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Single-cell genomics meets human genetics

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Abstract

Single-cell genomic technologies are revealing the cellular composition, identities and states in tissues at unprecedented resolution. They have now scaled to the point that it is possible to query samples at the population level, across thousands of individuals. Combining single-cell information with genotype data at this scale provides opportunities to link genetic variation to the cellular processes underpinning key aspects of human biology and disease. This strategy has potential implications for disease diagnosis, risk prediction and development of therapeutic solutions. But, effectively integrating large-scale single-cell genomic data, genetic variation and additional phenotypic data will require advances in data generation and analysis methods. As single-cell genetics begins to emerge as a field in its own right, we review its current state and the challenges and opportunities ahead.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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Introduction

Genome-wide association studies (GWASs) have uncovered hundreds of thousands of genetic variants associated with the risk of complex diseases and human traits. However, the majority of mechanisms linking these variants to their biological impact still need to be characterized, especially for variants found in non-protein-coding regions of the genome[1]. Expression quantitative trait locus (eQTL) mapping, which estimates the association between genetic variants (particularly SNPs) and RNA levels of either local or distal genes, can link variants to the putative target genes that they regulate. In addition, mapping of eQTLs, or other molecular QTLs[2], can help characterize the modes of action of disease-associated genetic variation. This approach can help identify the genes – and consequently, the pathways and processes – that may be involved in disease pathogenesis[3], which is a critical early step in identifying opportunities for therapeutic intervention.

For eQTL mapping to provide disease insights, changes in RNA expression levels must be assayed in the specific cell types and conditions relevant to the disease of interest, as the transcriptome and its regulatory mechanisms are dynamic and frequently context-dependent[4]. Seminal studies have demonstrated how eQTLs may only be detected in certain cell types[5] or upon stimulation (that is, response eQTLs[6,7]). Additionally, recent efforts have assayed eQTLs across many human tissues; most notably, the Genotype-Tissue Expression Consortium[8] has mapped eQTLs in more than 50 human tissues obtained from post-mortem donors. These traditional eQTL studies use bulk transcriptomes, which assess average expression levels across millions of cells from either whole tissues or cell-type samples. Using experimental (for example, fluorescence-activated cell sorting (FACS) and in vitro differentiation) and computational (for example, deconvolution) tools, bulk studies revealed some of the earliest insights into eQTLs specific to a cell type or transient state[9-11]. However, bulk studies are limited in their resolution of rare cell states or lack surface proteins with robust antibodies for FACS. Moreover, some transient or dynamic states cannot be recapitulated in vitro. These limitations reduce the utility of bulk eQTLs for understanding the biology of disease-associated variants: although tissue-level eQTLs are enriched for disease-associated genetic variants from GWASs, only 20–50% of common disease alleles colocalize with eQTLs[12-14], which suggests that many variants influence biology through cell-state-specific mechanisms that cannot be identified without fundamentally new approaches.

Single-cell genomic technologies, particularly single-cell transcriptomics (that is, single-cell RNA sequencing (scRNA-seq)), offer a solution. As these approaches, which measure expression levels in individual cells, have become prevalent in recent years, they have revealed unanticipated cellular heterogeneity in many biological systems[15-17]. In addition, recent advances in technology, algorithms and experimental design have reduced the cost of scRNA-seq, making it more comparable to bulk RNA-seq and thus feasible to deploy across thousands of individuals[18]. This approach allows researchers to combine the granularity of single-cell assays with the large sample sizes required for genetic association studies, enabling a new category of ‘single-cell genetics’ studies that most prominently feature single-cell eQTL (sc-eQTL) studies.

The number of published sc-eQTL studies has more than doubled between January and December 2022 (Fig. 1), and international initiatives such as the single-cell eQTLGen Consortium (established in 2020 (ref. 19)) are attempting to harmonize efforts in this space. sc-eQTL studies have started to tackle questions that could not be asked with bulk expression data, such as finding eQTLs that vary with the cellular context or identifying the cell states in which disease-associated variants modulate gene expression. Context-specific, high-resolution maps of expression across deeply phenotyped individuals will eventually be valuable for therapeutic development.

In this Review, we first briefly review single-cell genomics and human genetics, before focusing our attention on their intersection. Next, we review the first sc-eQTL studies, which demonstrate the feasibility of applying bulk analysis approaches to single-cell data. We discuss unanticipated challenges that become relevant when compared with traditional studies using bulk RNA-seq. Next, we highlight newer approaches using the single-cell resolution provided by scRNA-seq data, such as mapping eQTLs that vary along continuous trajectories. Finally, we provide an overview of key future directions for the field, including new data types and integration strategies, and translation to clinical and therapeutic applications.

A brief review of contributing fields

We define single-cell genetics as the emerging field at the intersection of single-cell genomics and human genetics. The two contributing fields each have opportunities, challenges and bottlenecks. Here, we review relevant gaps and synergies at this intersection (Fig. 2) and introduce concepts that provide the necessary context for this Review.

Single-cell genomics

Over the past decade, single-cell genomics has rapidly demonstrated its value for studying human biology[20]. scRNA-seq is the most common of the single-cell modalities, and it has scaled quickly[21] since its development in 2009: from only eight cells in the original publication[22] to over 4 million cells in a recent study[23]. The most popular methods today for capturing RNA from single cells are droplet-based techniques[24,25], which scale to tens of thousands of cells. Here, single cells are encapsulated inside microdroplets containing unique oligonucleotide-barcoded gel beads. When the cells are lysed, their mRNA molecules hybridize to the barcode and can be sequenced with a label corresponding to their cell of origin. Alternative methods are plate-based single-cell RNA-seq techniques (for example, Smart-seq3 (ref. 26)), in which cells are physically separated into 96-well or 384-well plates – with one cell per well – before library preparation and sequencing of full-length transcripts. Finally, in cases in which isolating viable single cells is technically challenging (for example, from frozen samples), single-nucleus RNA sequencing[27] is a valuable alternative (Box 1).

In the past 10–15 years, technological improvements in single-cell data collection have produced new analytical considerations distinct from those for bulk RNA-seq data: for example, the massive number of profiles generated by a typical experiment, the `sparsity` of the data and a spectrum of technical artefacts. Novel methods have been developed

to address these challenges. Single-cell-specific bioinformatics workflows such as Cell Ranger[24] perform raw data processing tasks, for example, read-level quality control, assignment of reads to their cell barcodes and RNA molecules of origin (that is, 'demultiplexing'), alignment to the reference genome and quantification. The data from an scRNA-seq experiment are typically represented as an integer matrix of the number of sequenced reads (or molecules, if `unique molecular identifiers (UMI)` were used) assigned to each gene in each cell[28]. For multi-individual pooled designs (particularly relevant for single-cell genetic studies), demultiplexing methods are necessary to assign cells to individuals of origin (for example, *demuxlet*[29] and *vireo*[30]). After generating these count matrices, the next common stage in an scRNA-seq analysis workflow[31-33] is pre-processing: for example, detection (and exclusion) of empty droplets, `doublets` and `ambient RNA` (which can confound associations with true single-cell expression measurements); normalization to adjust for total sequencing depth of cells (total number of reads); log transformation and correction for confounding factors including technical batch and cell cycle effects. Each of these steps is reviewed elsewhere[31-33].

Subsequently, downstream analyses can be applied to the preprocessed data. To reduce the computational burden, reduce noise and facilitate visualization, it is beneficial first to reduce the dimensionality of the data set. Feature selection reduces the data to, for example, highly variable genes[34,35]. Then, dimensionality reduction using linear methods such as `principal component analysis (PCA)` and `non-negative matrix factorization` is typically performed to aggregate signals across genes. These reduced dimensions can be used for visualization purposes either directly or via feeding to nonlinear transformations (for example, *t*-distributed stochastic neighbour embedding (*t*-SNE)[36] and uniform manifold approximation and projection (UMAP)[37]), which can further reduce dimensionality to two dimensions without the information loss that would occur if linear constraints were maintained.

Additionally, reduced dimensions can be used for subsequent downstream analyses. These include cell-level analyses to identify cell states and their dynamic relationships (for example, `clustering, cell-type annotation` or `trajectory inference`) and gene-level analyses to characterize the transcriptional profiles of these states (for example, differential expression or `gene regulatory networks`). Software to conduct these analyses is often available as part of extremely popular and comprehensive computational toolkits that create user-friendly single-cell workflows and consistent data objects. These toolkits are available in both R (for example, Seurat[38] and scran[39]) or Python (for example, Scanpy[40]). Recommended methodologies and parameters for these steps are reviewed elsewhere[31-33].

Impact of genetic variation on molecular phenotypes

In the two decades since the completion of the first human genome sequence[41], rapid advances in sequencing technology have enabled increasingly larger genome sequencing projects and the characterization of human genetic variation across hundreds of thousands of individuals[42-44]. For common (population `minor allele frequency` >5%) and near-common (1–5%) variation, genotype arrays provide a popular solution to measure genotypes

at approximately 500,000 ‘tagged’ loci systematically, and their low cost enables usage for large cohorts. DNA sequencing approaches additionally resolve rare (population minor allele frequency <1%) and structural genetic variation and can be applied to either protein-coding regions and their flanking sequences only (whole-exome sequencing) or the entire genome (whole-genome sequencing), using either cheaper short-read sequencing or more comprehensive, but substantially more expensive long-read approaches[45,46].

In the setting of severe monogenic diseases, the application of DNA sequencing methods in both research and clinical settings has improved the rate of genetic diagnosis and disease gene discovery[47,48]. In addition, for complex traits and common diseases, GWASs have led to the identification of more than 400,000 genetic associations[1] and the development of `polygenic risk scores (PRSs)`, which combine association signals across the genome to predict the risk of disease of an individual[49].

Studies of genetic variation can be combined with functional genomic assays to assess the potential biological impact of individual variants directly. The most popular approach is expression (e)QTL mapping, but similar frameworks can be used for DNA methylation, protein, histone modification, chromatin accessibility and splicing, reviewed elsewhere[2]. Because we expect most regulatory regions to be near their target, most QTL studies have focused on proximal (*cis*) mapping, for example, considering variants in and around the gene, methylation site or accessibility peak of interest. By contrast, *trans*-QTL mapping considers distal inter-chromosomal regulation but requires larger sample sizes[50].

At present, the sample sizes of QTL studies are several orders of magnitude smaller than those of GWASs (for example, ~30,000 in the largest blood eQTL study[51] versus >5 million individuals in the latest height GWASs[52]) owing to both cost considerations and the challenges of obtaining suitable tissue samples at the population scale. Fortunately, the magnitude of genetic effects on molecular traits is generally much larger than that on disease risk, and thus these sample sizes are sufficient to identify them. Although traditional QTL studies have considered common SNPs, approaches exist to interrogate the role of rare variants on, for example, the expression level. However, these remain largely limited to the study of rare variation in individuals with extreme phenotypes (that is, outlier analyses[53,54]), with few exceptions[55].

Linking QTL results to GWAS results can reveal the molecular function of disease-associated genetic variants, but this task remains nontrivial[56]. To better understand the disease relevance of QTLs, methods have been developed to assess whether they coincide with disease loci (statistical `colocalization`[57]) or whether their effect on an intermediate molecular trait is causal for disease (two-step `Mendelian randomization`[58]), which have been reviewed elsewhere[56]. `Transcriptome-wide association studies (TWASs)` leverage eQTL information to impute gene expression for GWAS cases and controls and then perform direct association of traits and genes without directly profiling gene expression in every individual[59,60].

Single-cell eQTL mapping using pseudo-bulk counts

Reduction in sequencing costs, well-established methodologies, processing pipelines, multiplexing techniques and batch-effect-removal methods enable the application of single-cell genomics (particularly transcriptomics) to large, genotyped cohorts. Furthermore, in single-cell genetics studies, using single-cell molecular profiling and genotypes from the same individuals enables the evaluation of the effects of genetic variants on molecular phenotypes at the level of a cell. Here, we focus on sc-eQTL studies, which test associations between genetic variants and changes in gene expression at single-cell resolution.

Proof-of-concept and early cell-type studies

In a 2013 study[61], sc-eQTLs were first mapped, motivated by the observation that averaging expression over many cells (as is done in bulk studies) would mask certain gene expression phenotypes such as transcriptional bursting, noise and dynamic expression fluctuation. Limited to *WNT* pathway genes in 15 lymphoblastoid cell lines, the demonstration of the authors that SNPs are associated with transcript variance and correlation across single cells, nevertheless, served as an initial proof of concept[61]. It was an early example highlighting the value of single-cell-resolved gene expression in genetic studies. Within the next 5 years, a few subsequent studies demonstrated the feasibility of transcriptome-wide sc-eQTL analyses[29,62]. These studies leveraged single-cell advances in assaying, demultiplexing and clustering cells and focused on well-delineated immune cell types within easily accessible human peripheral blood. Despite limited sample sizes (<50 individuals), these studies found tens to hundreds of eQTLs.

These studies established a preliminary approach for sc-eQTL analyses: measure single-cell gene expression in a genotyped cohort, cluster phenotypically similar cells and associate the aggregated expression of each gene in each cluster or cell type with genotypes of individuals at nearby variants. This approach, called the ‘pseudobulk’ eQTL analysis, which we discuss further in the next section, had the advantage of building on existing bulk eQTL pipelines, making it computationally scalable to progressively larger cohorts (the current largest sc-eQTL study considers nearly 1,000 individuals[63]). Moreover, this approach was compatible with more sophisticated methods to organize `single-cell phenotypes`, such as bins along a trajectory or high-resolution cell-state clusters, allowing the approach to be extended to more heterogeneous tissues and granular cell types, including immune cells[63-68] (with a particular focus on T cells[65,67,68]), induced pluripotent stem (iPS) cells and differentiating iPS cells[69-73] (including iPS cell-derived cardiomyocytes[72], dopaminergic neurons[70] and retinal ganglion cells[73]), fibroblasts[74] and brain cells[75].

Methods originally devised for bulk eQTL mapping

Initial sc-eQTL studies largely used association methods originally devised for bulk eQTL mapping and other association tests between genotypes and continuous traits (Box 2). These methods assume that (1) the distribution of a phenotype across all samples is approximately Gaussian and (2) only one phenotype observation is available for each individual. These two assumptions do not necessarily hold for single-cell expression data, which in general are much sparser, and contain multiple observations of each phenotype (that is, expression level

from multiple cells) per individual. To overcome this discrepancy, many studies have relied on pseudo-bulk strategies, in which gene expression levels are aggregated across multiple cells from a given individual to mimic a single bulk sample. The expression of a gene in the pseudo-bulk sample is typically either the sum of the raw counts of the gene or the mean of the normalized expression of the gene across the cells of an individual in the cell type of interest (more precisely defined using scRNA-seq data).

As in bulk studies, covariates may be confounded with allelic effects. Several approaches used to detect and correct for covariates affecting the expression of all (or a majority of) genes in bulk analyses can be extended to pseudo-bulk analyses. These include principal component analysis and probabilistic estimation of expression residuals (PEER), although the latter can perform suboptimally in some cases[76,77]. Single-cell studies have additional challenges, such as variable cell count per individual (inversely correlated with confidence in pseudo-bulk counts) or batch effects from multi-experiment study designs, which may create systematic differences in gene expression between experimental pools (Box 1). sc-eQTL models can increase power by accounting for these experimental factors with additional fixed or random effects[70]. There are many possible single-cell count normalization and aggregation and covariate correction strategies for pseudo-bulk sc-eQTL studies, which have been reviewed elsewhere[78].

Although these studies used pseudo-bulk scRNA-seq data for eQTL mapping, contemporary studies also began to explore ways to use additional information offered by single-cell profiles. For example, in principle, these data allow one to measure the association between genetic variation and cell-to-cell gene expression variability (Fig. 3). Increased variability may reflect a lack of expression stability and increased propensity to enter extreme, pathogenic states[79] or could uncover gene–environment (GxE) interactions with unmeasured environments and contexts[80]. Although a handful of studies have proposed methods to map such ‘variance eQTLs’ from single-cell data (borrowing from similar approaches in other settings[80-82]), they had limited success owing to insufficient sample sizes and the confounding correlation between the mean and variance of the expression of a gene[18,71]. As the size of single-cell genetic studies grows, and more sophisticated methods become available, we envision that single-cell variance eQTL studies will become more tractable. These early attempts to leverage single-cell-resolution data in genetic association models, nonetheless, have laid the foundation for new perspectives on modelling eQTLs, as well, with single-cell-resolution data.

Single-cell-resolution eQTL modelling

Cell types have historically been defined on the basis of discrete morphological and functional categories, and clustering scRNA-seq data work towards a similar ontological goal. To this end, early eQTL studies also discretized and aggregated cells of the same cell type to facilitate statistical modelling and interpretation. However, high-resolution single-cell data often reveal heterogeneity within discrete populations, which motivates modelling eQTLs at single-cell resolution. Here, we describe the second generation of sc-eQTL models, which adopt continuous frameworks to leverage granular single-cell-resolution data.

Single-cell models improve cell-state-dependent eQTL mapping

Recently, high-resolution molecular measurements (for example, transcriptomics) have been used to define and characterize single-cell phenotypes. They reveal not only discrete lineages but also continuous phenotypes and intermediate states. For example, scRNA-seq studies of human T cells have identified a continuum of cytotoxicity spanning multiple T cell sublineages[83,84]. During development, cells have been assayed in vitro and in vivo in intermediate differentiation states, such as the mesendoderm state preceding the determination of mesoderm or endoderm fate[69]. These continuous phenotypes sometimes reflect disease processes or pathogenic environmental signals, such as fibroblasts transitioning towards inflammatory states owing to NOTCH3 signalling in rheumatoid arthritis[85]. These examples highlight the need for more granular and continuous definitions of cell state (Box 3).

Once single-cell-resolution data are used to define these continuous states, we can model how genetic regulation varies dynamically along these trajectories. Rather than treating individuals as observations, these models treat each cell as its own observation of the expression of a gene. For example, one common model architecture is a mixed-effects `interaction` model, which includes random effects to account for the non-independence of cells from the same individual (which, if left unaccounted for, can inflate the false-positive rate[86]) and interaction terms between cell state and genotype to model state-dependent effects of genotype on expression[65,87,88]. These second-generation models map ‘dynamic’ eQTLs, assessing the effects of different genotype alleles on a trait that varies dynamically along a continuous axis. They have been successfully applied to continuous trajectories within differentiating iPS cells, T cells and other cell types[65,87].

Other single-cell-resolution methods have adopted different approaches. For example, Gewirtz et al.[89] used generative statistical (‘topic’) models to identify shared variation between genotypes and scRNA-seq profiles to identify both *cis*-eQTLs and *trans*-eQTLs across discrete cell types. As another example, Lu et al.[90] used decomposition approaches to identify genetic effects on expression that are shared or specific to discrete cell types.

However, these early applications have also revealed the challenges and limitations of these models, including the non-normality of single-cell expression counts and computational tractability. We discuss these in detail in the following sections.

Sparsity and non-normality of single-cell expression data

Single-cell data are sparse (containing many 0s), owing to incomplete sampling as well as genuine biological variation in transcript presence within cells. As a result, single-cell measurements are not well described by the Gaussian distribution that linear regression-derived models assume. The large number of cells that are assayed together in bulk transcriptomes (and, to a certain extent, pseudo-bulk aggregated measurements) meant that normalized expression profiles could be approximated as Gaussian, but this does not hold for single-cell profiles[91] (Box 2). Instead, discrete count distributions better describe these data. Despite their sparsity, single-cell profiles have been shown not to be zero-inflated[92]. Instead, a Poisson distribution offers an interpretable model of single-cell counts[91] that has

been used in recent studies, including the Poisson mixed-effect regression of Nathan et al.[65] and the Poisson reduced-rank regression model of Fitzgerald et al.[93]. In some cases, more parametrized negative binomial or multinomial models may be appropriate alternatives depending on the gene expression distributions[94]; null testing for *P*-value inflation can guide those choices.

Scalability and infrastructure as sample sizes grow

Modelling each cell separately – rather than aggregating cells into pseudo-bulk measurements – requires data sets on the order of hundreds of thousands of cells, instead of hundreds of samples as in a (pseudo-)bulk study for the same number of individuals. One solution is grouping small groups of <10 phenotypically similar cells into 'meta-cells'[95,96]. This type of aggregation is less disruptive than grouping thousands of cells in a cluster, or even hundreds of cells in a `pseudotime` bin, and is still usable for eQTL modelling[87].

Moreover, because effective sample size (number of unique individuals) is also expected to grow in future studies, methods must be scalable and compatible with high-speed computing and data storage infrastructure (Box 1). This is an area where sc-eQTL methods may learn from several previous genetics tools and infrastructures built to perform efficiently at scale, such as TensorQTL[97] (a graphics processing unit implementation of Matrix eQTL[98,99] for QTL mapping) and Hail (a cloud-based scalable implementation of several genetic tools[100]). Some sc-eQTL methods with more computationally expensive frameworks have already begun leveraging graphics processing units, such as scTBLDA[89], mentioned earlier. Methods may also benefit from parallelization across computing resources, cloud-based systems and algebraic and numerical approximations.

New opportunities

Current paradigms of sc-eQTL mapping offer a limited window into the overall picture of genetics and cell function. New technological advances, larger-scale studies and corresponding analytical and computational methods will be required to expand our view. In particular, we envision studies exploring more molecular traits (beyond gene expression), more types of genetic variants (beyond common SNPs) and more information about the individuals (for example, demographics, disease history and environmental exposures). Moreover, we expect data to be collected from progressively more diverse cohorts, including data from individuals of different ancestries, from individuals with diseases and from many different (disease-relevant) human tissues. As these rich data become available, new analytical and computational methods will be required to integrate information across data modalities (for example, chromatin accessibility, expression and protein level) and resolutions (from cell to tissue to individual), model context-specific and dynamic effects and predict outcomes relevant to human biology and health.

New data types

The molecular impact of DNA alleles can result in variation at the level of cells, tissues or whole organisms. A recent shift in human genetics has moved from variant discovery to exploring this multifaceted impact[101]. For any molecular phenotype we can measure,

we can integrate genotypic information to map QTLs associated with the phenotype. With early adoption of single-cell RNA-seq and robust analysis pipelines, sc-eQTLs have been an appealing area for the first single-cell genetic association studies. However, as we are able to more efficiently measure and computationally analyse more molecular traits at single-cell resolution, we can interrogate the genetics of more cell states and molecular processes at single-cell resolution (for example, single-cell chromatin accessibility QTLs[102]).

Multi-omics technologies allow us to assay more than one data modality within the same cell; for example, single-cell nucleosome, methylation and transcription sequencing (scNMT-seq)[103] measures chromatin accessibility, DNA methylation and expression, whereas cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq)[104] measures expression and surface protein level. Integrating multiple modalities provides multiple views on the phenotypes of the same cells, enabling higher-resolution definition of cell states to model dynamic sc-eQTLs and offering multiple phenotypes to model relationships with genetic variants. This integration task has been described as ‘vertical integration’[105], with cells being the common link across modalities.

Simultaneously, increasing sample sizes and newer technologies are making more classes of genetic variation amenable to analysis in single-cell cohorts, such as rare variants, repeats, insertions and deletions and structural variants. These have been associated with diseases[106-108], but there has been limited analysis of their effect on (whole-tissue) molecular phenotypes[109-111], and none at the single-cell level. More comprehensive and systematic association studies with single-cell models and precisely defined cell states may more fully capture the impact of these variants at the molecular level.

Diverse cohorts

Ideally, to understand the mechanisms underlying biology, we need to link genetics with molecular measurements and cell states in living humans under different natural perturbations. To do so, it is necessary to assay cells across thousands of individuals with known genotypes and at least partially characterized ‘environment’, including lifestyle (for example, smoking status, diet and pollution), demographics (for example, sex, age, geography and ethnicity) and other biomedical traits (for example, medical and vaccination history, disease state and progression and medications). Incorporating these different sources of variation into single-cell genetic studies provides a clearer picture of the interactions between genetics and factors underlying changes at the cellular level. Given the demonstrated relationships between these covariates and cell-state composition, incorporating these covariates into sc-eQTL models will provide richer context for dynamic eQTLs[112]. Cellular-resolved, large-scale and multifaceted data sets may also enable studies of GxE interactions and their effect on molecular traits.

In addition to environmental diversity, accounting for the effect of ancestry is important. Single-cell and genetic studies more generally have failed to include ancestral diversity for many reasons, including long-standing inequities and concentration of research funding in communities with predominant European ancestries[113-115]. Although diversity has been a growing priority in research studies, many institutions still lack adequate infrastructure and community engagement programmes to equitably recruit participants[116]. Most studies

continue to be conducted in European populations, and, as many have noted, genomic discoveries in Europeans are not always directly translatable to non-European individuals[114]. This limitation extends to sc-eQTL mapping studies, which largely consider samples of European ancestries. Yet, studying diverse populations is important, as they can have different causative alleles for diseases, different patterns of regulatory variation and different cell states and active pathways, together altering the context in which disease alleles act[117-119].

The sc-eQTL analysis in ancestrally diverse cohorts can help with `fine-mapping` and elucidate population-specific dynamic eQTLs and their relationship with disease, improving the translation of findings of genetic studies. A few studies have already been conducted in non-European populations (in Peruvian[65], Yoruban[71] and African American[82] populations), and large-scale cohorts from other geographical regions are being generated (for example, the Asian Immune Diversity Atlas, the African Ancestry Immune Cell Atlas and the Human Cell Map of Latin American Diversity). However, to maximize the findings that can be gleaned from these valuable data sets, it is essential to develop genetic algorithms for association testing, fine-mapping and meta-analysis that are robust to multi-ancestry data, which are currently lacking.

Studying disease tissue context

Many diseases have tissue-specific manifestations, making it critical to study the effects of genetic variation on gene regulation in disease tissue context. However, sc-eQTL studies to date have been largely limited to easy-to-access tissues (for example, skin and blood) or cell lines (for example, iPS cells), with only a minority of studies considering other tissues, such as the brain[75]. This limits our ability to learn about gene regulation in disease-relevant tissue (for example, colon for ulcerative colitis, or pancreas for type 1 diabetes mellitus). First, some disease-relevant cell types cannot be assessed at all in the absence of the relevant tissue. For example, neurodegenerative diseases such as Parkinson disease have proven especially difficult to study in part owing to the lack of access to data from the specific brain cells that are thought to be affected (dopaminergic neurons[120]). Second, even cell types that can be found, for example, in blood are found in a very different environment in tissue and thus may be subject to different context-specific genetic regulation. Finally, it is worth noting that tissues require handling, freezing and disaggregation, meaning that they are markedly more challenging to study. Moving forward, these are critical points that may be addressed by large-scale single-cell data generation projects such as the Human Cell Atlas[15,121].

Although most current studies have focused on ‘healthy’ individuals, another avenue to study disease-relevant gene regulation is to obtain single-cell profiling data from genotyped individuals with diseases and other traits of interest. For example, Perez et al.[64] mapped sc-eQTL in various blood cell types from patients with systemic lupus erythematosus. Additionally, the deficit of genotyped single-cell cohorts for hard-to-access tissues and people with a disease phenotype may be addressed by differentiating stem cells into cell types of interest and growing organoid models[122]. Recently, the concept of ‘`cell villages`’ has been introduced to help scale stem cell studies for larger numbers of

donor lines, providing power to explore gene regulation in disease-relevant cell types and genotypes[123].

Another promising avenue is to study spatial patterns of eQTLs to understand how gene regulation may interact with tissue structure to lead to disease. Spatial transcriptomics can record the in situ locations of cells along with their RNA expression profiles at near-cellular resolution[124]. These technologies are rapidly improving to become higher resolution, cheaper, higher fidelity and easier to implement[125]. In parallel, new mixture modelling strategies for spatial gene expression have already extended traditional analyses such as differential expression to spatial transcriptomics[126,127], and similar refinement may be useful for eQTL models[19]. With further development of computational tools and spatial technologies, there could be an that vary across spatial coordinates.

Enabling disease-relevant discoveries

eQTLs provide insight into the modes of action of disease-associated genetic variation – implicating genes they regulate, the direction of effect and cell states in which they have an effect – which has several important ramifications for understanding disease processes and, down the line, helping drug development.

Single-cell genetics for identifying disease-relevant cell types

Knowing the tissues, cell types and cell states most relevant to a disease phenotype can add to clinical understanding. With the development of sc-eQTL models that can identify cell-state-specific genetic effects on gene expression, we can now integrate existing knowledge about disease alleles with their predicted regulatory targets in each cellular context. This enables inference of the contexts in which the disease alleles may be most disruptive. Methods have been developed for complex traits affected by many genetic variants to integrate bulk tissue-specific eQTL effects and to prioritize the most relevant tissue[128,129]. For example, Kundu et al.[130] used eQTL mapping to fine-map causal disease-associated variants, finding, among other things, that the *ITGA4* locus for inflammatory bowel disease is active in monocytes. Single-cell-resolved eQTL maps will provide further granularity to these types of studies by enabling subcell-type resolution. For example, two distinct studies recently combined sc-eQTLs in (iPS cell-derived) dopaminergic neurons from 215 individuals[70] with GWAS results for Parkinson disease and schizophrenia, respectively, to confirm existing and identify novel genes that are likely to have a role in Parkinson disease and schizophrenia aetiology, using a Mendelian randomization approach[112,131].

Other methods using single-cell data can estimate more precise cell types relevant to disease, using variant-gene expression associations and other strategies to link disease-associated variants to genes[132-134] (Fig. 4). Some methods, such as single-cell disease relevance score (scDRS), estimate association of individual cells with the polygenic disease risk on the basis of their expression of genes proximal to GWAS variants[135]. This represents a step towards translating a PRS framework to single cells, aggregating SNP effects to predict heritable trait risk. Single-cell molecular QTL results may help construct similar predictors by further taking into account cell-type-specific regulatory effects of the genetic variants. For example, CONTENT (which stands for context-specific genetics) is an extension of

transcriptome-wide association study that uses context-specific eQTLs from either single-cell or bulk analysis to identify genes with context-specific expression associated with a disease, enabling quantification of the context-specific portion of disease heritability[136].

Moreover, these methods may benefit from more granular, single-cell data. When CONTENT was used to identify genes associated with systemic lupus erythematosus on the basis of eQTLs mapped in single-cell peripheral blood cells, it found twice as many genes when state-specific eQTLs were mapped using a single-cell-resolution decomposition method compared with pseudo-bulk meta-analysis[64]. This result highlights the importance of single-cell-resolution eQTL mapping approaches.

In addition to finding disease-associated genes, which may point to key pathways and drug targets, future extensions of similar methods may narrow down the cell context in which disease-associated genetics influences gene expression. This focus can also help us identify cell states to target with gene editing or other therapeutic molecules[137].

Future potential in the clinic

Importantly, although recent studies have shown that drug targets with genetic evidence are twice as likely to prove clinically effective[138,139], the translation of sc-eQTL results to the clinic is not a reality at present, and many critical steps are required to operationalize these data. Nonetheless, efforts using well-established data types provide hope that sc-eQTLs, too, may eventually have clinical utility.

First, complex disease heterogeneity may reflect underlying genetic and mechanistic differences. Genetic (PRSs[140,141]) and expression-based approaches (bulk[142,143] and single cell[144-148]) have been used independently to stratify patients on the basis of disease risk and into disease subtypes. A recent study[128] developed a method to prioritize disease-relevant tissues through Bayesian mixture modelling of the trait associations of tissue-specific bulk eQTL variants. They used this method to identify subgroups of patients with high body mass index whose genetic predisposition was most relevant to gene regulation in either brain, adipose tissue or muscle[128]. Using sc-eQTL studies and adapting bulk tissue methods may achieve similar results at cell-type and subcell-type resolution[149], potentially allowing patients with the same clinical disease to be stratified into subgroups with different disease prognoses and optimal therapeutic strategies.

Second, incomplete functional annotation of variants limits the utility of DNA sequencing to provide accurate diagnoses for patients with monogenic diseases. Functional genomic analysis of clinical tissue samples increases diagnostic rates above those provided by DNA sequencing methods alone, with bulk RNA-seq of disease-relevant patient tissue samples in particular now well-established as substantially improving diagnosis rates by identifying disease-causing changes in gene expression or splicing[150-152], leading to its incorporation into both research and clinical diagnostic workflows[153,154]. We can thus expect single-cell methods to increase diagnosis rates in two ways: first, by providing more accurate annotation of the genomic regions involved in the biology of specific disease-relevant cell states, leading to better in silico functional prediction for variants, and second, through direct

application to patient tissue to identify variants affecting transcript structure or expression in cell types that are rare in accessible tissue.

Conclusions and perspective

This article provides an overview of the nascent field of single-cell genetics, in which single-cell resolution molecular readouts are collected from hundreds or thousands of individuals and analysed in tandem with matched genotype data. sc-eQTL mapping, in which the effects of genetic variants on RNA levels are evaluated at single-cell resolution, is one of the most technically and algorithmically advanced approaches in this area; thus, it is where many of the first single-cell genetic studies have appeared and is the focus of this article.

The field of single-cell genetics (and single-cell technologies in general) is still in its infancy, and although it holds tremendous potential, there remain areas where bulk transcriptome approaches continue to have an important role. For example, in homogeneous cell types (for example, iPS cells), a bulk eQTL study may be better powered than an sc-eQTL study in the same cell type[78,155]. However, as technology improves and costs decrease, this gap will progressively diminish. Emerging technologies are becoming cheaper[156], require less specialized equipment[157], capture longer transcripts with higher fidelity[158] and may become amenable for large-scale single-cell studies in coming years.

As the second generation of eQTL mapping methods emerges, we can model regulatory differences at single-cell resolution and link them to differences in disease risk and heritability. This offers the promise of going beyond the conventional tissue and cell-type resolution that has, itself, still left the regulatory effects of many non-coding disease alleles unexplained[4,13]. Modelling cell-state-specific and context-specific eQTLs with single-cell data can also be used to improve inference of gene regulatory networks or haplotype-aware analyses of coordinated *cis*-regulatory effects on alleles[159,160]. However, as these single-cell data sets increase in size and algorithms seek to model heterogeneous, high-dimensional data, we face many challenges, as reviewed earlier.

Beyond these technical obstacles to implementing methods, there are additional barriers to clinical translation. Sample sizes for genetic studies are typically on the order of tens or hundreds of thousands, whereas single-cell studies have largely remained in the hundreds. Larger, more diverse cohorts of genotyped, single-cell-profiled individuals will be needed to conduct well-powered single-cell genetics studies with complex environmental or cell-state interactions. Additionally, this will enable GWAS-like studies linking genetic variants to cell-type composition and abundance estimated from scRNA-seq data (possibly adopting previous methods using FACS[161,162]), which are also genetically regulated and relevant to disease.

Moreover, eQTL studies often yield thousands of putative variant–gene expression associations. Although their results can be used as supporting evidence, experimental validation remains necessary to establish true causal relationships between variants and disease. This is an important open question, especially for dynamic eQTLs identified in rare or hard-to-isolate cell states. Replication in independent single-cell studies is possible,

but alternative molecular validation may be challenging. For sc-eQTLs and other single-cell genetic studies to be translated to the clinic, we need parallel development of experimental techniques to test the effects of variants in specific cell states at high throughput, such as CRISPR screens[163,164] or investigation in iPS cells or organoids[165,166]. Computational strategies that leverage the heterogeneity of other single-cell modalities measured across many individuals may also link eQTL variants to upstream regulatory elements[167,168] or downstream cellular phenotypes[169].

The existing and future studies described in this Review aim to provide novel insights and hypotheses into the mode of action of variants in gene regulation and disease pathogenesis. Understanding these causal pathways in a cell-state-specific manner may inform targeted therapeutic strategies.

Glossary

Allele
One of two or more alternative DNA sequences occurring at a particular genomic locus

Ambient RNA
Free-floating RNA captured in a single-cell RNA sequencing droplet or other reaction compartment

Cell-type annotation
Manual or algorithmic approach to assign labels (corresponding to cell type) to unbiasedly identified cell clusters

Cell villages
Cell lines derived from multiple donors cultured and differentiated together in a single dish. These are distinct from ‘uni-cultures’, in which each cell line is cultured independently. This makes the strategy particularly valuable for population-scale studies

Clustering
Algorithmic approach to group cells into clusters, which are groups of similar cells based on their transcriptomes

Colocalization
Statistical methods that aim to estimate the probability that the same genetic variant is causal for two different traits, for example, an organismal trait (for example, a disease in a genome-wide association study) and a molecular trait (for example, the expression level of a given gene in an expression quantitative trait locus study).

Doublets
Two or more cells (also called multiplet) captured and processed in the same droplet

Fine-mapping
The process of localizing association signals to causal variants using statistical, bioinformatic or functional methods

Fluorescence-activated cell sorting (FACS)
Experimental technique to select cells based on physical and chemical characteristics of individual cells. Single cells from a sample are suspended in a fluid and then injected into an instrument that uses lasers to detect cell morphology and fluorescently labelled features and sort cells based on these qualities.

Gene regulatory network (analysis)
A gene regulatory network is a set of interacting regulatory elements and genes that jointly control expression patterns that dictate a specific cell function.

Genome-wide association studies (GWASs)
Statistical procedure to identify associations between individual genetic variants and variation in continuous traits (for example, height) or risk of disease (for example, type 2 diabetes)

Interaction
Interplay between different sources of variation (for example, genetic variation and environmental exposure — GxE) that results in a joint effect on the trait of interest beyond the individual additive effects

Mendelian randomization
Statistical method using measured variation in an instrumental variable (for example, a genetic variant) to test the causal effect of an exposure (for example, the expression of a gene) on an outcome (for example, a common trait or disease)

Minor allele frequency
Population frequency for the least common (that is, minor) alleles within the population of interest

Non-negative matrix factorization
Dimensionality reduction method to decompose a matrix of non-negative values into two matrices of vectors capturing the essential features of a data set. Unlike principal component analysis, non-negative matrix factorization components are not orthogonal

Polygenic risk scores (PRSs)
Quantification of total risk of an individual for a given disease based on genetic contributors alone. PRSs are calculated by summing the dosage of an individual of thousands of variants weighted by the strength of their association with the trait (as estimated from a genome-wide association study for that trait).

Principal component analysis (PCA)
Dimensionality reduction method to identify main orthogonal axes of variation in a dataset, called ‘principal components’

Pseudotime
Approximate ordering of cells along a latent dimension based on single-cell RNA sequencing data. The ordering represents sequential changes along a transition (for example, during cell differentiation)

Response eQTL
An association between a genetic variant and RNA level (that is, an expression quantitative trait locus) that only becomes apparent when the cells the RNA is measured in are stimulated in some way (for example, immune activation)

Single-cell phenotypes
Cell characteristics (for example, function, gene expression and position along a transition) that can be estimated using single-cell-resolved molecular profiling (for example, single-cell RNA sequencing)

Sparse
Containing a large number of 0s. In single-cell data, sparsity is due to the combination of inefficient sampling and true absence of expression

Trajectory inference
Also known as trajectory mapping. A computational technique used in single-cell data to determine the form of a dynamic process experienced by cells (for example, lineage specification and differentiation) and then arrange cells based on their progression through the process, usually using a pseudotime approach

Transcriptome-wide association studies (TWASs)
Statistical method that uses estimated associations between variants and gene expression (for example, from expression quantitative trait locus studies) to infer expression for all individuals in a genome-wide association study and to identify associations between genes and traits/diseases.

Unique molecular identifiers (UMI)
Complex indices added to sequencing libraries before any PCR amplification steps, enabling the accurate bioinformatic identification of PCR duplicates. They are common in many single-cell RNA sequencing protocols

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Box 1

Experimental design trade-offs and considerations

As single-cell studies expand from hundreds or thousands of individuals to even larger cohorts, experimental design will have important implications on downstream analyses.

More individuals or more cells per individual?

Assuming budget constraints limit the total number of cells that can be assayed, researchers face a trade-off between maximizing the number of cells per individual or the total number of individuals. More unique, unrelated individuals will increase the power for genetic associations, especially with rarer variants. By contrast, more cells per individual may capture rarer cell types, although it increases the chance of doublets.

Multiplexing strategies

Large-scale single-cell experiments often multiplex samples in library preparation and sequencing and computationally assign cells to individuals a posteriori. This increases throughput and reduces cost and batch effects, while improving doublet detection. Yet choosing the optimal number of individuals per pool is not trivial. Combining more samples into one pool may mitigate batch effects, but can increase doublets and decrease sequencing coverage per individual.

Single cell versus single nucleus

Single-cell transcriptomic assays measure RNA either from whole cells (single-cell RNA sequencing) or from isolated nuclei (single-nucleus RNA sequencing). The latter is preferred for frozen or hard-to-dissociate tissues, where nuclei remain intact even under stress. The transcriptomic profiles are largely concordant, but there are inherent trade-offs. Single-nucleus RNA sequencing detects intronic pre-mRNA but cannot measure transcripts outside the nucleus, for example, mitochondrial genes. Cells that are more sensitive to the stress of dissociation, such as myocytes, are under-represented in single-cell RNA sequencing.

Scaling to large data sets

Scaling experiments to thousands of individuals requires logistical considerations. First, strategies to monitor the quality of cells and consistency of output (total number of cells, cell-type composition and doublet rate) across samples can minimize compounding effects of batch as well as human error. Analyses should consider scale to optimize memory and computations for increasingly large data sets by parallelizing, using graphics processing unit and storing data in sparse matrices

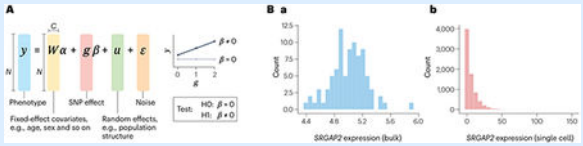
Box 2

Modelling considerations

Traditional genetic association testing for quantitative traits (be it gene expression or height) uses the linear mixed model. It tests for (additive) effects of the SNP on the phenotype while accounting for covariates and population structure. The effect size coefficient (β) provides both the magnitude and the direction of the effect.

The model in the figure (part A) assumes the phenotype ($\boldsymbol{y}$) to follow a Gaussian distribution, which is largely recapitulated when using bulk transcriptomics (see the figure, part Ba).

However, single-cell RNA sequencing data follow a distribution better described by a Poisson distribution (see the figure, part Bb). The histograms show the expression levels of the *SRGAP2* gene in induced pluripotent stem cells from the same ~100 individuals[78], considering bulk counts across individuals (see the figure, part Ba) and single-cell counts across cells (see the figure, part Bb).



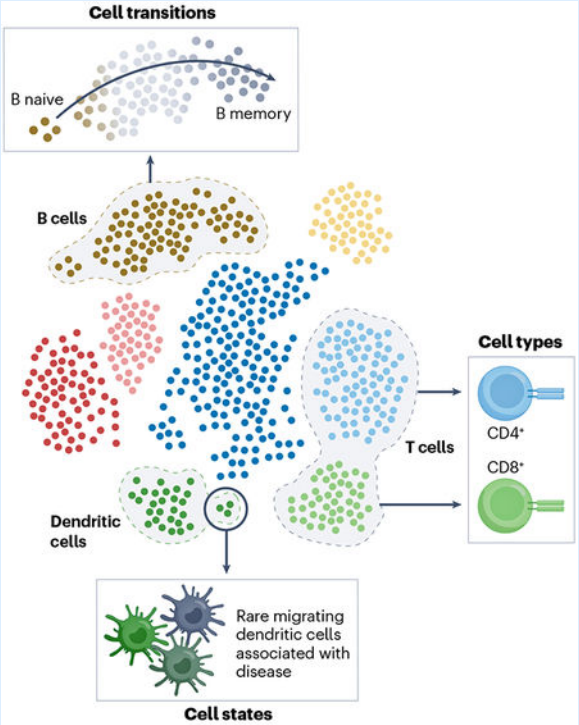
Box 3

Cell types and states

Single-cell genomics has introduced a paradigm shift in our understanding and definitions of cellular identity, type and state. In traditional bulk assays, discrete populations of cells have been defined and sorted a priori on the basis of extracellular markers. These correspond to cell types, which may be defined as groups of cells from distinct, irreversible developmental lineages.

With single-cell transcriptomics, we can define cell populations after assaying the cells on the basis of their expression of key marker genes (see the figure). These populations are more granular than what could have been sorted on the basis of extracellular markers and reveal cell states: functionally specialized, often plastic, subpopulations of cells. These states can be discrete (for example, T helper cells) or continuous (for example, developmental states).

Single-cell resolution allows us to then define the most disease-relevant populations of cells (which might be a whole cell type or might be a transient state).



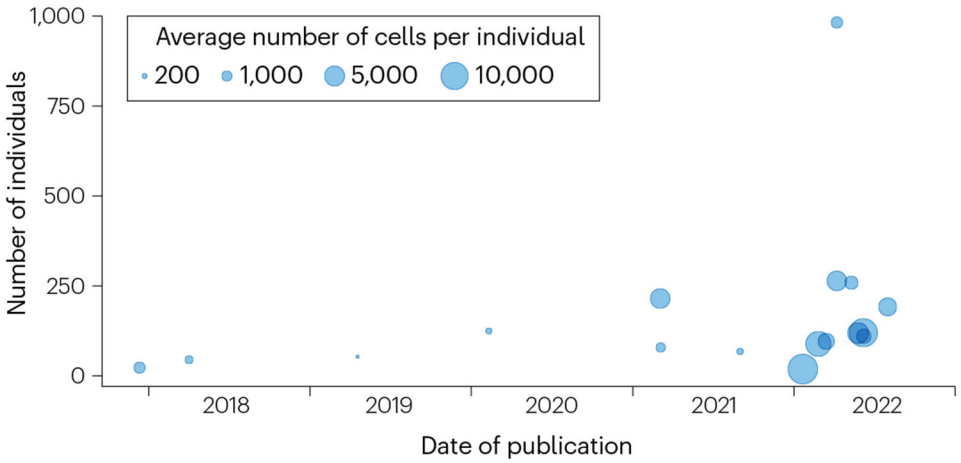


Fig. 1l. Overview of single-cell expression quantitative trait locus studies.

Single-cell studies published in the past 5 years. On the *x* axis is the date of publication, and on the *y*-axis is the number of unique individuals considered. The size of the dots represents the average number of cells per individual included in each study (when this number was not reported in this study, we estimated it as the total number of cells divided by the total number of individuals).

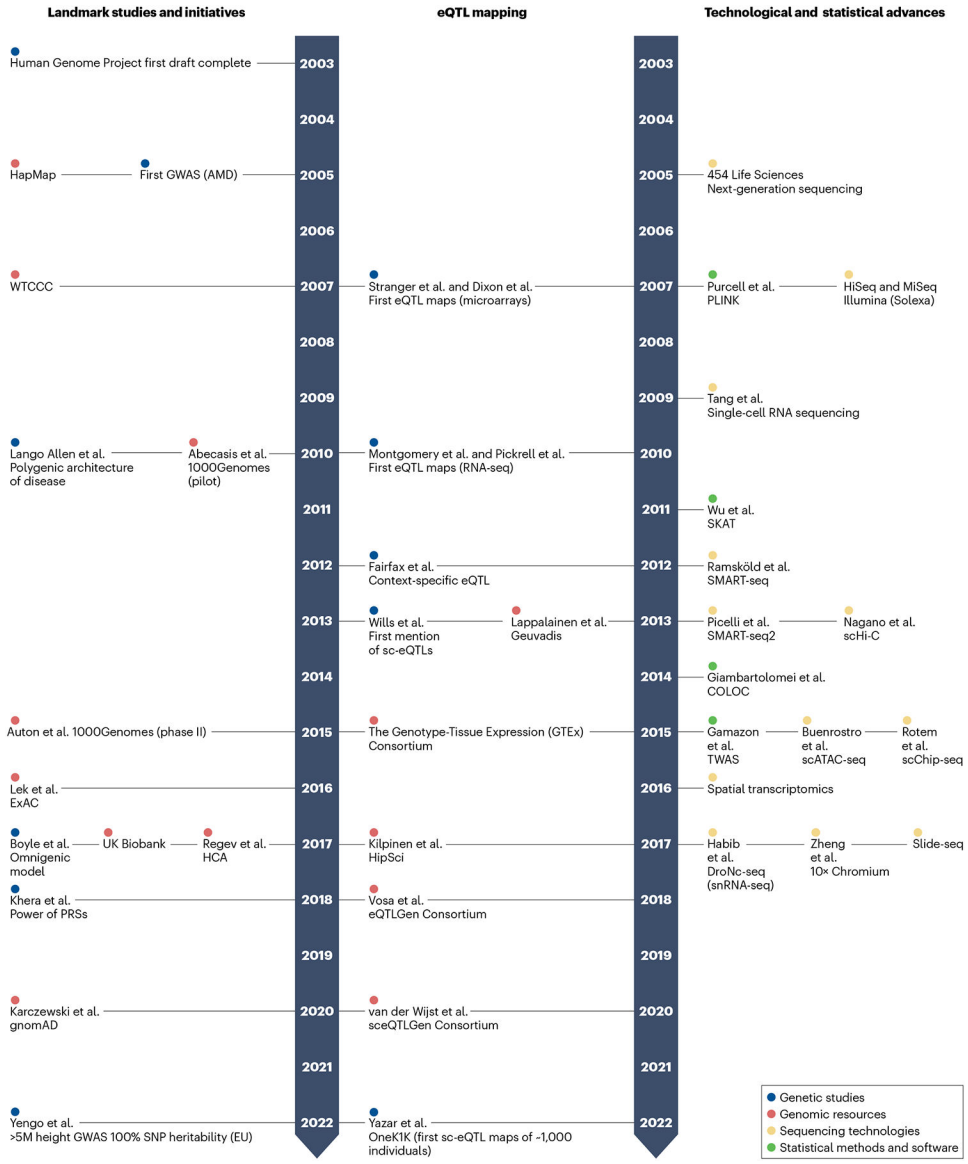


Fig. 2l. Human genetics and single-cell genomics, a 20-year timeline.

Fundamental genomic resources (red), genetic studies (blue), sequencing technologies (yellow) and statistical methods and software (green) have contributed to the current state of single-cell genomics and human genetics, including expression quantitative trait locus (eQTL) mapping studies. References 42-44,49,52 and 170-176 are for landmark studies and initiatives, respectively; refs. 22,24,27,57,58,124 and 177-183 are for technological and statistical advances, respectively; and refs. 5,19,51,61,63,155,184-189 are for eQTL mapping. GWAS, genome-wide association study; HCA, Human Cell Atlas; PRS, polygenic risk score; RNA-seq, RNA sequencing; snRNA-seq, single-nucleus RNA sequencing; TWAS, transcriptome-wide association study.

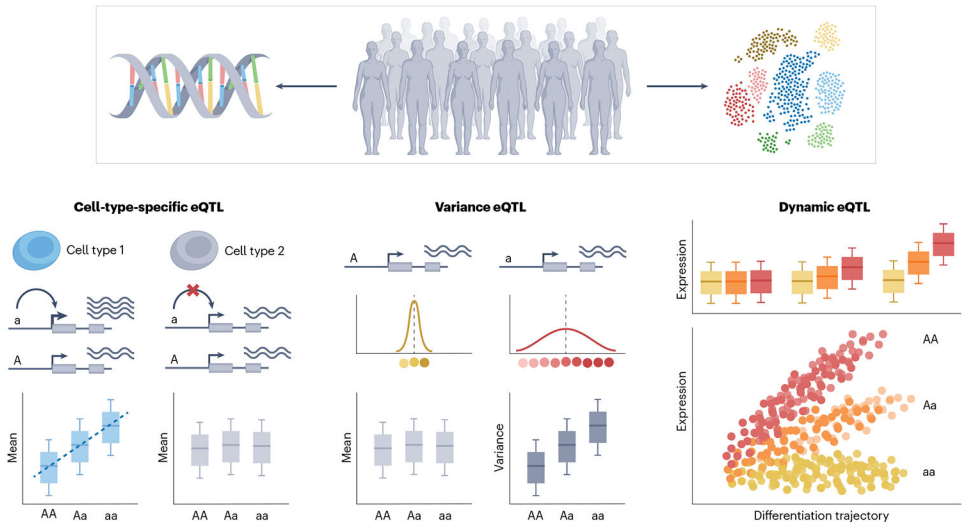


Fig. 3|. Types of single-cell expression quantitative trait locus.

Single-cell-resolved expression matched with genotype information allows one to consider different types of expression quantitative trait locus (eQTL) mapping strategies. When mapping cell-type-specific eQTLs, the single-cell resolution is exclusively utilized to more precisely characterize transcriptionally similar cells. Variance eQTLs test for genetic variants associated with cell-to-cell variability of gene expression (versus average expression level). Finally, to map dynamic eQTLs, single cells are ordered along a continuous trajectory, and the test consists in identifying eQTLs, the strength of which is modulated by such a trajectory.

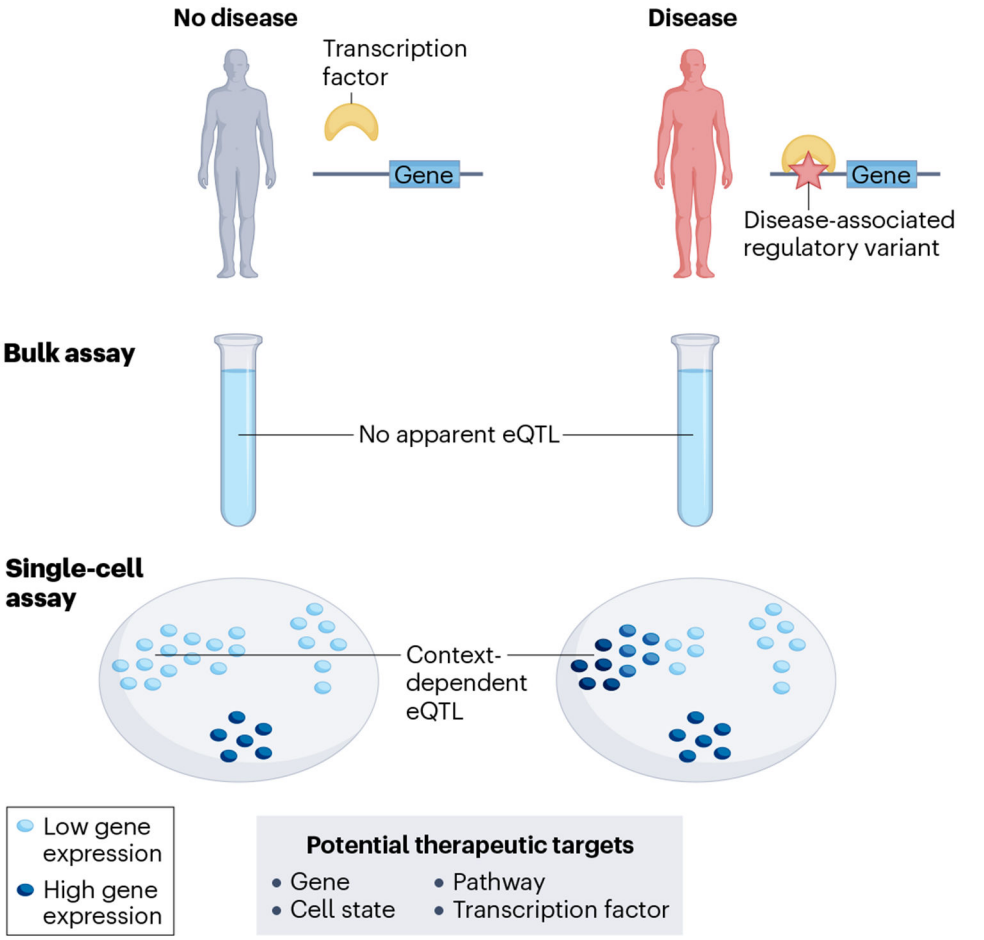


Fig. 4|. Downstream effect of context-dependent single-cell expression quantitative trait locus.
Identification of the specific contexts in which a disease-associated genetic variant regulates gene expression may ultimately lead to new therapeutic strategies. eQTL, expression quantitative trait locus.