DISEASE MECHANISMS

Integrative omics for health and disease

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Abstract | Advances in omics technologies — such as genomics, transcriptomics, proteomics and metabolomics — have begun to enable personalized medicine at an extraordinarily detailed molecular level. Individually, these technologies have contributed medical advances that have begun to enter clinical practice. However, each technology individually cannot capture the entire biological complexity of most human diseases. Integration of multiple technologies has emerged as an approach to provide a more comprehensive view of biology and disease. In this Review, we discuss the potential for combining diverse types of data and the utility of this approach in human health and disease. We provide examples of data integration to understand, diagnose and inform treatment of diseases, including rare and common diseases as well as cancer and transplant biology. Finally, we discuss technical and other challenges to clinical implementation of integrative omics.

Actionability
The property of a molecular finding that would result in a specific medical recommendation that is expected to improve a disease outcome.

Mendelian diseases
Diseases caused by a single locus or gene and that follow Mendelian patterns of inheritance (for example, dominant or recessive).

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The rapidly decreasing costs of high-throughput sequencing and other massively parallel technologies, such as mass spectrometry, are enabling their use in clinical research and clinical practice. Exome and genome sequencing are already being used to aid diagnoses, particularly of rare diseases[1–3], to inform cancer treatment and progression and, in early efforts, to create predictive models of disease in healthy individuals[4–6]. Numerous research efforts and companies are focusing on genome-wide profiles of genetic, gene expression and other omics data, such as the microbiome (BOX 1), as biomarkers for disease (see TABLE 1 for details). For instance, genome-wide association studies (GWAS) have been successful in identifying risk loci for disease. However, in many cases, the causal variant or gene is not identified[7]. Here, other omics technologies can provide a useful glimpse into the precise pathophysiology of the disease. Experiments generating data that are more proximal to an organismal phenotype, such as proteomics, can be expensive and are often not comprehensive, and a challenge remains to distinguish the causal origin of a disease. Thus, except in rare cases, no single technology can capture the complexity of the molecular events that lead to human disease.

Ideally, different technologies would be combined both to help diagnose disease and to create a holistic picture of human phenotypes and disease. However, implementation of multi-omics data introduces new informatics and interpretation challenges. Specifically, novel analytical and statistical methods are needed for combining disparate data sets, as well as standardized quality control metrics. Additionally, the field must address challenges in the interpretation of molecular events and, accordingly, their actionability and whether they can guide therapeutics and clinical care.

Below, we describe ways in which integrative omics can impact medicine by helping to manage health, as well as diagnose and treat disease. We discuss preclinical and clinical applications for rare Mendelian diseases, such as muscular dystrophy, and more common diseases, such as autism and Alzheimer disease. Furthermore, we investigate the use of multiple levels of omics technologies in cancer diagnosis and treatment. Throughout, we discuss the advantages of integrating multiple data sets, for instance, where one technology may address shortcomings of another to help provide insight into a mechanism of disease. Additionally, we discuss current methods as well as challenges in optimally combining and interpreting data from multiple sources, with some promising examples of their successful applications to elucidating mechanisms of human disease.

Dissecting Mendelian disease

In North America, approximately 10% of paediatric hospital admissions and 20% of infant deaths are attributable to Mendelian diseases[8–10]. In many cases, clinicians and families affected by Mendelian diseases are turning to exome and genome sequencing to find the causative mutations of their disease, which, depending on the disease and study design, has proved successful in 25–50% of cases previously not solved by targeted gene panels[3,11–13]. For diseases that typically act via a recessive mechanism,

Box 1 | **Incorporating the microbiome into integrative omics**

The microbiome has been associated with many common human diseases; however, an additional complication exists, as the direction of causality is not clear. Whereas causality is simple in genomic data, where (with the exception of cancer processes that cause mutations) DNA influences phenotypes, it is more difficult to disentangle whether microbiome composition is a cause or effect of disease, as these studies require expensive longitudinal or interventional experiments or mouse models that may not provide comprehensive insight into human biology. Nonetheless, it is very clear that patients with diseases, such as inflammatory bowel disease[110,111], type 2 diabetes[112] and obesity[113,114], have different microbiome profiles from those of healthy controls. In addition, the microbiome has a strong influence on immune function, which in some cases has been putatively causally linked to disease in animal models (reviewed in REF. 115).

As our understanding of the microbiome progresses, integrative analysis of this and other omics technologies is certain to advance our understanding of human disease. Recently, human genetic profiles have been shown to influence overall gut microbiota composition[116,117], which could suggest putative causal explanations for some disease-associated genetic loci[118] (for recent reviews, see REFS 119,120). Additionally, interactions between human genetics and microbiomes have been shown to influence disease, highlighting the potential for simultaneous interrogation of the two profiles[121]. Likewise, metabolic signalling between hosts and their microbiomes has become an area of active research, and there is increasing evidence that metabolite influences from gut bacteria may play a role in human disease[122]. Thus, it is likely that integrated analysis across genome, metabolome, microbiome and other omics profiles will prove beneficial for managing health and disease.

these investigations are most effective when the causal variant is either already in a variant–disease database, such as Clinvar, or a protein-truncating (for example, stop-gain, frameshift or essential splice site) variant in a known disease gene. However, in some cases, the effect of the variant may be more subtle (for example, an intronic variant creating a cryptic splice site), the variant may be difficult to detect owing to somatic mosaicism or several candidates are equally likely to be deemed causal. Furthermore, such diagnoses are additionally complicated when the genetic aetiology is not well known or when the candidate variants fall in genes that are less well described. Integrating additional information, such as RNA sequencing (RNA-seq) or network analyses, can be useful for detecting molecular events that prioritize among likely causal variants or provide additional evidence that a candidate mutation is causative. For instance, in a multi-omics analysis of patients with uncharacterized Fanconi anaemia, DNA sequencing and array comparative genomic hybridization (aCGH) were effective in identifying the mutations that were eventually deemed causal, whereas RNA-seq provided evidence of pathogenicity for some unsuspecting variants, including intronic and synonymous variants that affect splicing patterns, as well as a deletion of a non-coding exon and upstream region that resulted in ablated expression of a transcript[14].

More recently, two systematic studies of approximately 50 patients each have provided estimates of the additional gain in diagnosis rate using RNA-seq and other technologies (FIG. 1), ranging from 10% to 35%[15,16]. In one of these studies, a diagnostic investigation of patients with muscular dystrophy (MD), no causal variants were identified through whole-exome sequencing (WES), but RNA-seq data identified splice anomalies that revealed variants with cryptic splicing effects. Notably, even if whole-genome sequencing (WGS) were performed on these patients, these variants would have been identified but likely not flagged as causal, as many of them were intronic or otherwise not predicted to affect splicing. Given its rapidly decreasing costs and substantial information gain, RNA-seq is likely to become a powerful tool in characterizing disease pathophysiology in clinical practice. Similarly, as proteomics technologies become cheaper and more accessible, they may be used to identify protein level changes brought about, for instance, by missense variants that affect protein stability or post-translational modifications.

Genetic aetiology
The genetic factors that cause a particular disease.

Genetic architecture of common disease

Most common diseases such as diabetes[17], obesity[18], schizophrenia[19,20] and autism[21] are complex and a result of a combination of multiple genetic and environmental factors. Thus far, thousands of genomic loci have been significantly associated with human diseases (for a recent review, see REF. 22); however, once established as bona fide associations, the difficult task remains of characterizing the genes in the context of the molecular pathophysiology of the disease and its interacting genes and pathways. To this end, a number of methods have arisen to analyse multiple omics data sets, including network and enrichment analysis.

Network analyses. Integration of multiple orthogonal data types can be used to narrow the search space for disease genes and identify causal mechanisms of disease. Specifically, network models, including protein–protein interaction, regulatory and co-expression networks, have proved to be a valuable resource for prioritizing and identifying disease genes and pathways (for recent reviews, see REFS 23–26). These networks can be used with any genome-scale data set, including single-nucleotide polymorphism (SNP) or gene expression data, to investigate the topological properties of the most significantly disease-associated genes in a study, particularly when no or few hits reach genome-wide significance. In the case of genetic variation data, a challenge exists in mapping SNPs to the affected gene: in some cases, the effect of the variant is clear — such as a frameshift variant in an immune-response-related gene, *NOD2*, in Crohn's disease[27] — but more often, the affected gene for a variant may be ambiguous[28]. Additionally, SNPs may be grouped into genes to increase power, but patterns of linkage disequilibrium must be addressed[29].

Despite these challenges, network methods have yielded successful insights into human disease. For instance, in patients with autism spectrum disorder (ASD), genes harbouring de novo missense or nonsense mutations are enriched for genes with high degrees of connectivity in protein–protein interaction networks to all other genes and particularly previously ASD-implicated genes[30]. In this way, such approaches provide a mechanism to prioritize among putative disease genes, either by suggesting a greater functional impact due to their presence as a hub gene in a network or through guilt-by-association with previously associated genes.

Additionally, two recent studies from our laboratory integrating genomic, RNA-seq and proteomic data have identified new genes and complexes involved in autism

Table 1 | **Data types for integrative omics**

Data type	Large-scale research efforts	Utility and advantages	Major caveats
Genetic variation	Many GWAS consortia, 1000 Genomes, gnomAD and UK Biobank	Unbiased source of genetic basis of disease and direct inference of causality	At least one step removed from the phenotype
Epigenetics	ENCODE and Roadmap Epigenomics Project	Functional impact and typically easy to infer causality	Not applicable for all phenotypes
Gene expression	GTEx and GEUVADIS	Inexpensive assay for an intermediate step towards the phenotype	Not applicable for all phenotypes
Proteomics and metabolomics	CPTAC, EDRN and Common Fund	Likely to be very close to the phenotype	Expensive and difficult to scale (proteomics)
Microbiome	Human Microbiome Project	Likely to be very close to the phenotype and measures a combination of genetic and environmental influences	Combination of genetic and environmental influences makes it difficult to infer the direction of causality

In this table, 'phenotype' refers to an organismal phenotype. CPTAC, Clinical Proteomic Tumour Analysis Consortium; EDRN, Early Detection Research Network; ENCODE, Encyclopedia of DNA Elements; GEUVADIS, Genetic European Variation in Health and Disease; gnomAD, Genome Aggregation Database; GTEx, Genotype–Tissue Expression; GWAS, genome-wide association study.

and characterized their function[31,32]. Specifically, analysis of protein–protein interaction networks revealed a module (or coherent community of interacting genes) that was enriched for known genes involved in autism, as well as genes harbouring copy number mutations and rare mutations in autism cases. This module was enriched for genes involved in synaptic transmission, and RNA-seq revealed that many of the genes in a submodule were differentially expressed in the corpus callosum in patients with ASD, providing a putative molecular explanation for the observation that many individuals with ASD have a smaller corpus callosum than controls[32]. Similarly, mapping of rare variants in patients with autism onto protein complexes revealed both novel proteins and novel molecular machinery involved in autism, including the histone deacetylase (HDAC) chromatin remodelling complexes and other protein complexes[31]. Thus, integrating protein interaction data with WGS and WES data can provide new insights into important diseases, including autism, type 2 diabetes[33] and heart disease[34] (additionally reviewed in REF. 35).

Enrichment analysis. Recently, numerous large-scale enrichment analyses have been performed in order to understand the global mechanisms of information flow from DNA to physiology. Protein-coding variation is fundamental to many traits and, as such, associated loci from GWAS for many traits are enriched for protein-sequence-disrupting (non-synonymous) variation[36]. However, only a small fraction of associations fall into this category and, therefore, integration of non-coding regulatory annotations with disease association data can be valuable for identifying disease genes and disease aetiology (reviewed in REF. 37). In particular, assays for measuring gene expression (RNA-seq) as well as regulatory activity in regions that control gene expression (such as chromatin immunoprecipitation followed by sequencing (ChIP–seq) for transcription factor binding sites or DNase-seq for detecting regions of open chromatin) have been valuable in identifying tissue-specific signatures of genomic regulation. Accordingly, disease-associated variants are enriched among expression quantitative trait loci (eQTLs) as well as in transcription factor binding sites[38–41] and, thus, it is likely that many disease aetiologies may act through regulatory mechanisms. Indeed, a recent study of 108 loci associated with schizophrenia provided evidence for 20 of these loci having changes in gene expression that could at least partially explain their associations[20].

Recently, partitioning heritability methods using GWAS summary statistics and functional annotation data elucidated the relative contribution of coding and regulatory variants, suggesting that the bulk of heritability of many common traits stems from variants in regulatory regions (regions of open chromatin as measured by DNase hypersensitivity)[42], as well as many cell type-specific enhancers[43]. Additionally, such enrichment information can be used to discern causal variation as well as to identify novel genes for diseases and traits by increasing the weight of annotations that are specific to each trait[36]. As of this writing, such methods are not yet in clinical practice but have been invaluable in revealing the aetiology of many common diseases.

Narrowing causal mechanisms in common disease

As previously mentioned, GWAS have been successful in identifying loci that are statistically associated with disease, but they rarely identify causal variation. Integration of multiple data types, such as functional annotation data, can also provide insight into the potential function of specific disease-associated variants.

Indirect integration across individuals. Currently, a cost-effective method to ascertain the causality of variants associated with a trait is using multiple independent data sets to pinpoint causal mechanisms from a set of candidate loci with biological evidence[44]. Such a process may begin with a GWAS, after which, a set of genome-wide significant loci are assayed for functional follow-up; the specific experiment may depend on the types of loci identified or the genetic architecture of the disease. For coding variants, follow-up experiments that ascertain

Expression quantitative trait loci
(eQTLs). Genetic variants that are statistically associated with gene expression.

Heritability
The fraction of phenotypic variability of a trait that can be attributed to additive genetic variation.

DNase hypersensitivity
A measure of openness of chromatin, as measured by its sensitivity to cleavage by DNase I.

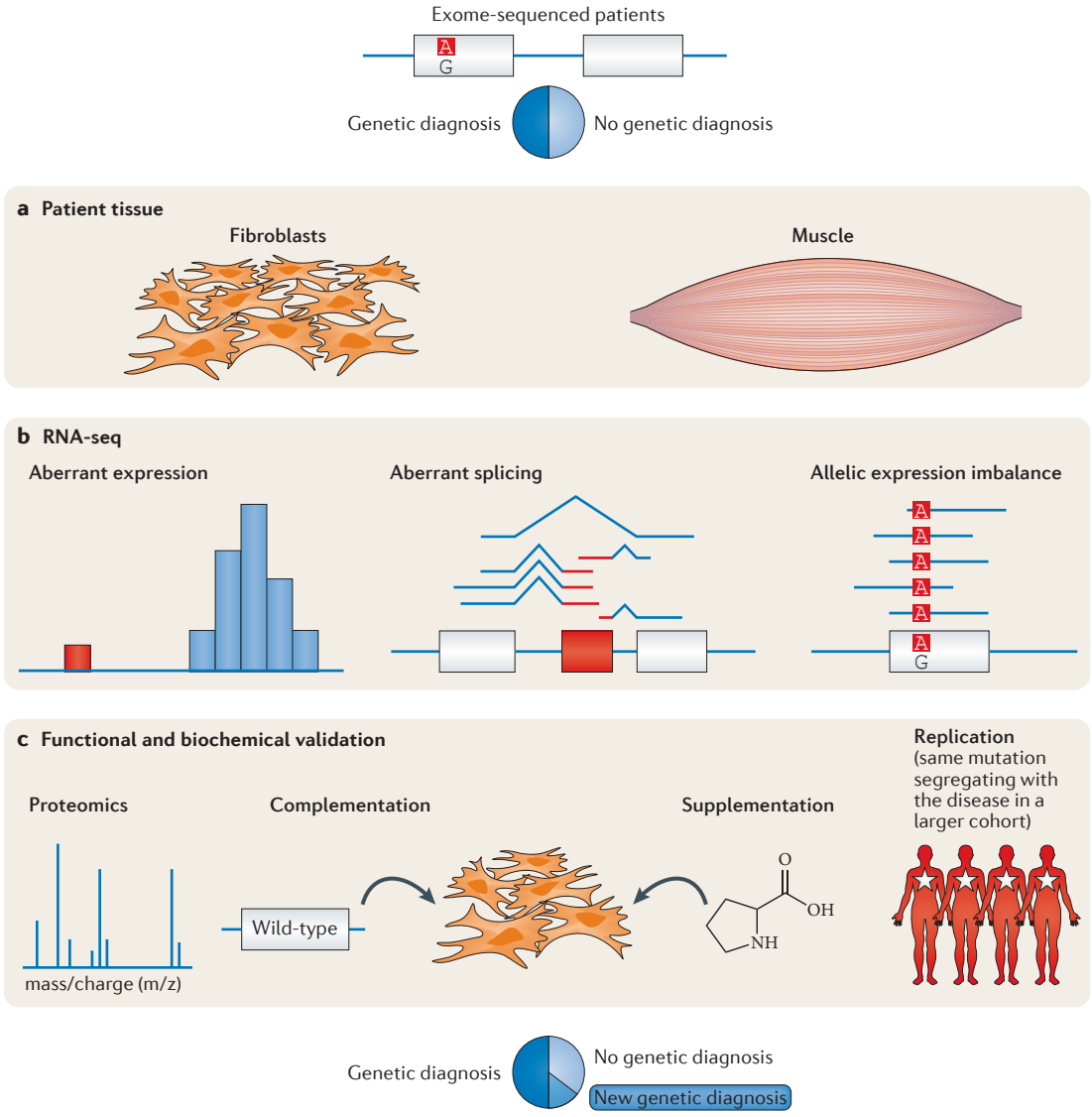


Figure 1 | **Identifying a causal variant to diagnose a patient with a rare disease.** In Kremer et al.[15] and Cummings et al.[16], multi-omics approaches were used to aid in the diagnosis of patients with undiagnosed disease. Although exome and genome sequencing can be effective in identifying causal genetic variation between 20% and 50% of the time, depending on the mode of inheritance and phenotype, the majority of cases cannot be solved by these technologies alone. **a,b** | Using RNA sequencing (RNA-seq) data from patient tissue, these approaches were able to make a molecular diagnosis for many patients, identifying genes with aberrant expression, splicing or allele-specific expression, which would suggest a molecular mechanism for the disease progression. **c** | In some cases, functional validation, such as proteomics, can lend additional support to these diagnoses. Figure is adapted from REF. 15, Macmillan Publishers Limited.

the effect of the variant on protein structure or function are ideally performed to demonstrate causality[45]. For non-coding variants, the effects are often more difficult to interpret, but recent large-scale epigenetic studies, such as the Encyclopedia of DNA elements (ENCODE)[46] and the Roadmap Epigenomics[47] projects, can suggest possible mechanisms for regulatory control, as well as transcription factors to target for follow-up experiments. For instance, a detailed study on a variant associated with systemic lupus erythematosus (SLE) showed that the variant also affects nuclear factor-κB (NF-κB) binding and is associated with expression of tumour necrosis factor-α induced protein 3 (TNFAIP3) at both the mRNA and protein level[48].

Recently, two investigations from Manolis Kellis and colleagues integrating multiple data types have yielded fruitful insights into the molecular pathology of Alzheimer disease and obesity. First, combining gene expression and epigenomic data, the group showed that genes that are upregulated in an Alzheimer disease mouse model show immune cell enhancer signatures[49]. Crucially, whereas a link between immune system genes and Alzheimer disease had long been previously established, multiple omics data types proved useful in this scenario to establish a direction of effect, showing that there is a concerted increase in expression and regulatory activity at immune system genes in Alzheimer disease.

Similarly, integrating epigenome and chromosomal conformation data, as well as expression information from patients with an *FTO* obesity allele and a number of other data types, provided a mechanistic explanation for the risk allele[50] (FIG. 2). Genome editing of the risk allele using CRISPR–Cas9 restored aberrant expression and thermogenesis, suggesting a potential therapeutic avenue for obesity phenotypes.

Direct integration within an individual. Whereas synthesizing data from multiple disparate technologies can create a link between layers of biological mechanism, characterizing multiple omics profiles in a single individual will be a powerful tool for creating a holistic view of the molecular effects that lead to physiological phenotypes. However, these approaches can be expensive, as they require multiple interventions and technologies on the same individual and, as such, thus far have had limited sample sizes. The first such study was performed in our laboratory and followed a single individual for over 7 years[6] (and M.P.S., unpublished observations), whereas a similar study followed another individual for 1 year[51]. In Chen et al.[6], genomic analyses predicted an elevated risk of type 2 diabetes, which was subsequently revealed through detailed omics analyses, including transcriptomics, proteomics, metabolomics and other measurements. In particular, genes involved in insulin signalling and response were found to be downregulated by RNA-seq and by liquid chromatography–tandem mass spectrometry (LC–MS/MS) proteomics during a respiratory syncytial virus infection, which coincided with increased blood glucose concentration to diabetic levels. These approaches are advantageous in their ability to track a mechanistic link across a shared genetic and individual background, as one can follow a progression of molecular events, such as the differential expression of a GWAS-identified disease-associated gene leading to differences in RNA and protein levels and their corresponding metabolites.

However, as omics profiling experiments have a high multiple hypothesis testing burden (for example, across all genes in the genome or thousands of metabolites), larger sample sizes will be useful to determine the generality of such correlations. A recent study monitoring various omics profiles across 23 individuals identified inflammatory signatures during weight gain, and found that certain metabolic pathways did not return to baseline after subsequent weight loss[52]. This analysis highlights the extent of similarities in longitudinal omics profiles across individuals, as well as individual-specific signatures at steady state and under experimental perturbations. To further qunatify these differences, projects have been initiated to extend such analyses to thousands of individuals, characterizing preterm births, inflammatory bowel disease and type 2 diabetes[53]. In a similar vein, two separate groups recently profiled genetic and metabolomics data: one of these calculated polygenic risk scores for over 100 individuals and correlated these with measurements of metabolites[54], whereas the other identified rare deleterious variants in healthy volunteers that correlated with outliers of individual metabolites and metabolic pathways[55]. Additionally, as reference databases of omics data for healthy individuals become available (as are already available for exome[56], genome (for example, the Genome Aggregation Database (gnomAD)) and RNA-seq[57] data), it will become easier to interpret individual-level data in the context of these control cohorts.

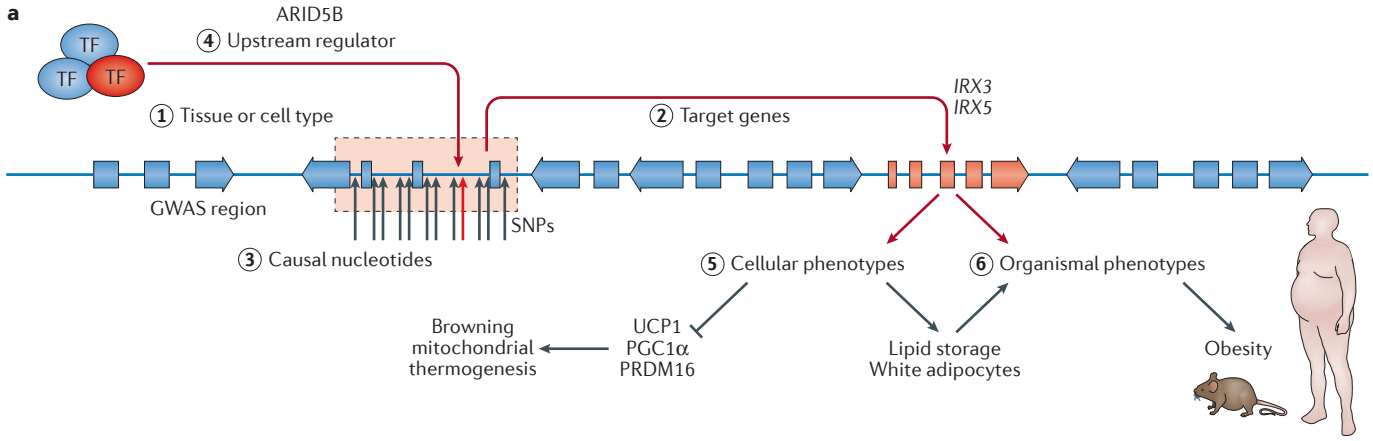
Other efforts include the Framingham Heart Study and genome characterization studies, such as the Genotype–Tissue Expression (GTEx)[57] project, with its proposed extension to analyses beyond gene expression in the enhanced GTEx (eGTEx) project[58]. These projects have adopted a breadth-first strategy for omics profiling, in which a high number of individuals are characterized with a limited panel of technologies that assay a single set of molecular markers (for example, whole-genome DNA methylation assays).

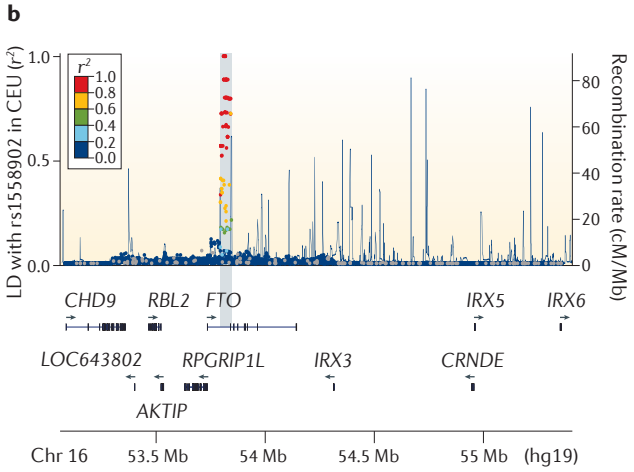
Cancer

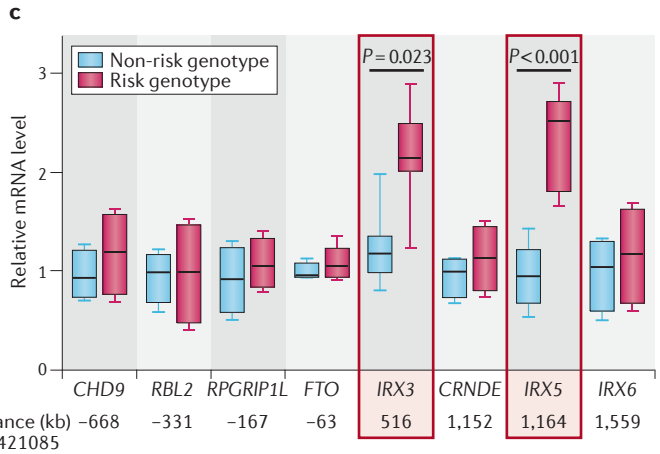
One area where multiple omics analyses have had and will continue to have enormous impact is in cancer profiling, diagnosis and treatment. Indeed, many of the previously discussed strategies (for example, network methods) will be effective in identifying genetic mechanisms of cancers. However, there are conceptual differences in cancers that complicate their analyses and require special handling. In addition to the technical challenges of calling somatic variants (see the 'Accuracy and validation' subsection in the 'Challenges' section below), the majority of genetic changes evident in cancer cases are benign and do not drive cancerous cell growth; therefore, determining which mutations are drivers or which pathways are involved remains a considerable challenge. Additionally, although some cancers share genetic signatures across individuals, there is still a high level of diversity among driver mutations, which can lead to differences in prognosis and therapeutics.

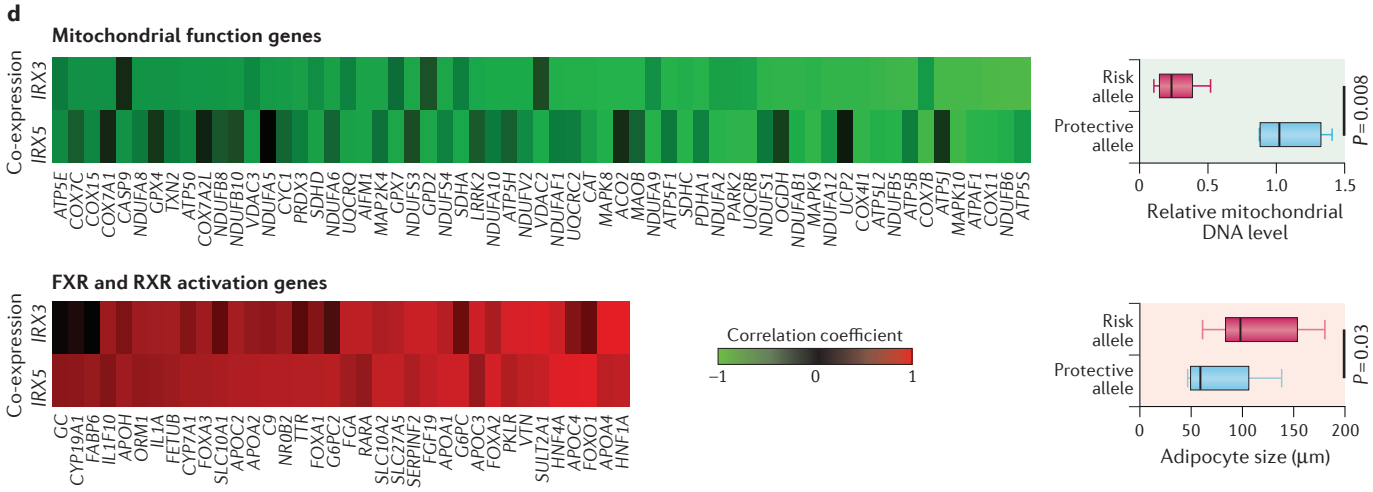
Identifying driver mutations. A typical process to identify driver mutations involves WGS of multiple tumours to identify recurrently mutated genes[59]. Overlaying functional data can help to prioritize this information, as driver mutations are more likely to be in genes that are expressed in a given cancer. For instance, in an analysis of driver mutations identified using WES coupled with copy number variation (CNV) microarray data, RNA-seq data were used to identify an expressed gene fusion of *EGFR–SEPT14*, which was functionally validated to affect glioma growth[60]. In a different analysis using similar technologies, the driver mutations and processes underlying multiple metastases within an individual were shown to be largely similar across metastases, suggesting that a single metastasis is sufficient for downstream analysis[61]. In this way, using additional omics data complements genetic data, providing a mechanism to filter the deluge of genetic variation to functionally relevant causal variants.

Molecular signatures of cancer. In addition to identifying driver mutations, multiple types of omics data can reveal general biochemical pathways that are active









in individual cancers and classify them into subtypes. As such, this can be a valuable tool for ascertaining which pathways to target within a patient, even if strong candidate mutations are not detected in those pathways — for example, owing to difficult to characterize non-coding mutations or indirect effects. For instance, clusters of transcriptomics and DNA methylation patterns have been used to identify subtypes of cancers, which have varying survival prognoses[59,62].

More recently, three studies of the Clinical Proteomic Tumour Analysis Consortium (CPTAC) have used proteomic approaches to identify cancer subtypes for colorectal, ovarian and breast cancer based on protein expression signatures[63–65]. Importantly, the proteomics data revealed overlapping but not identical correlation with the transcriptome and genetic data, indicating that the different data types expose different types of information. These studies demonstrated the distinct

◀ Figure 2 | **From genome-wide association studies to mechanism.** In a recent study, Claussnitzer and colleagues present a comprehensive approach[50] to identifying a causal mechanism for an obesity-associated variant in the *FTO* gene. Part **a** shows an overview of the deciphered biological mechanisms and the numbered steps of the strategy referred to below. From the initial genome-wide association study (GWAS), the significant association of the *FTO* region with obesity is shown in the Manhattan plot (part **b**). First, the researchers established the relevant tissue or cell type (step 1) as well as the downstream target genes using regulatory genomics, including chromatin state information and chromosomal conformation (Hi-C) data. Here, they established the variant as an expression quantitative trait locus (eQTL) for the developmental genes iroquois homeobox 3 (*IRX3*) and *IRX5* (step 2), where the risk allele shows increased expression of these genes but not others in the vicinity (part **c**). They demonstrate that expression of *IRX3* and *IRX5* is anti-correlated and correlated with genes involved in mitochondrial function and adipocyte size, respectively (part **d**). Next, they established the causal nucleotide variant (step 3) in an AT-rich interactive domain-containing protein 5B (ARID5B) motif (step 4) using CRISPR–Cas9 to show its molecular effects, including altered signatures of expression and phenotypic effects on the regulation of energy balance (step 5). Finally, they establish causality of the variant on an organismal level using mouse models (step 6). *AKTIP*, AKT interacting protein; CEU, Utah residents (CEPH) with northern and western European ancestry; *CHD9*, chromodomain helicase DNA binding protein 9; *CRNDE*, colorectal neoplasia differentially expressed; FXR, farnesoid X-activated receptor; LD, linkage disequilibrium; PGC1α, peroxisome proliferator-activated receptor-γ co-activator 1-α; PRDM16, PR domain zinc-finger protein 16; *RBL2*, RB transcriptional co-repressor like 2; RXR, retinoid X receptor; SNPs, single-nucleotide polymorphisms; TF, transcription factor; TSS, transcription start site; UCP1, mitochondrial brown fat uncoupling protein 1. Figure is adapted from *The New England Journal of Medicine*, Claussnitzer, M. et al., FTO obesity variant circuitry and adipocyte browning in humans, **373**, 895–907, Copyright© (2015) Massachusetts Medical Society, REF. 50. Reprinted with permission from Massachusetts Medical Society.

genetic and transcriptional processes that translate into proteomic alterations. Finally, integration of imaging information with omics information is expected to be valuable in cancer diagnosis and prognosis[66,67].

Recent developments in characterizing the non-coding regions that regulate gene expression have become increasingly valuable for understanding the regulatory landscape of cancer. Studies integrating reference data sets of regulatory information[46,47] with WGS data from The Cancer Genome Atlas (TCGA) revealed a number of regulatory regions that are enriched for mutations in patients with cancer[68–71]. In these cases, causal genetic variation in these non-coding regions is still difficult to pinpoint, highlighting the continuing need for research into prioritizing such variation; nevertheless, shared network topology across individuals with the same cancer can inform cancer subtypes that may have different prognoses and therapeutic strategies. Finally, given the strong dependence of cancerous growth on metabolic changes, it is likely that metabolomics will also play an important role in cancer diagnostics or prognosis in the future.

Challenges

Until now, most integrative models have been reported and published in research settings. However, the adoption of clinical genomics has expanded rapidly over the past few years from the first successful diagnosis[1] to multi-institutional and international adoption[72]. In the same vein, longitudinal multi-omics profiling, with its first recent research examples[6,54], may similarly emerge as a clinical tool.

However, for clinical adoption of any technology to occur, high specificity and sensitivity are required, both in detection and interpretation. At present, aside from the use of WES or WGS in exceptional cases, such technologies are not regularly used across clinical practices because for many diseases, they have not been proved superior to current tests. Going forward, clinical guidelines must be established to ensure accuracy and efficacy, and tests to show non-inferiority and cost-effectiveness must be performed.

Nonetheless, omics profiling can be an effective way of detecting large-scale or pathway-level alterations — cheaper and often more comprehensive than performing thousands of individual tests — and longitudinal profiling can show patient-specific trends and add statistical support through repeated measurements[6]. Although challenges remain in establishing clinical guidelines, many of the concepts surrounding the interpretation of genetic variants (particularly rare or novel variants) may apply to a general molecular event (such as a differentially expressed gene, novel protein phosphorylation or unique metabolome signature) as our understanding of the biology and reference databases mature.

Analytical challenges. There are various analytical challenges that must be addressed to enable the widespread adoption of integrative omics in clinical practice, particularly those of statistical methods for data aggregation, scalability and integration into electronic health records (EHRs). Most importantly, a robust and reproducible statistical framework is needed to properly analyse multiple disparate data sets, each with their own variances and biases. Multi-omics data can be analysed in a multi-stage or meta-dimensional fashion (reviewed in REF. 73). Briefly, one option for drawing inferences from these data involves pairwise analyses of data sets, mounting evidence to support a signal. However, analysing three or more data sets simultaneously requires more sophisticated multi-dimensional methods, such as Bayesian models[74], neural networks[75] or dimensionality reduction[76]. This is further complicated by the fact that various omics data types are fundamentally different: for instance, genetic variation data are discrete and static, whereas RNA-seq measurements are continuous and can provide longitudinal information.

Although the data analysis methods described above are effective for learning about biology and disease, they are not specifically designed to apply this information to individual-level data for clinical purposes. In the genomics space, with an individual's genotype and a database of results from GWAS, one can compute a polygenic risk score to assess an individual's risk of disease[4,77] (for recent reviews on methodology, see REFS 22,78). A major obstacle remains in building such frameworks for multiple omics profiles, which is likely to face some of the same challenges, such as the difficulty in applying results discovered in one population to individuals in another[79,80].

In addition to challenges with analytical approaches, these analyses and the storage of all associated data will require tremendous computational resources: although

the amount of data for multiple omics technologies on a single individual may be manageable (for example, terabyte-scale (10^{12} bytes)), these data must be put into a larger context to understand deviations from the background distribution, which requires data from thousands of samples (exabyte-scale (10^{18} bytes)). Fortunately, cloud-computing-based options have begun to alleviate these concerns[81], providing elastic computation and storage facilities based on specific requirements from each hospital or healthcare provider system while simultaneously promoting reproducibility in computational processes[82].

At present, such integrative data sets often do not have a standard format for research use, let alone for use in a structured clinical system; therefore, the infrastructure to house and manage these data will be required, which introduces financial and administrative burdens. In particular, health informaticians will be tasked with building a robust infrastructure for storing genetic and transcriptomic data in the EHR. Moreover, determining which information will be reported back to a patient and incorporated into an EHR will require concerted efforts from clinicians and researchers.

Accuracy and validation. Individually, genome-wide data sets carry inherent error rates[83], and structural variants are still difficult to detect and, as such, are rarely called. The accuracy of more continuous and longitudinal data, such as mRNA expression and proteomic data, may be more difficult to assess depending on the specific tissue assayed, but these technologies are highly reproducible for technical and biological replicates[84,85]. In some situations, these technologies independently identify different aspects of the same biological process and thus can validate each other: for instance, RNA-seq can internally replicate exonic variants identified through WES or WGS, whereas proteomic expression can validate expression from RNA-seq. However, in a clinical setting, where high confidence is required, these tests are currently validated by independent technologies, potentially including established clinical tests, such as enzymatic or single-assay tests.

For cancer genomics, disentangling heterogeneous data is a substantial challenge. As each tumour is a mosaic of cells with varying degrees of somatic mutation, variant detection is difficult, even before attempting to discern driver mutations from passenger mutations. In particular, cancers display signatures of somatic mutations that are clonal or found in only a subset of cells in a tissue, complicating their discovery, and high-coverage and high-quality data are necessary to distinguish these from sequencing errors (for a recent review on the computational methods to do so, see REF. 86). Ultra-deep sequencing of cell-free DNA to follow the presence of cancer mutations and single-cell sequencing to detect cancer heterogeneity are emerging as powerful methods. However, cell-free DNA for early cancer detection requires robust methods to distinguish genuine low-frequency events from sequencing errors, and single-cell sequencing is still expensive. Nonetheless, such methods have already been used to disentangle tumour heterogeneity[87] and identify a secondary finding of cancer in a prenatal test[88]. As additional omics data sets are integrated with ultra-deep sequencing, we expect the advantages of each of these methods to complement each other and provide a uniquely powerful method for molecular interrogation in the clinic.

Interpretation. Even with highly accurate data, another difficulty lies in the interpretation of genome-scale results, particularly rare and novel molecular events, which often vastly outnumber the number of events that can be reasonably functionally validated. Many variants in an individual genome, especially if they have not been seen before, do not have a clear functional effect and are known as 'variants of uncertain significance' (VUS)[89]. This problem is compounded for other data types, such as transcriptomic or proteomic data, and decisions for what constitutes a clinically significant molecular event, for example, an RNA expression threshold, are difficult to determine across disparate data types. Fortunately, large reference population data sets, which are already available for exome[56] and genome sequencing (gnomAD) and gene expression[57,90], will aid in the interpretation of rare events by providing a quantitative context as to their actual frequency in a population. In particular, a causal variant would be expected to have a significantly higher frequency in affected individuals than in a wider asymptomatic population, which can lend support to or negate previous suggestions of pathogenicity[91,92]. Additionally, physicians may discover additional pathogenic molecular events for unrelated conditions, which are known as secondary or incidental findings[93], over which there is still considerable debate as to the extent to which results should be returned to patients (for a recent review, see REF. 94).

When integrating multiple omics technologies, these problems are occasionally ameliorated, particularly for rare and novel molecular events for which statistical analysis is not feasible. In particular, direct integration of omics technologies that expose orthogonal information may provide additional evidence for a molecular event: for instance, if a VUS is shown by RNA-seq to affect splicing of a key disease gene, this can corroborate a potential pathogenic mechanism[16]. This way, multiple technologies can establish a chain of causality that a single technology cannot.

Finding the relevant tissue. In order to maintain consistency across samples, many large-scale research studies have been performed on readily available samples, such as blood or cell lines, including transformed lymphoblastoid cell lines[90,95]. However, for clinical applications, it is ideal to study tissues that are relevant for a particular disease as gene expression varies considerably across tissues[96,97] (FIG. 3). The GTEx, Roadmap Epigenomics and Functional Annotation of Mammalian Genome 5 (FANTOM5) projects provide reference data sets for multi-tissue gene expression and epigenomics data[47,57,98]. In many cases, the disease-relevant tissue may be well described, such as muscle tissue for MD; however, if the disease is less well defined or the tissue is not available,

Structural variants
A class of genetic variation that is typically 1 kb or larger, which includes copy number duplications, insertions or deletions, as well as translocations and inversions.

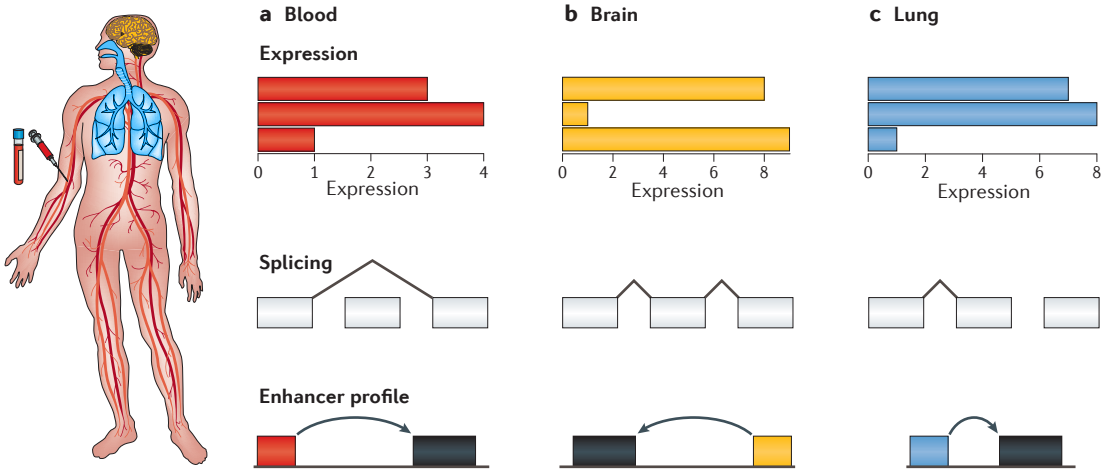


Figure 3 | **Finding the relevant tissue.** Although blood (part **a**) is often the most convenient tissue to assay owing to its availability and ease of procurement, it is often not the ideal tissue for observing a molecular phenotype for a given disease, which may primarily affect other tissues such as brain (part **b**) or lung (part **c**). In particular, its transcriptional landscape, including expression levels, splicing patterns and enhancer usage, may not be amenable to detecting differential uses of these patterns compared with a tissue that is more proximally affected by a disease, such as muscle tissue in muscular dystrophy.

a tissue may be identified from a network analysis of the disease[99]. Indeed, using the disease-relevant tissue has proved beneficial in diagnosis of patients with MD, where transcriptome analysis of the muscle tissue resulted in diagnoses that would not have been made via easily accessible proxy tissue, such as blood or fibroblasts, owing to the relatively low expression of disease-relevant genes[16]. In using such data for clinical utility, care should be taken to ensure that data from patient samples are comparable to reference data sets, which will be crucial going forward for additional omics data, such as metabolomics and proteomics. Of course, such analyses are further complicated where there is substantial cellular heterogeneity in the tissue, such as the brain: in these cases, technologies with single-cell resolution will provide valuable insights into resolving each individual cell type. In cases where primary tissue is difficult to obtain or maintain in culture, introduction of a mutation into induced pluripotent stem (iPS) cells using CRISPR systems can provide a powerful framework for molecular validation[100].

Actionability and therapeutics. Perhaps most important to the discussion of the use of any technology in the clinic is that of actionability. Indeed, a piece of information does not need to inform a course of action to be useful: having the knowledge of a diagnosis and ending a diagnostic odyssey can be invaluable to patients and families[101] (for a thorough perspective on the purpose of genetic testing for diagnoses, see REF. 102). However, data that can inform an intervention are additionally beneficial, in a framework that has been termed 'precision medicine' or 'personalized medicine'. In particular, classifying a patient's subtype of a disease to recommend a specific drug, determining whether a potential transplant is a good match on the basis of omics profiling (BOX 2) or identifying a causal mechanism for a novel disease (and developing a therapeutic that can target the direct molecular outcome) can improve outcomes and prolong the lives of patients. However, even non-causal molecular events that are statistically associated with an outcome can be actionable, particularly in the form of lifestyle change recommendations, including diet, monitoring and preventive treatments; indeed, individuals with high genetic risk of coronary heart disease experience greater benefits from statin treatment[103,104].

Conclusions and future perspectives

At present, only in very few cases have omics technologies (particularly genome sequencing and, to a lesser extent, RNA-seq) been shown to outperform traditional clinical tests and, therefore, substantial technical and regulatory hurdles exist to incorporating these technologies into clinical practice. However, as the use of multiple technologies enables a clearer picture of health and disease, it is likely that integration of these technologies will become commonplace in future clinical practice. Additionally, as recent large biobank initiatives, such as the UK Biobank, Million Veterans Project and All of Us, collect biological data and perform multiple layers of omics assays on millions of individuals, they will yield profound insights into human disease and serve as valuable reference databases for additional studies and clinical applications.

Predictive models of disease risk for healthy individuals and early detection of disease. As with traditional clinical tests, molecular measurements from large-scale omics data can be integrated into models of disease risk. In particular, recently, a set of methods has been developed for calculating the genetic risk of a particular disease, known as a polygenic risk score (recently reviewed in REF. 105). These methods have been successful in stratifying patients into high-risk and low-risk categories

Box 2 | **Multiple omics profiling of transplant donors and recipients**

Every year, thousands of patients are given organ and haematopoietic stem cell transplants. However, mortality among transplant patients remains very high. A standard practice for matching donors with recipients involves human leukocyte antigen (HLA) typing, for which methods have been recently developed using high-throughput sequencing technologies[123,124]. However, it is becoming increasingly clear that non-HLA factors can considerably affect prognosis and development of graft-versus-host disease (GVHD), as HLA-matched sibling donor transplants convey a lower risk of GVHD than HLA-matched but unrelated donor transplants[125], and common non-HLA polymorphisms have been associated with GVHD[126].

Accordingly, many omics applications may be used to determine optimal donor–recipient matches, as well as to monitor markers of rejection[127]. For instance, sequencing cell-free DNA can detect circulating donor DNA[128], the levels of which are correlated with the severity of organ rejection[129]. Additionally, sequencing this cell-free DNA can simultaneously detect viral DNA to indicate a marker of infection[130]. Additional omics data, such as RNA or protein expression, may also be used to assess compatibility of donor–recipient pairs, as well as monitor for markers of rejection (for a recent review, see REF. 131). Integration across multiple omics technologies may well emerge as a useful tool for transplant biology.

for diseases such as cardiovascular disease[77], as well as for predicting traits such as educational attainment[106]. Single-assay tests are often performed to follow up the results of predictions of disease risk, whether derived from genetics or family history. For example, if a patient is predicted to be at risk of type 2 diabetes, then assays for glucose and glycosylated haemoglobin (HbA_{1c}) levels and other tests, such as a glucose tolerance test, are performed. However, in the future, if a metabolomics panel could be performed simultaneously at high quality and low cost, this would obviate the need for the single follow-up assay. In addition, data from wearable devices that continuously collect data are likely to be very powerful in combination with omics data for early detection of disease before symptom onset[107].

Disease management. In addition to prediction and early diagnosis, integrative omics is expected to become increasingly powerful for disease treatment and prognosis. Information from the transcriptome, epigenome, microbiome, proteome and metabolome as well as imaging and wearable data will all be used to help decipher disease to facilitate prognosis and thereby guide treatment. In cancer, DNA and RNA sequencing of tumour–normal pairs has identified translocation and gene expression signatures, which has suggested targeted therapies that resulted in disease regression[108,109]. In the future, as multiple omics measurements are associated with prognosis in other diseases, it is likely that such data-driven paradigms will be powerful tools for medical research and also facilitate clinical diagnosis and treatment.

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