additional examples are discussed in Appendix B. Unless otherwise specified, all associations described have meta-analysis FDR corrected p-value below 5%, and we provide their nominal p-values in parentheses.

CFAB and RARR2, mediators of adipogenesis are under genetic control. CFAB (complement factor B) and RARR2 (Retinoic acid receptor responder protein 2) levels associate with distinct

Protein	Protein name	Clin.	SNP	Chr	Position	LOCUS PPI	pQTL valid. p-value
CADH5	**Cadherin-5**	**L**	**rs8176741**	**9**	**136131461**	**1.00**	$\mathbf{4.68 \times 10^{-30}}$
CD209	**DC-SIGN**	**L/V**	**rs8176741**	**9**	**136131461**	**1.00**	$\mathbf{7.74 \times 10^{-10}}$
			rs2519093	**9**	**136141870**	**1.00**	$\mathbf{6.24 \times 10^{-26}}$
CFAB	Factor B	G/L	rs7772063	6	31906334	0.85	9.61×10^{-4}
			rs641153	6	31914180	1.00	3.92×10^{-12}
CNTN2	CNTN2	L	rs11240396	1	205205081	1.00	6.82×10^{-14}
CO7	C7	L	rs71623870	5	40966676	0.83	4.03×10^{-4}
ECM1	ECM1	L/V	rs34964511	1	150298015	1.00	3.77×10^{-6}
			rs71578487	1	150340059	1.00	1.07×10^{-11}
			rs72696900	1	150425256	0.82	1.5×10^{-6}
			rs11802612	1	150427279	1.00	3.7×10^{-6}
			rs35094010	1	150449557	1.00	3.76×10^{-6}
ESTD	Esterase D	L	rs73193065	13	47383681	0.90	2.31×10^{-15}
FA12	Coagulation factor XII	L/V	rs55785724	5	176817583	1.00	1.34×10^{-5}
FA7	Coagulation Factor VII	L/V	rs3093233	13	113758130	1.00	3.11×10^{-88}
FCN2	FCN2	L/V	rs3811140	9	137772111	1.00	9.66×10^{-14}
FCN3	Ficolin-3	L/V	rs10902652	1	27558522	1.00	1.62×10^{-3}
FETUA	a2-HS-Glycoprotein	G	rs2593813	3	186332571	1.00	2.47×10^{-10}
			rs2593813	3	186332571	1.00	4.51×10^{-8}
HEMO	**Hemopexin**	**L**	**rs10801560**	**1**	**196714600**	**1.00**	$\mathbf{2.36 \times 10^{-26}}$
I17RA	IL-17 sR	G	rs738035	22	17594886	1.00	1.48×10^{-20}
I17RB	IL-17B R	L/V	rs35518479	3	53873814	0.76	9.98×10^{-6}
IDUA	IDUA	L/V	rs10017289	4	943534	1.00	1.22×10^{-11}
IL18R	IL-18 Ra	L/V	rs3836108	2	103037742	1.00	5.22×10^{-26}
IL1AP	IL-1 R AcP	G/L/V	rs724608	3	190348810	1.00	8.7×10^{-114}
IL6RA	IL-6 sRa	G	rs4845372	1	154415396	1.00	1.72×10^{-81}
ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3	L/V	rs736408	3	52835354	0.97	1.46×10^{-6}
KAIN	Kallistatin	L	rs5511	14	95033595	1.00	9.9×10^{-24}
KLKB1	Prekallikrein	L	rs80177406	4	187166024	0.99	3.54×10^{-6}
KNG1	Kininogen HMW	L	rs1621816	3	186439173	1.00	1.44×10^{-13}
KYNU	KYNU	G/L/V	rs6741488	2	143793701	1.00	3.22×10^{-20}
LYAM2	**sE-Selectin**	**L/V**	**rs2519093**	**9**	**136141870**	**1.00**	$\mathbf{6.81 \times 10^{-62}}$
LYSC	Lysozyme	L	rs71094714	12	69790495	1.00	8.41×10^{-19}
MPRI	IGF-II receptor	L	rs3777411	6	160476945	1.00	4.95×10^{-11}
PA2GA	NPS-PLA2	G/L/V	rs6672057	1	20293791	1.00	3.86×10^{-15}
PCSK7	PCSK7	L/V	rs11216284	11	117003060	1.00	8.17×10^{-31}
PROC	**Protein C**	**L/V**	**rs141091409**	**20**	**33739915**	**0.43**	$\mathbf{1.66 \times 10^{-18}}$
RARR2	TIG2	G/L/V	rs1047586	7	150035459	0.96	2.39×10^{-11}
SIGL6	Siglec-6	L	rs8101887	19	52029477	1.00	3.39×10^{-14}
SPRL1	SPARCL1	L/V	rs7681694	4	88462729	0.99	5.70×10^{-14}
TXD12	**TXD12**	**L**	**rs13062429**	**3**	**49559485**	**1.00**	$\mathbf{2.26 \times 10^{-5}}$
			rs34519883	**3**	**49575831**	**1.00**	$\mathbf{5.39 \times 10^{-33}}$
WFKN2	WFKN2	G/L/V	rs9303566	17	48922281	1.00	3.38×10^{-11}

Table 1: Proteins associated with clinical parameters (Figure 4A) and controlled by pQTL variants. All associations were detected at FDR $< 5\%$. Associations with glycemic traits (fasting glucose, insulin, HOMA-IR) are indicated by G, with total lipid traits (HDL, LDL, triglycerides, total cholesterol), by L, and with visceral fat (visceral adiposity index), by V. *Trans*-pQTL associations are in bold.

clinical parameters (Figures 4A and 5), yet both play a role in adipogenesis and hence are particularly interesting in the context of obesity and related co-morbidities.

The CFAB protein controls the maturation of adipocytes in rat[38] and has a determinant role in metabolic and cardiovascular dysfunctions linked with the Metabolic Syndrome.[41] In our study, both the MS and SomaLogic measurements were positively associated with BMI (MS: $p = 2.08 \times 10^{-8}$, SomaLogic: $p = 2.23 \times 10^{-13}$) and with fasting insulin (adjusting for BMI; MS: $p = 4.45 \times 10^{-5}$, SomaLogic: $p = 3.44 \times 10^{-4}$). The CFAB SomaLogic levels were negatively associated with cholesterol ($p = 1.43 \times 10^{-3}$), LDL ($p = 1.30 \times 10^{-5}$), and with HDL at higher FDR (nominal $p = 1.47 \times 10^{-2}$, corrected $p = 0.11$). This is consistent with previous work linking *CFB* gene expression from different human adipose tissue fractions with insulin resistance and lipid levels.[42]

Our MS and SomaLogic analyses independently highlighted the same *cis*-acting locus as putative regulator of the CFAB protein. In particular, the sentinel pQTL SNP detected in the SomaLogic analysis, rs641153, is a missense variant located in the MHC region, 180 base pairs away from a transcription binding site (significantly closer than other SNPs, $p = 1.16 \times 10^{-2}$). Further investigation

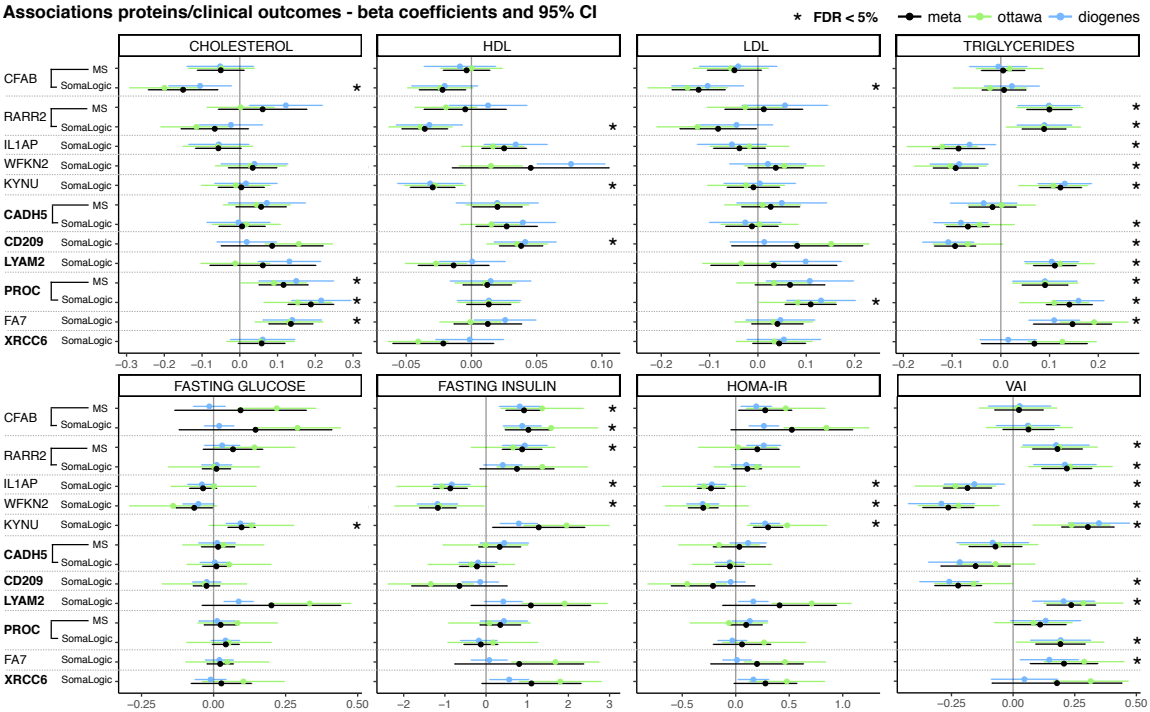

Figure 5: Forest plots for associations between a proteins under genetic control and clinical parameters, adjusting for age, gender and BMI (Material and Methods); the selection of proteins shown covers the examples discussed in the main text and the Appendix B. All endpoints are measured in both the Ottawa and DiOGenes cohorts; they correspond to total lipid levels (first row: total cholesterol, HDL, LDL, triglycerides), glucose/insulin resistance (second row: fasting glucose, fasting insulin, HOMA-IR) and VAI. In each case, regression coefficients with 95% confidence intervals are shown for the Ottawa and DiOGenes analyses, and for the meta-analysis. The stars indicate associations with meta-analysis FDR $< 5\%$ (correction applied across all proteins under genetic control, not only those displayed; see Figure 4A). For proteins with measurements in the MS and SomaLogic platforms, association results are displayed for both; *trans*-regulated proteins are in bold.

using JASPAR and SNP2TFBS indicated that rs641153 may affect the binding sites of four transcription factors (EBF1, TFAP2A, TFAP2C and HNFA), and this SNP has indeed been reported as both e- and pQTL.[4,43]

RARR2 (Chemerin protein) is encoded by an essential adipogenesis gene, *RARRES2*. This adipokine plays an important role in inflammation, adipogenesis, angiogenesis and glucose homeostasis.[44,45] Our pQTL analyses indicated a *cis* association between a missense variant, rs1047586, and RARR2 protein levels, in line with previous findings reporting this SNP as e- and pQTL (Supplementary Tables S4 and S6). Moreover, our analyses of protein levels revealed significant associations with triglycerides, fasting insulin and HDL (Figure 5, Supplementary Table S8), which is consistent with previously described pleiotropic associations of *RARRES2* variants with circulating RARR2, triglyceride levels and diverse measurements related to inflammation,[46] and findings from animal models.[47,48] Moreover, MS and SomaLogic RARR2 levels were strongly associated with visceral fat, even when controlling for BMI (Figure 5; Supplementary Table S8), further strengthening the relevance of this protein in the development of the Metabolic Syndrome.[45,49]

Pleiotropic effects from the *ABO* locus onto CADH5, CD209, INSR, LYAM2 and TIE1. *ABO* is a well-known pleiotropic locus associated with coronary artery diseases, type 2 diabetes, liver enzyme levels (alkaline phosphatase) and lipid levels.[2–4] Our analyses highlighted two independent sentinel SNPs in the *ABO* region: rs2519093 and rs8176741 ($r^2 = 0.03$). The former SNP is *trans*-

acting on E-selectin (protein LYAM2 encoded by SELE), the Insulin Receptor and the CD209 antigen. The latter SNP is *trans*-acting on the Tyrosine-protein kinase receptor (Tie-1), Cadherin-5 and CD209. Both SNPs were reported as *cis*-acting eQTL variants for *ABO*, *OBP2B* and *SURF1*, and further queries in public databases indicated that rs8176741 may affect the binding sites for three transcription factors (Myc, MYC-MAX and Arnt), suggesting a complex gene regulation circuitry.

Our clinical analyses indicated associations of CD209 and LYAM2 with triglycerides and visceral fat, and CADH5 with triglycerides only (Figure 5). The LYAM2 levels were associated with all the glycemic variables in the Ottawa cohort (fasting glucose: $p = 6.43 \times 10^{-6}$, fasting insulin: $p = 3.54 \times 10^{-4}$, HOMA-IR: $p = 1.8 \times 10^{-4}$), but only with fasting glucose in the DiOGenes cohort ($p = 8.91 \times 10^{-4}$), although we observed a trend for HOMA-IR (nominal $p = 0.02$, corrected $p = 0.15$). Since the Ottawa subjects are more insulin-resistant than the DiOGenes subjects (average HOMA-IR with standard deviation: 4.97(3.88) versus 3.00(1.71), $p = 2.52 \times 10^{-18}$; Supplementary Table S1), LYAM2 might represent a marker of insulin-resistance severity. Consistent with this hypothesis, the plasma levels of LYAM2 are employed as a biomarkers of endothelial dysfunction and risk of type 2 diabetes.[50]

We found that CD209 circulating levels were positively associated with HDL, negatively with triglyceride levels, and, consistently with these effects, negatively with visceral fat index, suggesting beneficial effects of high CD209 levels. Further investigation using deconvolution of adipose tissue gene expression profiles showed that CD209 is predominantly secreted by M2 macrophages.[51] These cells are involved in extracellular matrix remodelling and secrete cytokines with an anti-inflammatory role, counteracting the effect from pro-inflammatory macrophages M1. Interestingly, other adipose cell types, including M1 macrophages display little, if any, CD209 expression. M1 and M2 macrophages have been extensively discussed in the context of obesity, and it is well established that M2 macrophages have a protective role against obesity and insulin resistance[52] and increase fatty oxidation and oxidative phosphorylation.[53] In our data (both the Diogenes and Ottawa cohorts, and at FDR 5%), we found that CD209 levels were positively correlated with M2 secreted proteins (IL10, CSF1, ARGI1) and negatively correlated with M1 pro-inflammatory markers, such as TGF-beta, IL6 and interferon-gamma (Supplementary Figure C.1). Importantly, CD209 was positively associated with circulating levels of adiponectin, an hormone secreted in adipose tissue which plays a key role in glucose regulation, fatty acid oxidation and triglycerides clearance. This lends support that CD209 could be a secreted protein, released by M2 macrophages from adipose tissue, with a beneficial role in controlling lipid levels, thereby possibly protecting from developing dyslipidemia and related metabolic complications.

XRCC6, a DNA repair protein as putative biomarker for metabolic disorders. We identified rs4756623, as a novel *trans* pQTL for XRCC6 (X-Ray Repair Complementing Defective Repair In Chinese Hamster Cells; also known as Ku70). Proxy searches (down to r-square 0.5 in European ancestry panels) did not reveal any tag SNP previously reported as a QTL (including e-, p-, or m-QTL). The *XRCC6* gene activates DNA-dependent protein kinases (DNA-PK) to repair double-stranded DNA breaks by nonhomologous end joining. DNA-PKs have been linked to lipogenesis in response to feeding and insulin signaling.[54] DNA-PK inhibitors may reduce the risk of obesity and type 2 diabetes by activating multiple AMPK targets.[55] A recent review discussed the role of DNA-PK in energy metabolism, and in particular, the conversion of carbohydrates into fatty acids in the liver, in response to insulin.[56] It described increased DNA-PK activity with age, and links with mitochondrial loss in skeletal muscle and weight gain. Finally, *XRCC6* functions have been reported as associated with regulation of beta-cell proliferation, islet expansion, increased insulin levels and decreased glucose

levels.[55,57]

We observed significant associations between the XRCC6 protein levels and several clinical variables in the Ottawa cohort (FDR $< 5\%$). Higher expression was associated with decreased HDL ($p = 5.83 \times 10^{-4}$), as well as with higher triglycerides ($p = 4.39 \times 10^{-4}$), insulin levels ($p = 4.50 \times 10^{-4}$) and visceral adiposity ($p = 5.94 \times 10^{-5}$; Figure 5). We only found marginal associations using the DiOGenes data for insulin levels (nominal $p = 0.02$, corrected $p = 0.14$) and HOMA-IR (nominal $p = 0.02$, corrected $p = 0.16$). The directionality of these effects was consistent in both cohorts. As the Ottawa subjects were more severely obese, the effects might be larger for subjects with pronounced Metabolic Syndrome.

Our pQTL sentinel SNP, rs4756623, is intronic and located within the *LRRC4C* gene, a binding partner for Netrin G1 and member of the axon guidance.[58] To our knowledge, *LRRC4C* has not been previously described in the context of obesity, insulin resistance or type 2 diabetes. However, its partner Netrin G1 is known to promote adipose tissue macrophage retention, inflammation and insulin resistance in obese mice.[59] The underlying regulatory mechanisms between rs4756623 and the *XRCC6* locus should be clarified, and functional studies will be required to understand their physiological impact.

Discussion

Despite important technological advances, large-scale pQTL studies remain infrequent, owing to their high costs.[2–6,30] To date, most studies have focused on data from the general population with limited access to clinical parameters and have assessed links with diseases by relying on information from different studies.

Here we described the first integrative pQTL study that relates the associations discovered to metabolic disorders, such as insulin resistance and dyslipidemia, in the obese population considered. Our Bayesian method LOCUS confirmed 93 pQTLs (75 distinct proteins) highlighted in previous studies,[2,4–6,30] despite our sample sizes 2.5 to 18 times smaller, and revealed 20 novel pQTLs (18 distinct proteins, see Supplementary Table S4), with sound evidence for functional relevance and implications for the development of the Metabolic Syndrome. Our two-stage approach achieved very high replication rates ($> 80\%$), and validated findings which standard univariate designs would have missed (e.g., the *trans* associations with INSR, PROC, SEM3A, TENA and XRCC6 would have been missed). This corroborates our simulation study, which demonstrated the increased statistical power of LOCUS over univariate approaches on synthetic data mimicking the real data. Owing to its joint modelling of all proteins and genetic variants, LOCUS both accounts for linkage disequilibrium and exploits the shared regulatory architecture across molecular entities; this drastically reduces the multiplicity burden and enhances the detection of weak effects. Finally, our analyses indicated that proteins under genetic control are enriched in associations with clinical parameters pertaining to obesity co-morbidities, which further supports a genetic basis of these parameters and emphasizes the advantages of pQTL studies for elucidating the underlying functional mechanisms. Our complete pQTL and clinical association results offer opportunities to generate further hypotheses about therapeutic options; they are accessible from the searchable online database `https://locus-pqtl.epfl.ch`.

The work presented in this paper is at the interface of methodological developments for pQTL mapping and concrete biological findings that take advantage of our tailored statistical approach in a thorough analyses of two obesity cohorts and two independent proteomic technologies. A central ambition was to showcase that LOCUS can bridge the gap between Bayesian multivariate inference and its practical use for analyzing current molecular QTL data. Indeed, the applicability of LOCUS

goes beyond pQTL studies, as it is tailored to any genomic, proteomic, lipidomic or methylation QTL analyses and can be used for genome-wide association with several clinical endpoints. Its joint framework is made efficient at a genome-wide scale thanks to a scalable batch-wise variational algorithm and an effective C++/R implementation. Performance profiling for our method demonstrated that it is applicable to the analysis of millions of SNPs and thousands of molecular levels (Appendix A). To our knowledge, no other fully multivariate method is applicable to large molecular QTL studies without drastic preliminary dimension reduction; LOCUS therefore opens new perspectives for uncovering weak and complex effects.

Web ressources

ECHOSEQ: `https://github.com/hruffieux/echoseq`
Ensembl: `http://grch37.ensembl.org/index.html`
GEMMA: `http://www.xzlab.org/software.html`
GTEx: `https://gtexportal.org/home`
GWAS Catalog: `https://www.ebi.ac.uk/gwas`
IMPUTE2: `http://mathgen.stats.ox.ac.uk/impute/impute_v2.html`
JASPAR: `http://jaspar.genereg.net`
LOCUS: `https://github.com/hruffieux/locus`
Metafor: `https://cran.r-project.org/web/packages/metafor/index.html`
PhenoScanner: `http://www.phenoscanner.medschl.cam.ac.uk`
PLINK: `http://zzz.bwh.harvard.edu/plink`
ProteomeXchange: `http://www.proteomexchange.org`
R: `https://www.r-project.org`
SHAPEIT: `https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html`
SNP2TFBS: `https://ccg.vital-it.ch/cgi-bin/snp2tfbs/snpviewer_form_parser.cgi`
UCSC: `https://genome.ucsc.edu`
UniProt: `https://www.uniprot.org`

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Author contributions

HR designed and developed the LOCUS method with input from AD and JH. AV supervised the omics data generation and preprocessing. JC contributed to data processing. HR and AV designed the pQTL study. HR implemented statistical analyses with input from AV and AD. WS and AA designed the DiOGenes clinical study; BD and MEH designed the Canadian program. HR and AV interpreted the results, wrote the manuscript with input from all authors, and have primary responsibility for final content.

Declaration of interest

HR, JC, JH and AV are full-time employees at Nestlé Research. WS reports research support from several food companies (Nestlé, DSM, Unilever, Nutrition et Santé and Danone), and pharmaceutical companies (GSK, Novartis and Novo Nordisk). He is an unpaid scientific advisor for the International Life Science Institute, ILSI Europe.

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A Computational performance of LOCUS

The runtime of LOCUS for the simulations presented in the paper was similar to that of GEMMA: on average, for one replicate, LOCUS took 5 minutes and 26 seconds to complete, while GEMMA took 7 minutes and 4 seconds, running in parallel on four cores of an Intel Xeon CPU, 2.60 GHz.

Figure A.1 presents runtime profiling for LOCUS when applied to different numbers of SNPs and molecular traits. All runs completed within hours.

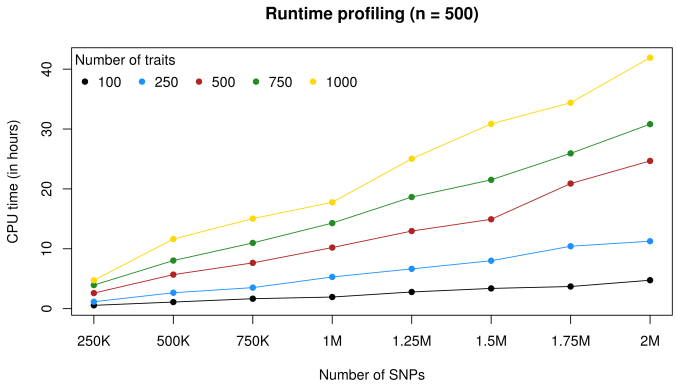

Figure A.1: Runtime profiling in CPU hours, for 2.5×10^5 to 2×10^6 SNPs and 100 to 1000 traits, on an Intel Xeon CPU at 2.60 GHz with 256 Gb RAM. For each case, the average runtime of five replications is displayed. The same annealing settings as in the MS and SomaLogic analyses were used (50 geometrically-spaced temperatures and initial temperature $T = 20$).

B Further examples of pQTL loci with probable implications in metabolic disorders

The importance of IL1AP for Metabolic Syndrome. The IL-1 pathway plays a critical role in the immune-response associated with obesity and type 2 diabetes;[60] other IL-1 related cytokines, such as IL-1ra, are also well documented in the context of type 1 and type 2 diabetes.[61] The IL1AP (IL-1 receptor accessory) protein is a co-receptor of the IL-1 receptor, and its soluble levels were found reduced in obese subjects.[62] Our analyses found an association between rs724608 and IL1AP, corroborating previously identified associations with SNPs in LD ($r^2 = 0.93$).[62]

We found associations between IL1AP expression and measures of fasting insulin levels ($p = 3.88 \times 10^{-5}$), HOMA-IR ($p = 3.89 \times 10^{-4}$), triglycerides ($p = 1.61 \times 10^{-3}$) and visceral fat ($p = 2.1 \times 10^{-4}$) (Figures 4A and 5). Moreover, worsened Metabolic Syndrome scores[63] were associated with lower protein levels ($p = 1.20 \times 10^{-3}$ in Ottawa and $p = 2.50 \times 10^{-4}$ in DiOGenes).

WFKN2, a TGFβ-activity protein with protective effect against metabolic disorders. The role of the WFKN2 protein and of its coding gene, *WFIKKN2*, in regulating TGFβ activity has been extensively studied in muscle and skeletal muscle,[64] but, to our knowledge, not in other tissues. We describe it for the first time in the context of obesity and metabolic disorders. We found that higher protein levels were associated with lower levels of fasting insulin, triglycerides, HOMA-IR and visceral fat (Figure 5), suggesting a protective role against metabolic dysregulation.

Our analyses suggested that the WFKN2 levels are controlled by rs9303566, which is consistent with other p- and eQTL studies (Supplementary Tables S4–S5). This SNP was found to be associated with DNA methylation and histone marks,[65,66] and is located within 100 base pairs of a transcription

factor binding site, with numerous factors such as MYBL2, NFIC, EP300 and MXI1. It is in strong LD with other SNPs with potential regulatory impact; for instance, it is located 9Kb upstream to rs8072476 ($r^2 = 0.97$), which overlaps another cluster of transcription factor binding sites (FOXA1, ESR1, USF1 & 2, TFAP2A & 2C).

Inflammation mediated proteins and their role in insulin resistance. We found a *cis* effect of rs6741488 on KYNU (Kynureninase) plasmatic levels. KYNU is an enzyme involved in the biosynthesis of nicotinamide adenine dinucleotide (NAD) cofactors from tryptophan. This protein and its pathway have been found to be particularly relevant for obesity and associated metabolic disorders. KYNU was found to be up-regulated by pro-inflammatory cytokines in human primary adipocytes, and more so in the omental adipose tissue of obese compared to lean control subjects.[67] Other studies indicated that the kynurenine pathway (KP) may act as an inflammatory sensor, and that increased levels of its catabolites may be linked with several cardiometabolic defects, including cardiovascular disease, diabetes and obesity.[68] In our cohorts, higher KYNU levels were associated with decreased HDL levels ($p = 6.66 \times 10^{-4}$), and increased triglycerides levels ($p = 3.43 \times 10^{-8}$), visceral fat ($p = 2.51 \times 10^{-8}$) and insulin resistance (marginally, nominal $p = 2.53 \times 10^{-2}$, corrected $p = 0.17$), see Figure 5; as expected, higher protein levels were associated with a worsened Metabolic Syndrome score (Ottawa $p = 8.23 \times 10^{-5}$; DiOGenes $p = 3.62 \times 10^{-6}$).

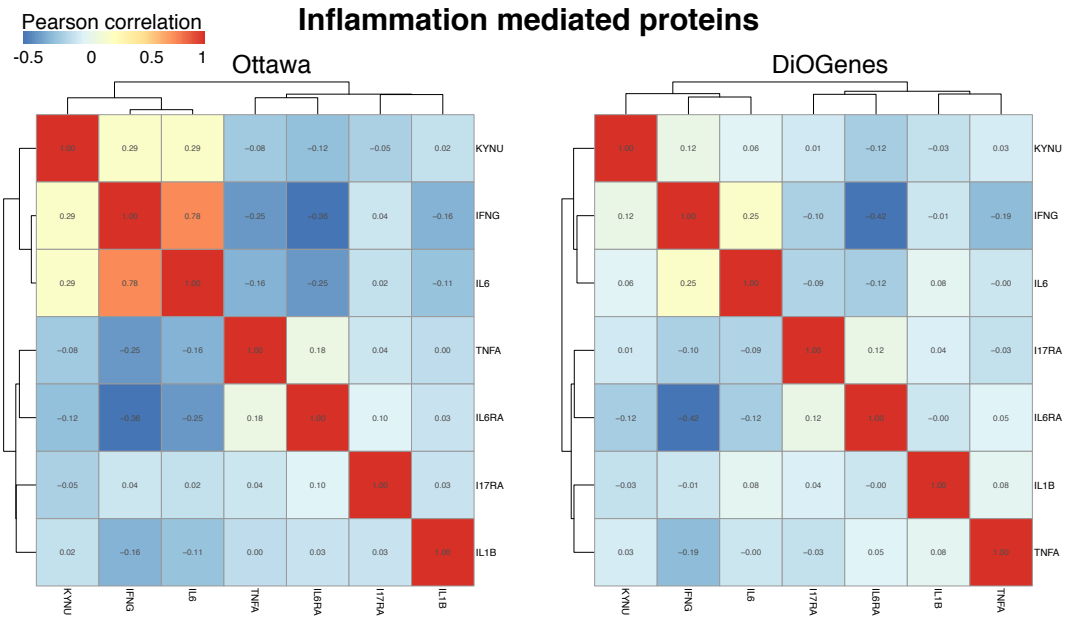

Figure B.1: Correlation of KYNU, IFNG, IL6, THFA, IL6RA, I17RA and IL1B in Ottawa (left) and in DiOGenes (right).

Recent work suggested a causal link between obesity and cancer, mediated by KP activation through inflammatory mechanisms.[69] Interestingly, our analyses highlighted two soluble interleukin receptor antagonist proteins, namely IL6RA and I17RA, that were both under genetic control and associated with insulin resistance (Figure 4A). We did not find significant correlation between the I17RA and KYNU protein levels, but we did observe a significant negative correlation between IL6RA and KYNU (Ottawa $p = 0.01$ and DiOGenes $p = 4 \times 10^{-3}$). We found a link between the plasma levels of KYNU and pro-inflammatory molecules, namely, IL6, IFNG and TNFα. In the Ottawa cohort, where subjects displayed high low-grade inflammation status, KYNU was positively associated with IL6 and IFNG at FDR 5%, while in DiOGenes, we found a positive association with IFNG only (see

Figure B.1). Finally, metabolic dysfunctions mediated via KP may relate to another inflammatory pathology, namely, psoriasis,[70] a skin disease aggravated by obesity and improved by weight loss.[71,72]

Our results thus highlighted pQTLs with probable roles in inflammation and subsequent metabolic dysfunctions, reinforcing previous discussion[68,73] of the potential of KP therapeutic inhibitors against cardiovascular disease and metabolic disorders.

Complement/coagulation: a *trans*-acting insertion linking PROC and its receptor. PROC (Protein C, coding gene PROC on chromosome 2) and its paralog protein FA7 (Coagulation Factor 7, coding gene F7 on chromosome 13) regulate the complement and the coagulation systems. Both systems promote inflammation[74] and contribute to metabolic dysfunction in the adipose tissue and liver.[75] Our analyses suggested novel pQTLs for these proteins (Supplementary Table S3): FA7 was associated with rs3093233, which is a known eQTL of *F7* and *F10* in several tissues (Supplementary Table S5). PROC may be controlled by *trans*-regulatory mechanisms, initiated in its receptor gene, *PROCR*, on chromosome 20; it was indeed associated with an insertion, rs141091409, located 20Kb upstream of *PROCR*, an association observed with both our proteomic platforms. Previous studies found associations between cardiovascular disease and variants located in the *PROC* or *PROCR* genes.[76,77] Interestingly, our hit, rs141091409, was in strong LD ($r^2 > 0.95$) with the missense variant rs867186, previously identified as associated with coronary heart disease.[77]

Our clinical analyses support the relation of PROC and FA7 levels with lipid traits: both were positively associated with cholesterol, triglycerides and visceral fat (Figures 4A and 5). PROC levels were quantified by both platforms, and displayed consistent results. The SomaLogic measurements of PROC were positively associated with LDL ($p = 5.39 \times 10^{-5}$). The role of these proteins for cardiovascular disease and NAFLD diseases in the overweight/obese population would merit further investigation.

C Correlation between CD209 and other macrophage protein levels

Figure C.1 displays the correlation between the CD209 levels (controlled by $rs8176741$, see main text) with macrophage protein levels.

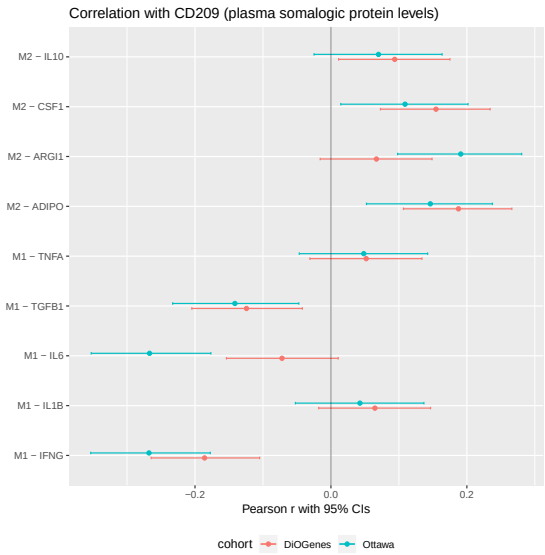

Figure C.1: Pearson correlation coefficient for the association between CD209 protein levels and M1-M2 macrophage protein levels in Ottawa and in DiOGenes.