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High-throughput mouse phenomics for characterizing mammalian gene function

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Abstract

We are entering a new era of mouse phenomics, driven by large-scale and economical generation of mouse mutants coupled with increasingly sophisticated and comprehensive phenotyping. These studies are generating large, multi-dimensional gene–phenotype datasets, which are shedding new

Further information

International Mouse Phenotyping Consortium (IMPC): http://www.mousephenotype.org
International Mouse Phenotyping Resource of Standardised Screens (IMPReSS): http://www.mousephenotype.org
Online Mendelian Inheritance in Man (OMIM): http://www.omim.org
Orphanet: http://www.orpha.net
Hybrid Mouse Diversity Panel (HDMP): https://systems.genetics.ucla.edu/
Edinburgh Mouse Atlas Project (EMAP): http://www.emouseatlas.org/
Phenotype And Trait Ontology (PATO): https://bioportal.bioontology.org/ontologies/PATO
Genotype–Tissue Expression (GTEx) Project: https://www.gtexportal.org/home/
Search Tool for the Retrieval of Interacting Genes/Proteins (STRING): https://string-db.org/
Monarch Initiative: https://monarchinitiative.org/
100K Genomes Project: https://www.genomicsengland.co.uk/
NIH Undiagnosed Diseases Network (UDN): https://undiagnosed.hms.harvard.edu/
Mouse Genome Informatics: http://www.informatics.jax.org

Competing interests statement

The authors declare no competing interests.

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light on the mammalian genome landscape and revealing many hitherto unknown features of mammalian gene function. Moreover, these phenome resources provide a wealth of disease models and can be integrated with human genomics data as a powerful approach for the interpretation of human genetic variation and it relationship to disease. For the future, the development of novel phenotyping platforms allied to improved computational approaches, including machine learning, for the analysis of phenotype data will continue to enhance our ability to develop a comprehensive and powerful model of mammalian gene–phenotype space.

Table of contents blurb:

Although the field of functional genomics is increasingly adopting genome-scale approaches, a comprehensive understanding of gene functions requires the parallel development of deep phenotyping platforms. This Review discusses strategies for broad-based mouse phenomics, applied both to gene-knockout collections and to diverse strains harbouring natural genetic variation. The authors discuss technical challenges, analysis pipelines and insights into human disease genetics.

Introduction

Deciphering the genetic networks underlying disease and biology will require a comprehensive multi-species approach that combines an increasingly sophisticated genetic analysis with deep and robust phenotyping approaches and advanced analytics. This paradigm is well exemplified by the use of the mouse as a key model organism for gene function studies and the elucidation of disease mechanisms.

The mouse provides a highly advanced tool box for genome manipulation that enables the generation of a wide variety of genetic variants and alleles at each and every locus across the mouse genome [123]. The advent of CRISPR–Cas9 technology is a further step forward in the rapid and economical generation of complex and targeted mouse mutations[4,5,6]. Coupling large-scale mutagenesis programmes with broad-based phenotyping pipelines offers the possibility of generating multi-dimensional datasets that relate mutant alleles to biochemical, physiological and developmental changes across the phenotype landscape[7–1112]. This provides a fundamental knowledge base on which we can dissect and analyse genetic variation in the context of functional mechanisms using new statistical approaches and machine-learning algorithms. As such the generation of a comprehensive picture of gene function in the mouse has a natural synergy with the development of comparable multi-dimensional datasets and biobanks in humans, which involves the study of the relationship between genetic variation and phenotype across diverse cohorts and populations.

However, the history of mouse genetics and the application of mouse genomic tools to date highlights the many challenges that we face in delivering comprehensive and robust datasets that inform on gene function. These include, firstly, the relatively poor state of legacy data on gene function in mouse genetics that often arises from a failure to record detailed experimental procedures or metadata, and at the same time underlines future requirements for standardization. Second, investigations of mouse mutants often reflect the interests and

expertise of the investigator, thus failing to identify pleiotropy (the multiple functions of a gene) to its fullest extent.

Uncovering pleiotropy is central to elaborating gene function and the pathological basis of disease (Fig. 1). There is an increasing recognition that pleiotropy is ubiquitous [13]. Pleiotropy is visible in numerous genetic phenomena, from variable expressivity[14], phenotypic expansion[15], to the genetic networks revealed through genome-wide association studies (GWAS)[16] and phenome-wide association studies (PheWAS)[17] (Fig. 1). It is possible that pleiotropy emerges from the highly interconnected gene networks in cells and tissues that extend beyond core loci, which have direct roles in traits and disease in particular tissues, to the modest regulatory effects of many peripheral genes. This might be termed network pleiotropy [16]. The implication is that there are few genes expressed in a particular tissue that do not have some effect on a disease phenotype associated with that tissue. Whatever the mechanism, pleiotropy is a fundamental property of genetic networks and disease, and the manifestation of pleiotropy will vary with the genetic context. Extending phenotyping approaches to capture the full panoply of pleiotropic effects will be key to revealing a profound understanding of gene function and the genetic bases for disease. Moreover, the acquisition of more extensive phenotyping datasets in any species will improve our ability to compare phenotypes across species, to identify causal associations and to dig deeper into pathobiological mechanisms using a comparative approach.

Ultimately, the development of comprehensive catalogues of mouse gene function and the integration of those phenotype datasets with other species requires a step change in phenotype approaches, as well as novel informatics tools and approaches, in order to realise the value of the extensive genome-wide analyses that are being undertaken across human and other model organisms. For both these aspects there is enormous current activity and interest in fundamental and novel developments that build upon international consortia and collaborations, and these alliances are laying the foundation for a new phenomics in the mouse.

This article describes the ongoing and future developments in genetic and phenotyping analysis in the mouse, including the development of new phenotyping technologies, which will enhance a comprehensive, systems-wide view of gene function. We discuss the key challenge of developing new analytical tools to enable data integration across species, particularly for integration of phenotype data. We also highlight how a comprehensive catalogue of gene function in the mouse will underpin the dissection of the parallel multi-dimensional datasets generated in human.

Phenotypic screens of laboratory mice

Over the past century, the characterization of phenotypes and the identification of the underlying genetic bases of spontaneously occurring variants in colonies of laboratory mice has contributed greatly to our current knowledge of gene function. Early detection of spontaneous variants depends upon careful observations of breeding colonies for the identification of phenodeviants demonstrating visible phenotypes such as eye anomalies, changes in coat colour or texture, and behavioural changes (such as hyperactivity or

circling)[18]. Meticulous inspection of mouse stocks still remains an important tool for identifying new mutants in laboratory colonies [19] and an essential universal test for high-throughput phenotyping screens [2021]. However, assessing gene function through the manifestation of physical or behavioural anomalies in mouse colonies will only dissect a very limited segment of the genome landscape. The recent history of mouse genetics over the past few decades is characterized by the implementation of a range of mutagenesis tools — radiation [22], chemical [238] and genetic [2] — which enable efficient induction and recovery of mutants. High-throughput mouse mutagenesis in combination with increasingly sensitive phenotype screens is delivering a plethora of mouse mutant strains and adding substantially to the gene function catalogue. Increasingly, the focus has been on ever more sophisticated phenotyping platforms covering all body systems in order to deliver insights into a diverse range of biological mechanisms.

Hypothesis-free phenotyping screens

Mouse genetics was transformed by the implementation of high-throughput mutagenesis programmes utilizing two contrasting approaches: random *N*-ethyl-*N*-nitrosourea (ENU) chemical mutagenesis[7,8] and gene-trap and transposon technologies[9]. For both modalities, it is feasible to generate large numbers of mutant animals that can be screened by a range of phenotype tests. Even for gene-based technologies the screens are applied in a hypothesis-free and unbiased manner without making any *a priori* assumptions about the function of the underlying gene that was mutated. The expectation was that novel aspects of gene function would be efficiently revealed. Indeed, this promise was fulfilled and the first hints of extensive pleiotropy in the mammalian genome uncovered[7,8]. Importantly, the identification of phenotypes for genes of unknown function gathered pace, and henceforth it was recognized that an important limitation to discovery lay with the availability, sensitivity and logistics of phenotyping screens.

The majority of all mouse screens involve elements of cage-side observations that reveal visible phenotypes including dysmorphologies and size differences[2425]. For example, many neurological disorders can be identified by experienced mouse handlers noting differences in movement and activity[26], and the appearance of unusual behaviours such as fitting[27], jerky movements[28] and circling[24]. Building on these simple observational protocols, a whole plethora of phenotyping tests have emerged over the past few decades in parallel to the emergence of the mouse mutagenesis toolkit. There is not the space here to exhaustively cover the full range of phenotyping platforms (many of which are illustrated in Fig. 2), but several areas serve to illustrate the pace of change. Neurological and behavioural systems have been an areas of focus, with a variety of test environments employed for the identification of novel mutants with defects in characteristics such as motor function[2930], balance [31] and various behavioural measures such as anxiety [3233]. More complex test environments such as wheel running activity to measure circadian profiles[34], pre-pulse inhibition (PPI) profiling[35] and neurohistopathology[36] have enabled the discovery of complex behavioural phenotypes modelling many human neurological[28] and neuropsychiatric disorders[37]. Sensory systems have been investigated by extending the test environment to include the optokinetic drum [38] and auditory brainstem response [39]. Furthermore, imaging systems have been applied to allow the capture of diverse parameters

on bone structure and body composition[40]. Clinical chemistry and haematological analyses have developed to allow the assessment of a wide range of parameters relating to multiple organs and systems including blood chemistry and urinalysis[7,8,25,41,42,43], haematology[44] and components of the murine immune system[45,46,47]. Importantly, each of these phenotyping platforms has a relatively low impact on the mouse tested and can therefore be used in combination with other platforms and also repeated during the animal's lifetime. Ultimately, phenotypic screens end with terminal assays, which include histopathology [48] or terminal imaging, and the assessment of morphology by a variety of modalities at embryonic stages for lethal mutations[49–51,52]. In addition, tissues can be banked and utilized for a variety of omics studies; however, partly due to cost, these omics analyses have not featured extensively in the past or current phenotyping pipelines. Finally, broad-based phenotyping would be most efficiently and economically delivered at mouse genetics centres with a diverse range of skills and equipment, the so-called mouse clinics[53]. The mouse clinics would take advantage of the inherent economies of scale and operate a phenotyping pipeline that delivers a comprehensive assessment of gene–phenotype relationships at reasonable cost. The economies of scale also speak to the extensibility of the pipeline; incorporating new tests and paradigms (as discussed below) can be delivered at modest expense.

Launch of multi-centre broad-based phenotyping

The increasingly diverse phenotyping platforms that emerged for use in large-scale mutagenesis screens required a critical appraisal of both the utility of the phenotyping procedures and how they might be employed. First, it is desirable to assess and, if necessary, improve procedures to ensure reproducibility across laboratories and across time. The European Union Mouse Genetics Research for Public Health And Industrial Applications (EUMORPHIA) programme tested and developed a robust set of phenotyping standard operating procedures (SOPs) that is available through the European Mouse Phenotyping Resource for Standardized Screens (EMPReSS) database[54] (Table 1). Importantly, the standardised SOPs incorporate the capture of metadata (including feed, environment, person performing the test etc.) that takes into account possible confounders and gene–environment interactions[55], and is critical for reproducibility as well as improving the statistical power to detect significant associations. Second, it is recognized that the application of a full range of tests to each mouse line is essential to reveal pleiotropy and provide a more profound understanding of gene function in the context of diverse biological systems.

It was appreciated that mouse clinics also provide the infrastructure for large-scale mutagenesis, the ability to generate and breed many mouse lines simultaneously, that when allied to broad-based phenotyping pipelines could analyse hundreds, perhaps thousands, of genes over reasonable timescales. The activity of the clinics need not be focused entirely on single gene mutations, but could also assess any genetic resource from inbred lines to outbred populations. The aim would be to gather multi-dimensional genetic and phenotype data that would reveal the genetic networks at play across diverse systems.

Phenomics for every mouse gene

Critical to the emergence of a comprehensive catalogue of mammalian gene function is the generation of a mutant resource for every gene in the mouse genome. The International Knockout Mouse Consortium (IKMC) set out to generate mutations in embryonic stem cells for every gene in the mouse genome[56] (Table 1). Using both high-throughput gene trapping and gene targeting approaches[57], they developed mutant embryonic stem (ES) cell lines for more than 18,500 genes representing over 90% of mouse protein-coding loci. Most of the mutations are conditional, where mutations can be induced in a temporal or spatial manner.

The focus of mouse mutagenesis and phenotyping pipelines on null and severe loss-of-function mutations raises the question of the relevance of these alleles and their phenotypes to our understanding of the contributions of human genetic variants (which are probably alleles of modest effect) at these loci to complex disease. The primary rationale for the use of null mutations is to reveal important functional contributions to phenotypes across diverse biological and disease systems and to provide a fundamental baseline for gene function. Indeed, it is clear from studies in the human population that there are thousands of associations between Mendelian and complex diseases that are manifest in a phenotype code linking Mendelian loci to complex disorders[58]. As such, human variants associated with complex disease are enriched in genes that encapsulate this Mendelian code. This underlines the rationale and utility of analysing and cataloguing mouse null mutations. In a similar vein, there are numerous examples where for human complex disorders the generation of null mutants for strong candidate disease genes has proven valuable in validating genes and elucidating constituent endophenotypes that may contribute to the pathology of a complex disease. To take one example, null mouse mutations for autism candidate genes *Shank1*[5960], *Shank2*[61] and *Chd8*[6263] result in autistic-like behaviour, which exemplifies the utility of mouse knockouts in assessing fundamental and relevant disease phenotypes.

The IKMC mutant ES cell resource was the basis for two major pilot programmes to assess broad-based phenotyping across hundreds of mouse lines (Table 1). At the Sanger Institute, UK, the Mouse Genetics Programme (MGP) undertook the generation of hundreds of mutant lines and applied a diverse range of phenotype screens[10]. At the same time, the European Mouse Disease Clinic (EUMODIC) programme undertook a multi-centre programme (involving MRC Harwell, Helmholtz München, ICS Strasbourg and the Sanger Institute) generating and analysing hundreds of lines through a common phenotyping pipeline employing standardized EUMORPHIA procedures[11]. The use of common reference lines in the EUMODIC programme allowed an assessment of the reproducibility of phenotype outcome across centres, and demonstrated the robustness of the phenotype platforms employed. Importantly, both the MGP and the EUMODIC programmes revealed extensive pleiotropy across hundreds of genes and uncovered phenotypes for many genes with hitherto unknown function. These programmes underlined the critical role for broad-based screens in any future initiative to scale to a genome-wide effort.

Current systematic phenotyping efforts

International Mouse Phenotyping Consortium.

In 2011, the International Mouse Phenotyping Consortium (IMPC) was formed with the goal of generating a comprehensive catalogue of mouse gene function by generating and characterizing null mutations for every mouse gene[12]. Until recently, the IMPC has generated mouse mutants using the tm1b null mutant allele from IKMC [57] (Table 1). IMPC now employs CRISPR–Cas9 to generate null mutations by the deletion of an early critical exon. Importantly, all mutants are generated on a coisogenic C57BL/6N background. Homozygous mutants enter a broad-based, standardized adult phenotyping pipeline[64] (Fig. 2). Cohorts of male and female mutant mice undergo a wide range of phenotype tests from 9–16 weeks, followed by a variety of terminal tests. The tests cover a wide range of system areas including neurological, behavioural, metabolic, cardiovascular, pulmonary, fertility, sensory, and musculo-skeletal function. Homozygotes that are embryonic lethal undergo characterization through an embryonic pipeline[14] that assesses the stage of lethality, as well as applying various high-resolution imaging modalities (such as optical projection tomography (OPT), micro-computed tomography (μCT) and high-resolution episcopic microscopy (HREM)) to elaborate morphological defects[49–51,52]. In the case of embryonic lethal mutants, heterozygotes enter the adult phenotyping pipeline. The tm1b allele carries a lacZ reporter that enables the determination of tissue expression patterns of each disrupted gene. In IMPC, both embryonic and adult expression profiles have been acquired for many lines. To date, IMPC has generated over 7,000 mutant lines and phenotype data has been collected on over 5,000 lines.

The value of a broad-based phenotype approach is strongly supported by the global analysis of the multi-dimensional datasets that are emerging. A number of key findings are transforming our view of the mammalian genome landscape. First, an extraordinary number of novel phenotypes and models are revealed [64]. 90% of the gene–phenotype annotations described by IMPC have not previously been reported, emphasizing the noteworthy and widespread pleiotropy. For the first 3,328 genes analysed, 889 known human disease genes in Online Mendelian Inheritance in Man (OMIM) and Orphanet have an orthologous IMPC mouse strain with at least one phenotype. Given that the IMPC pipeline is broad but shallow, not focussed on particular disease areas, and incomplete for some of the lines, it was remarkable that 360 IMPC lines (40%) have phenotypic overlap with the 889 human disease genes, and the majority (279, 78%) are the first reported mouse model for these diseases. A major analysis of embryonic lethal lines from the first 1,751 knockouts provided several profound, novel insights into essential (lethal) genes [14]. The analysis confirmed a strong enrichment for human disease genes within the set of embryonic lethal, essential genes. But perhaps the most intriguing finding was the high degree of variable expressivity observed in embryonic lethals, despite the uniform, co-isogenic background of the mutant lines. These observations were also reflected in the significant number of subviable lines that demonstrate variable lethality. Mutant genes resulting in subviability, as with standard non-essential genes, were significantly more likely to have a paralogue compared to essential genes. The implication is that subviability reflects stochastic variation that is induced in normally buffered pathways[65]. This is another example of pleiotropy where differing

components (and phenotypes) of the disrupted genetic network are variably manifest in the mutant. It might be termed ‘stochastic pleiotropy’.

The phenotyping of both male and female cohorts (mutant and wild-type) through the IMPC pipeline has enabled for the first time an in-depth analysis of the extent of sexual dimorphism across the entire mammalian genome [66]. Analysis of 2,186 mutant lines for up to 234 traits found that nearly 18% of mutant phenotypes are influenced by sex, demonstrating that sexual dimorphism is extraordinarily pervasive. This is an important finding as, in the past, for many individual mutants and traits phenotype analysis was confined to one sex.

Finally, in two disease areas, deafness and metabolism, analysis of relevant disease-specific phenotypes for a large number of IMPC mutant lines has uncovered numerous novel genetic loci. These findings substantially expand our understanding of the genetic landscape associated with these disease states [6768].

Inbred, recombinant inbred and outbred lines.

In addition to the analysis of single-locus, typically null mutations, mouse geneticists have employed the considerable genetic variation between inbred strains to study genetic systems, and in particular to dissect the genes involved in complex traits. These complex trait resources have been analysed through many phenotyping platforms, and it is very pertinent to consider their application to high-throughput phenomics. There are essentially two classes of mouse resources for the analysis and mapping of complex traits. The first class includes the huge panel of extant inbred strains and the Recombinant Inbred (RI) lines[69], which includes the Collaborative Cross (CC) lines[70]. Inbred and RI lines have been phenotyped incrementally for multiple traits over many years, and together these resources have been an important tool for quantitative trait locus (QTL) mapping[71,72]. The Hybrid Mouse Diversity Panel (HDMP) combines inbred strains and RI lines for fine mapping of diverse phenotypes [7273]. The CC lines have also been extensively phenotyped at a variety of centres for a number of traits [7475]. However, there is considerable potential and economies to be gained by a comprehensive analysis of all the CC lines through a high-throughput, broad-based pipeline similar to that operated by IMPC.

The second class of mouse resource for QTL mapping includes various outbred populations of mice generated by pseudo-random breeding from inbred strains. This class covers the Heterogeneous Stock (HS) and Diversity Outbred (DO) populations. A high-throughput phenotyping pipeline for HS populations has already been developed[76] and applied to the genetic analysis of diverse traits including asthma, type 2 diabetes, obesity, anxiety, immunological, biochemical and haematological phenotypes[77]. DO mice are derived from outbreeding of different CC lines and are a potent tool for high-resolution trait mapping[78,79] and a resource that would be amenable to analysis in the high-throughput pipelines currently being applied to single-gene mutations. Lastly, commercial outbred mice have been used for fine mapping of specific traits[80]. Recently, the same outbred population (CFW) has been employed coupled to a high-throughput phenotyping pipeline for large-scale trait discovery and mapping[81]. From the analysis of more than 1,800 animals from the outbred population, 156 unique QTLs for 92 phenotypes were discovered, of which around a fifth were mapped

at gene-level resolution. Thus broad-based phenotyping and the development of standardized, high-throughput pipelines is an important tool not only in the analysis of specific gene variants, but also for the discovery and dissection of complex traits.

Current challenges in mouse phenotyping

Two of the most demanding challenges with respect to mouse phenotyping are reproducibility and inter-species comparability between mice and humans. In order to address these issues it is necessary to overcome both genetic and species differences, thereby ensuring the data collected from mouse models is increasingly biologically relevant to human clinical studies. By delivering robust and comparable phenotype data in both mice and humans we will increasingly build upon our understanding of genetic and disease systems. At the same time, it is imperative to develop new modalities for phenotyping mice that address avenues for revealing hitherto unexplored physiological and behavioural areas.

Standardized genetic systems for reproducibility.

Inconsistencies in mouse phenotyping data between laboratories can often be attributed to uncontrolled genetic backgrounds, arising from differences between the background strain used to generate a mutant and the strain used in subsequent breeds [82]. In recent years this has led to the demand to generate co-isogenic strains of genetically altered (GA) mice, delivering experimental cohorts where the genetic background is standardized and phenotyping data are reproducible. Phenotyping co-isogenic mutants provides robust data in the context of a single defined genome and is the essential foundation for the investigation of gene function [1110]. However, progress in gene editing and the development of extensive series of recombinant inbred lines [8370] will enable a wider range of standardized genetic backgrounds in which a mutation can be examined. The analysis of individual CRISPR mutations on a diversity of inbred backgrounds is likely to uncover further pleiotropic diversity (in a similar manner to phenotypic expansion in humans), elaborating the biology of gene function and complexity of gene interactions [84].

Whereas for inbred strains of mice the issue of genetic variation has been all but eliminated, this does not remove other unwanted sources of variation, which need to be assessed (as discussed above). Increasingly, we need to pay attention to the mouse microbiota — the commensal, symbiotic and pathogenic microorganisms found living in or on laboratory mice — and understand how they contribute to phenotypic variation. Undoubtedly the microbiota is influential in many[85] but all not phenotyping tests. The logistics of characterizing and stabilizing the microbiomes of an experimental cohort of mice and developing experimental mouse models of the human microbiome represent considerable challenges for the future [86], including new statistical methods that can adjust for the microbiome variation.

Humanizing the test environment.

One of the most exciting prospects is the development of new phenotyping technologies that ‘humanizes’ the testing environment. For example, metabolic phenotyping has begun to address the comparative differences in surface area to volume ratio of mice and humans by establishing testing paradigms that run at thermoneutrality, thereby circumventing

differences in the regulation of heat production in the mouse [87]. In addition, the use of home-cage monitoring systems to detect changes in feeding patterns [88] is likely to replace more conventional metabolic cages as they offer improvements in the complexity of data acquisition including duration, number of feeding bouts and timing of feeds in combination with activity data, replacing the rather crude single measurement of food intake.

Arguably the most controversial field in terms of reproducibility in mouse phenotyping data has been behavioural assays [89]. A future goal is not just to reduce variability caused by subjectivity or non-standardized testing (largely addressed by the widespread use of automation such as tracking systems) but also to increase the sensitivity and diversity of parameters measured. Extrapolating mouse behaviour to humans is problematic, with many behavioural tests assessing simple paradigms that fail to exemplify the complexity of human behaviour. More-complex protocols often require weeks of training and acclimatization. However, new home-cage monitoring technologies bring extraordinary opportunities in behavioural phenotyping that will enable researchers to explore new but different complex paradigms and improve the assessment and development of behavioural models (Fig. 3). In addition, they are adaptable to high-throughput phenotyping pipelines. By recording data including video from the home cage, it is possible to avoid issues of anxiety in the test environment and explore sequences of voluntary activity longitudinally[90] over many days including behaviours associated with well-being[91]. In contrast to some conventional apparatus, it is now possible to monitor activity continuously and during the entirety of the dark/active phase of the mouse[92] and to reveal previously unrecognized behaviours[93], including with cage-mates present[94]. The specialist equipment required for such analysis is costly and requires both substantial infrastructure and skills to handle the large multidimensional datasets generated. However, the rapid expansion of both supervised and unsupervised machine-learning technologies in animal behavioural studies [95] promises to deliver objective, longitudinal and sensitive data and a rich stream of novel behavioural phenotypes (see below and Fig. 4).

Environmental perturbations.

There is considerable interest in the application of environmental perturbations or challenges to large-scale phenotyping screens, thus enriching the phenotypes that might be revealed. Challenges might include diet (such as high fat [96]), noise [97], infection [98] or other immunological perturbations [99], although inevitably careful attention is required to consider potential indirect and confounding effects of a challenge across multiple phenotypic domains. As such it will often be prudent to utilize separate cohorts of animals for some challenges.

Age-related phenotypes.

The assessment of phenotype throughout the animal's lifetime is the focus of an increasing number of mouse genetics studies. There have been systematic efforts to determine the phenotypes of aged inbred lines and GWAS to reveal loci involved with age-related disease [100101]. In addition, a recent report describes a large-scale phenotype-driven ENU mutagenesis screen for age-related disease loci involving the recurrent screening of mutagenized pedigrees as the mice aged [38]. A considerable number of novel age-related

phenotypes and genes were uncovered that would not have been revealed from screens at earlier time-points. Fig. 4 illustrates a typical pipeline that might be used for the discovery of age-related phenotypes from large-scale screens, involving early-adult phenotype screens of mouse cohorts followed by an intervening period while mice are aged, and a subsequent round of late-adult phenotyping at one year of age or more.

Analysis of multi-dimensional phenotype data

High-throughput, broad-based phenotyping pipelines present a number of profound challenges in terms of phenotype analysis. First, the diverse nature of the data, from categorical to continuous measures, requires differing presentational and statistical approaches that are tuned to the data and analysis requirements. Second, metadata parameters need to be incorporated within the defined SOPs for each phenotyping test to ensure standardization and reproducibility. Metadata should also include the production and entry of control cohorts into the phenotyping pipeline. Moreover, it is important to document randomization and blinding schemes. Overall, it is critical that statistical models are developed that capture the experimental design and any significant sources of variation. It is also important that the reporting of experiments meets the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for animal experimentation, providing transparency that underpins reproducibility[102]. Finally, the use of phenotyping pipelines in a multi-centre programme benefits from the incorporation of common reference lines, which enables measures of phenotype concordance to be calculated and provides confidence in standardization and reproducibility[11].

The high-throughput, multi-centre nature of the IMPC exemplifies the challenges that are faced and the extraneous factors that must be incorporated into the data analysis. Initial approaches with t-tests and Mann–Whitney tests have given way to more sophisticated linear regression and Bayesian approaches that model the impact of other sources of variation beyond genotype and phenotype[11103]. As might be expected, evidence has emerged of significant batch effects, litter effects and interday variability that have the potential for confounding effects on phenotype readouts. For example, female *Expi*$^{tm1a(KOMP)Wtsi}$ mutant mice were initially observed to have significantly decreased circulating chloride levels. Further investigation showed that all the knockout females were phenotyped on a single day without concurrent controls, and all data collected on that day were low compared to other test days. It is difficult and costly to test littermate controls in a high-throughput pipeline, and also not always feasible to test controls concurrently with mutants. Thus centres tend to use a multi-batch workflow. As a consequence IMPC employs a linear mixed model, which has been shown to have the appropriate power and sensitivity for multi-batch workflows, minimizing the risk of genotype effects being confounded by temporal variation [103].

The interpretation of data from the wide array of image data generated from adult and embryonic pipelines remains a substantial roadblock, but progress is being made. For example, in the current IMPC pipelines, 2D and 3D images are generated ranging from lacZ expression patterns to μCT data. The manual annotation of these data by specialists is time consuming and prone to bias or error so it is therefore crucial to develop automated image analysis approaches that can robustly identify phenotypes. The development of

methodologies to date has largely focused on the 3D data, which is nearly impossible to manually annotate. However, the data are amenable to image registration methods. Automated pipelines have been developed [49,5051] which allow anatomical differences in embryonic day (E) 15.5 micro-CT data to be detected. Computer-automated image registration algorithms consist of three analyses (intensity, deformation and atlas-based) which detect missing anatomical structures and differences in volume of whole organs. These initial efforts provide a platform on which to develop tools for other embryonic stages and to manage the large volumes of data that will emerge from comprehensive embryonic phenotyping at multiple developmental time-points. Nevertheless, progress in the methods for the analysis of imaging data from other phenotyping modalities such as X-ray and high-resolution scans of histopathology slides is a priority. Novel techniques such as deep convolutional neural networks, a supervised machine learning method, are demonstrating applications in mouse phenotyping and have to date robustly segmented a number of mammalian cells. Methods such as this can be applied to automatically annotating slides in high-throughput phenotyping [104]

These multi-dimensional data sets and associated analysis from high-throughput mouse phenotyping are disseminated by the FAIR principles of data management — making data Findable, Accessible, Interoperable and Reusable [105]. The application of these principles builds off the experience from other large, collaborative biological projects that include biobanks as well as the Roadmap Epigenomics[106] and Functional Annotation of the Mammalian Genome (FANTOM)[107] consortia that have had their data reapplied in thousands of published studies[108] (Fig 5). A key process in adhering to FAIR data principles is annotation of data with community developed ontologies to facilitate data discovery. The key ontology used by the high-throughput mouse phenotyping groups is the mammalian phenotype (MP) ontology[109], with other integrated ontologies such as the Edinburgh Mouse Atlas Project (EMAP)[110] and the Phenotype And Trait Ontology (PATO)[111]. These ontology annotations allow integration with the diverse array of functional datasets in the public domain and opens up the potential for assigning function to poorly characterized genes in the mouse genome. By taking the annotated gene–phenotype data from knockouts and leveraging datasets such as the Genotype–Tissue Expression (GTEx) project [112], Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) [113], and Orphanet[114], we can look to build new pathways based on network analysis of our phenotype data, informing us on potential disease mechanisms and therapies based on orphan drugs

New statistical machine learning methods will be developed that can integrate the multiple phenotype data-modalities into a single joint model. In human genetics, these multiple-phenotype models will increase statistical power to detect genetic variants by averaging out confounding variation found in any one phenotype, similar to ‘enrichment analysis’ used in GWAS[115], thus enhancing comparisons with mice. Latent-factor models and related methods will help to identify low-dimensional structure within the high-dimensional phenotype data[116], providing novel statistical targets for association testing. Moreover, the use of molecular phenotypes from omics data including RNA expression and proteomics will facilitate the identification of causal genetic variants of unknown function. Real-time cage monitoring will demand scalable computational methods for processing and compressing hours of video capture of home-cage environment[117]. New bioinformatics

techniques will collate video recordings of group-housed mice in their home-cage environment and use advanced visualization tools and machine-learning analytics to extract comprehensive datasets and automatically classify behaviours from a spectrum of mouse strains and mutants (Fig 3). Classification and mapping of phenotypes into networks and hierarchies — e.g. of behavioural types, and sub-phenotypes — has the potential to increase the power to detect variants affecting biologically related traits [118].

Integration with human clinical genetics

The extensive multi-dimensional datasets emerging from large-scale mouse phenotyping programmes are an important foundation for the analysis of mammalian gene function. As we elaborate above, these datasets are already resetting our view of the mammalian genomics landscape. In addition, they are a powerful tool for cross-species analysis and integration that will shed further light on gene–phenotype relationships in diverse species. This is particularly true for human and clinical genetics and the community's ability to assess gene–phenotype relationships in humans, and to relate DNA variation in humans to disease outcome. Advances in DNA sequencing technologies have revolutionized diagnosis and discovery of new gene associations in human disease since the first successes in 2010, particularly for rare conditions involving Mendelian inheritance[119,120]. Based on these successes, numerous national programmes have been or are being initialized to perform large-scale whole-exome or whole-genome sequencing of patients alongside comprehensive collections of clinical data to address the long diagnostic odysseys that many patients with rare diseases undergo, as well as personalized treatments of cancer patients. For example, in the UK the 100,000 Genomes Project [www.genomicsengland.co.uk] is embedding genomics into the mainstream of a national healthcare system for the first time. In the US, the NIH Undiagnosed Disease Network [121], Centers for Mendelian Genomics[15] and Precision Medicine Initiative [122] are leading the way, and major national initiatives are underway in Japan, China, Singapore, Canada, Australia, Denmark, Norway, the Netherlands and France.

Despite all these advances, most large-scale projects can provide a genetic diagnosis for only around 25% of cases[123124125] with many patients presumed to carry as yet uncharacterized variants within, or regulating, known or novel disease genes. Here, the potential of mouse phenomics programmes to shed light on genes carrying rare, potentially pathogenic variants but with little or no previous functional knowledge can be critical. Clinical geneticists routinely interrogate such data through searching the literature as well as model organism databases and integrated portals provided by the IMPC[126], model organism aggregated resources for rare variant exploration (MARRVEL) [127] and the Monarch Initiative [128]. However, manual searches are not scalable for routine healthcare pipelines such as that of the 100,000 Genomes Project, hence it is valuable to have programmatic access as provided by the Monarch Initiative through their portal or automated variant prioritization tools that include comparison of a patient's clinical phenotypes to those observed in model organisms including the IMPC (Figure 6) [129]. Examples of such approaches used in the 100,000 Genomes Project, and other disease sequencing efforts, include Exomiser [130], Genomiser [131] and Phevor [132]. Routine inclusion of mouse and fish model organism data in Exomiser and application to the NIH Undiagnosed Disease Programme has been demonstrated to increase

diagnostic yield by 10–20% in the NIH Undiagnosed Disease Programme[133]. Collections of deep patient phenotype data and reproducible mouse phenotypes in a standardized structure using ontologies such as the Human Phenotype[134] and Mammalian Phenotype Ontology [109] are critical to these approaches, as well as the semantic similarity algorithms to compare and rank phenotypic profiles between human and mouse developed by the Monarch Initiative [135]. Similarly, standardized collections of other multi-dimensional datasets, such as transcriptomics, is emerging as a critical step as researchers seek to further improve variant analysis.

As well as variants in unknown disease genes, variants of uncertain significance (VUS) in known disease genes often overwhelm disease sequencing studies. Here, the tremendous advances in gene engineering through CRISPR–Cas9 [56] are lowering the barrier to rapid, efficient and cost-effective validation of such variants. Moreover, despite the numerous successes of genomics in identifying causative disease variants, an understanding of the pathobiological mechanisms and development of therapeutic options for rare disease patients remains as slow as ever. The ready availability of well-characterized and reproducible mouse models of human disease from large-scale mouse phenomics programmes is key to addressing this challenge at the basic research and translation level. Personalized mouse lines containing the same functional variants as human patients will accelerate the investigation of mechanisms and pre-clinical screening of therapeutics. The single gene, mouse KO resources such as IMPC are obviously most applicable to the study of Mendelian disorders, especially where the mechanism is loss of function and pathogenic variations in the human gene have not been previously described. Overarching the single-gene mutant resources are mouse inbred and outbred resources and their power to identify the loci and networks involved in complex traits, which can be explored in more depth by the generation and phenotyping of mutations at individual loci. Importantly, however, it will be insufficient to simply generate a comparable allele in the mouse genome. High-throughput and broad-based phenotyping pipelines will need to be utilized to reveal comprehensive phenotype data, uncover pleiotropy across diverse disease systems, and enable clinical geneticists to use the available tools to assess phenotype matches and compare pathobiology.

Outlook

High-throughput mouse phenomics has been a powerful engine for biology and biomedical sciences, with a number of transformative impacts on genetics and genomics. Increasingly, however, mouse mutagenesis and phenotyping will be integrated with the demands and strategic directions of human biology and disease studies, including Mendelian disorders, precision medicine and complex disease. Already the power of linking rich, multi-dimensional mouse phenomics data with that of humans is persuasive. Moreover, the ability through CRISPR to alter the mouse genome at will highlights the opportunities to utilize the vast expertise of the mouse genetics community and its facilities and pipelines in the functional analysis of human variation, including point mutations both in coding and regulatory sequences. The functional analysis of non-coding sequences remains a formidable challenge, but one that will need tackling by both mouse and human geneticists in the drive to understand the totality of human variation in complex disease. The determination of the function of non-coding sequences, including regulatory and non-coding transcripts must be a

critical ambition for mouse phenomics. The scale of the endeavour is enormous, but initially over the next few years we expect mouse genetics to turn its attention in this direction, undertaking comprehensive studies into the phenotypes of a substantial number of non-coding variants and transcripts.

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Glossary terms

Variable expressivity
Differing phenotypic features among individuals with the same genotype

Phenotypic expansion
The expanding array of phenotypes that may be associated with mutations in a specific gene

Genome-wide association studies
(GWAS). Genome-wide analyses of single nucleotide polymorphisms (SNPs) in human cohorts to test for association between SNPs and traits

Phenome-wide association studies
(PheWAS). Testing genetic variants for an association with multiple phenotypes or traits (the phenome) in human cohorts

Pre-pulse inhibition
(PPI). Used to assess sensorimotor gating. In the PPI test, sensorimotor gating is assessed by measuring the innate reduction of the startle reflex induced by a weak prestimulus (prepulse) prior to a subsequent strong startle stimulus (pulse). Deficits in PPI responses are noted in patients suffering from a range of illnesses including schizophrenia

Optokinetic drum
Assesses the threshold of visual acuity by placing a mouse in the centre of a rotating drum and measuring reflexive head turning in response to the rotation of stripes which subsequently decrease in width and distance of separation

Auditory brain stem response
(ABR). Measures the electrical response in the auditory nerve and brain stem to either a defined frequency, or a longer, complex auditory stimulus. This allows frequency-specific auditory thresholds to be determined

Gene trapping

A random insertional mutation into an intron or exon of a gene that disrupts expression of the trapped gene

Gene targeting
Targeting by homologous recombination into embryonic stem (ES) cells to introduce mutations ranging from single base-pair substitutions to large deletions

Endophenotype
A heritable and measurable component of a phenotype, intermediate between gene and disease.

Coisogenic
Isogenic strains differing only at a single locus are coisogenic strains. Thus, all International Mouse Phenotyping Consortium (IMPC) lines are coisogenic on the C57BL/6N background

Optical projection tomography
(OPT). An optical computed tomography technique that is used to acquire 3D images of early embryo morphology in the mouse

Micro-computed tomography
(μCT). High-resolution X-ray computed tomography to acquire 3D images of embryo morphology in the mouse, usually during later stages of development

High-resolution episcopic microscopy
(HREM). A method for the determination of the 3D structure of embryos using recurrent block surface (episcopic) imaging of sections from histological samples

Subviable lines
Mouse mutant lines for which some individual mice show embryonic lethality, whereas others of identical genotype survive

Paralogue
Paralogues are pairs of genes that derive from a common ancestral gene, and may undertake similar functions

Recombinant inbred
Recombinant inbred (RI) mouse lines are derived by the intercrossing and subsequent inbreeding of two distinct inbred lines. Each line carries a differing patchwork of chromosome segments from the two parental lines, allowing us to relate phenotypic differences between the parental inbred strains to the underlying genetic loci involved

Collaborative Cross
Collaborative Cross (CC) lines are a multi-parental recombinant inbred panel derived from crosses between 8 inbred lines (including 3 wild-derived inbred strains), capturing a greater genetic diversity more evenly spread across the genome

Quantitative trait locus

(QTL). A locus that contributes some proportion of the total phenotypic variance of the quantitative trait. Many quantitative traits are determined by multiple genes (or QTLs), each of which may have small or large effects on the phenotype

Heterogeneous Stock
(HS). HS populations enable fine-resolution mapping of traits, and are created by the intercrossing of inbred or recombinant inbred lines followed by mating schemes that minimize inbreeding

Diversity Outbred
(DO). The DO population is a Heterogeneous Stock that was derived by random mating of 144 partially inbred Collaborative Cross lines, providing single-gene mapping resolution

Ontology
Phenotype ontologies encompass the naming, description and interrelationship of phenotypes

Orphan drugs
Drugs that are developed to treat a rare medical condition, an orphan disease

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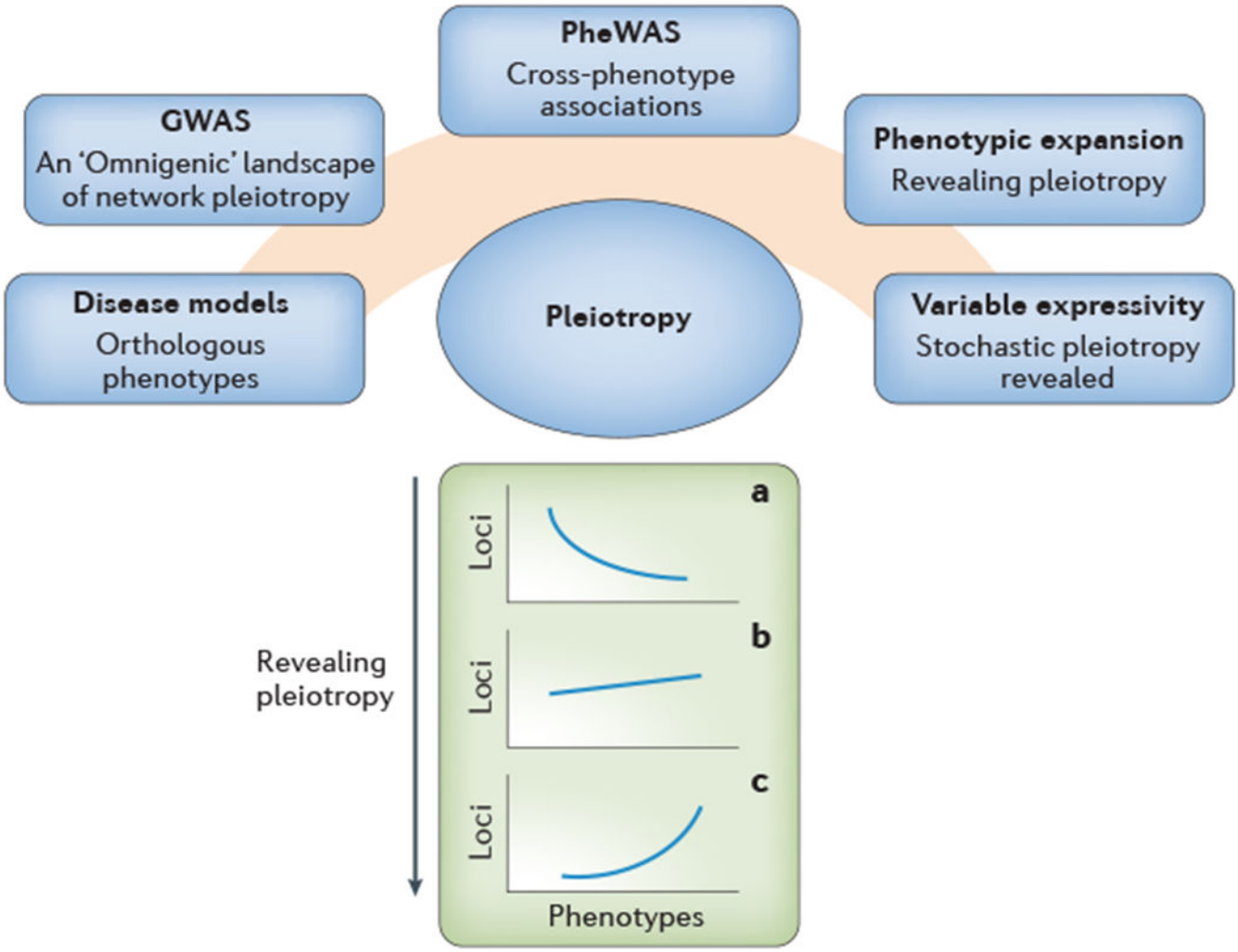

Fig. 1. Pleiotropy is central to our understanding of mammalian gene function.

Pleiotropy, the multiple functions of a gene, is manifest through the exploration of disease models and a variety of other phenomena. These include genome-wide association studies (GWAS) where for complex traits the association signals are widely spread across numerous genes and not simply in core disease pathways. The implication is that network pleiotropy is rife and that all genes expressed in a particular tissue are likely to affect phenotype outcome – the "omingenic" hypothesis[16]. Pleiotropy is also revealed in phenome-wide association studies (PheWAS) where the associations of individual genetic variants with multiple phenotypes, known as cross-phenotype associations, are uncovered[17]. The well-known phenomenon of phenotypic expansion in human genetics also exemplifies the pervasiveness of human pleiotropy[15]. Finally, the well-known phenomenon of variable expressivity by which the expression of different aspects of phenotype varies across individuals with identical genotype is also revealing of pleiotropy. Uncovering pleiotropy to its fullest extent is a critical ambition for high-throughput mouse phenomics with the aim of improving our knowledge of pleiotropy and developing datasets where multiple functions are documented. Currently, for most loci we have limited knowledge of pleiotropy and for most genes our knowledge of phenotypes is limited (**a**). The challenge for genetics is to extend our

knowledge of the multiple functions of genes to an increasing number of loci (**b**) and ultimately to most of the genes in the genome (**c**).

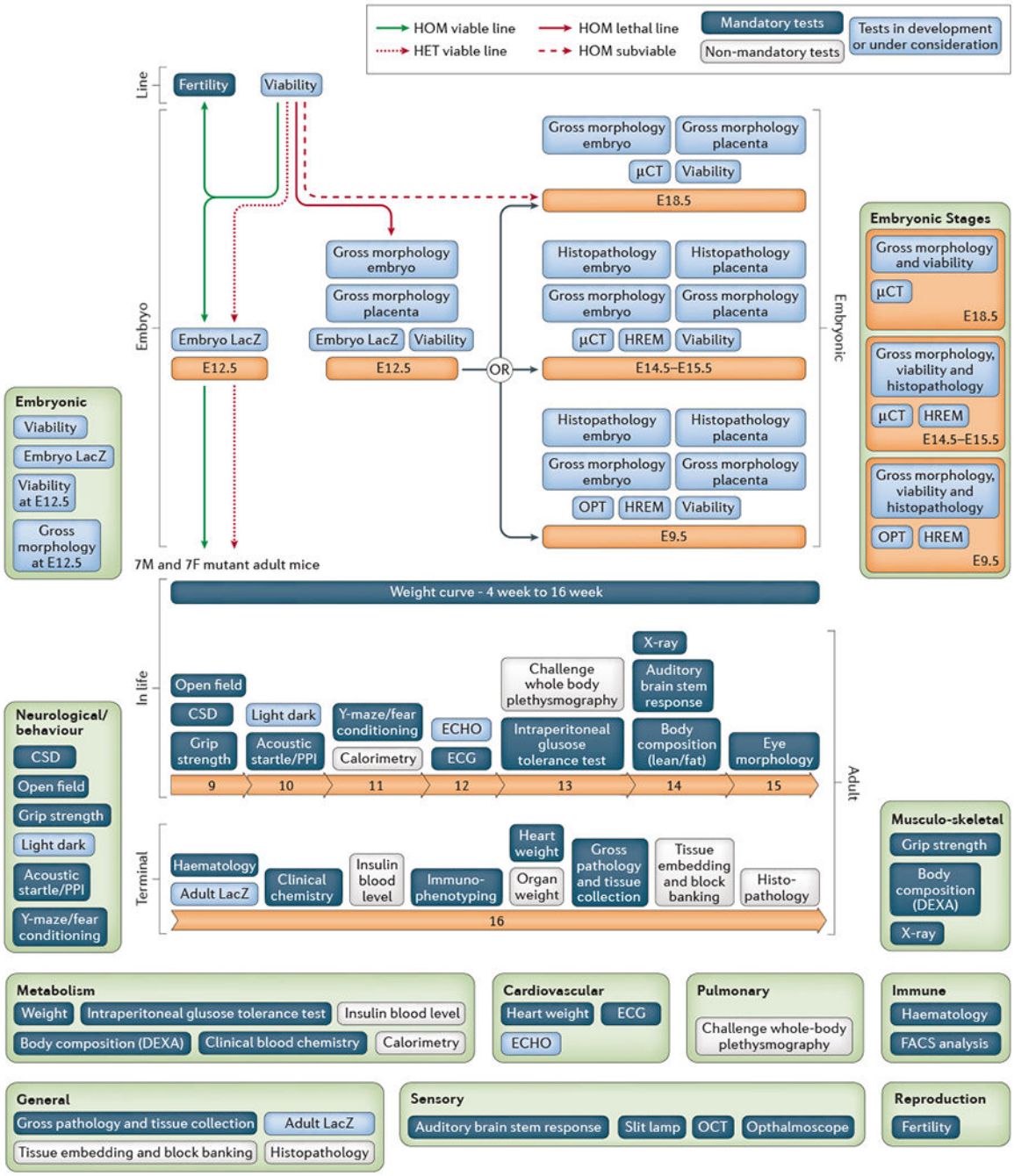

Fig. 2. The IMPC phenotyping pipeline.

The International Mouse Phenotyping Consortium (IMPC) pipeline provides an exemplar of the potential of high-throughput pipelines for the acquisition of broad-based phenotype data at both embryonic and adult time-points. The range of phenotyping platforms ensures the recovery of phenotype data across multiple systems and disease states. The key systems areas that are analysed are indicated along with the relevant phenotype tests that impact upon that area. Each phenotyping test is underpinned by a standard operating procedure (SOP) in the International Mouse Phenotyping Resource of Standardised Screens

(IMPReSS) database (www.mousephenotype.org/impress) that defines the phenotyping procedure and the associated metadata that is required.

μCT, micro-computed tomography;

CSD, combined SHIRPA (SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment) and dysmorphology;

DEXA, dual-energy X-ray absorptiometry;

E, embryonic day,

ECG, electrocardiography;

ECHO, echocardiography;

FACS, fluorescence-activated cell sorting;

HREM, high-resolution episcopic microscopy;

OCT, optical coherence tomography;

OPT, optical projection tomography;

PPI, pre-pulse inhibition.

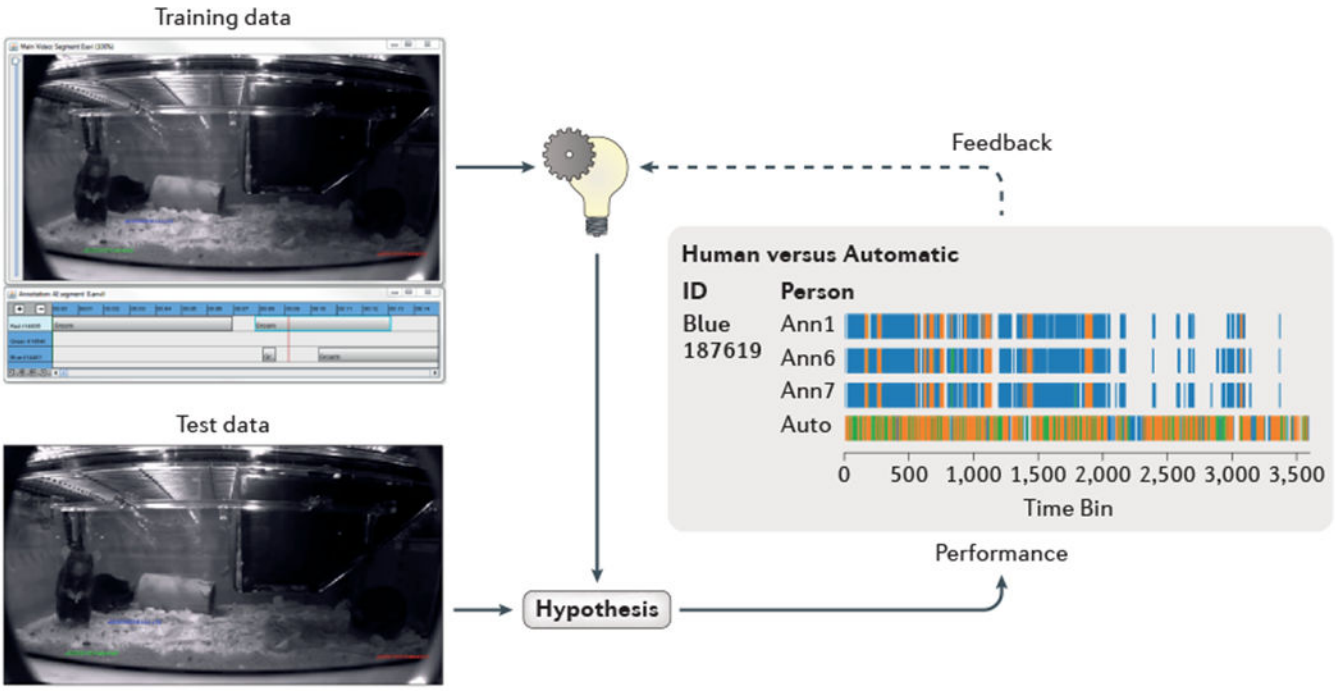

Fig. 3. Home-cage monitoring and machine learning.

The figure illustrates a supervised learning feedback loop. This type of automation is essential in order to analyse longitudinal changes in the patterns of behaviour in genetically altered (GA) mice, potentially extending into months and years. Experienced animal researchers and technical staff watch many hours of video recording during which time they record the specific behaviours of individual mice (such as climbing, feeding and drinking). Subsequent machine learning from the manual annotation data generates algorithms that are validated by using test data and performance analysis. This is represented by the data plot (bottom right), where the pattern of behaviours detected by human annotation is compared to those of the machine-learning algorithms. Where the data is non-comparable, further refinement of the algorithms ensues.

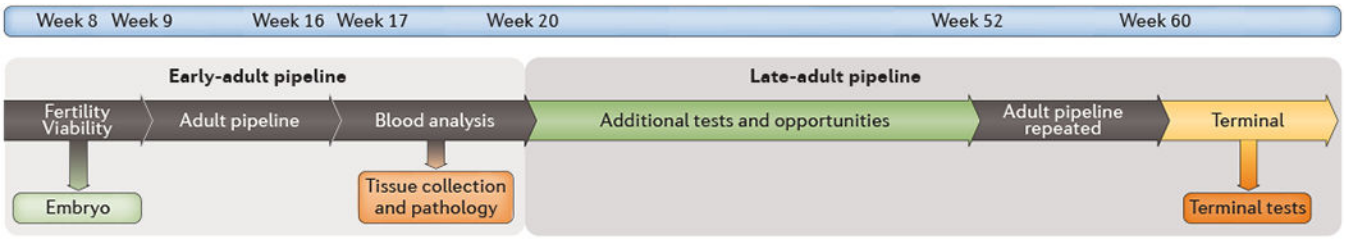

Fig. 4. Ageing as a new dimension of high-throughput mouse phenotyping.

There is increasing interest in the use of ageing pipelines to reveal novel phenotypes, particularly those that might model age-related disease. We illustrate a typical plan for an ageing phenotyping pipeline (mouse age: week 8 to week 60). Cohorts of mutant mice would as usual enter a phase of early-adult phenotyping, including where appropriate embryo analyses. At the end of early-adult phenotyping, some mice from the cohort may be removed from the pipeline for terminal assays. The remaining cohort proceeds to ageing, and subsequently, at around one year or older, adult phenotyping is repeated (late-adult phenotyping) followed by terminal tests. The intervening period between early and late adult phenotyping provides a window for additional phenotyping tests that might not be part of the standard adult phenotyping pipeline.

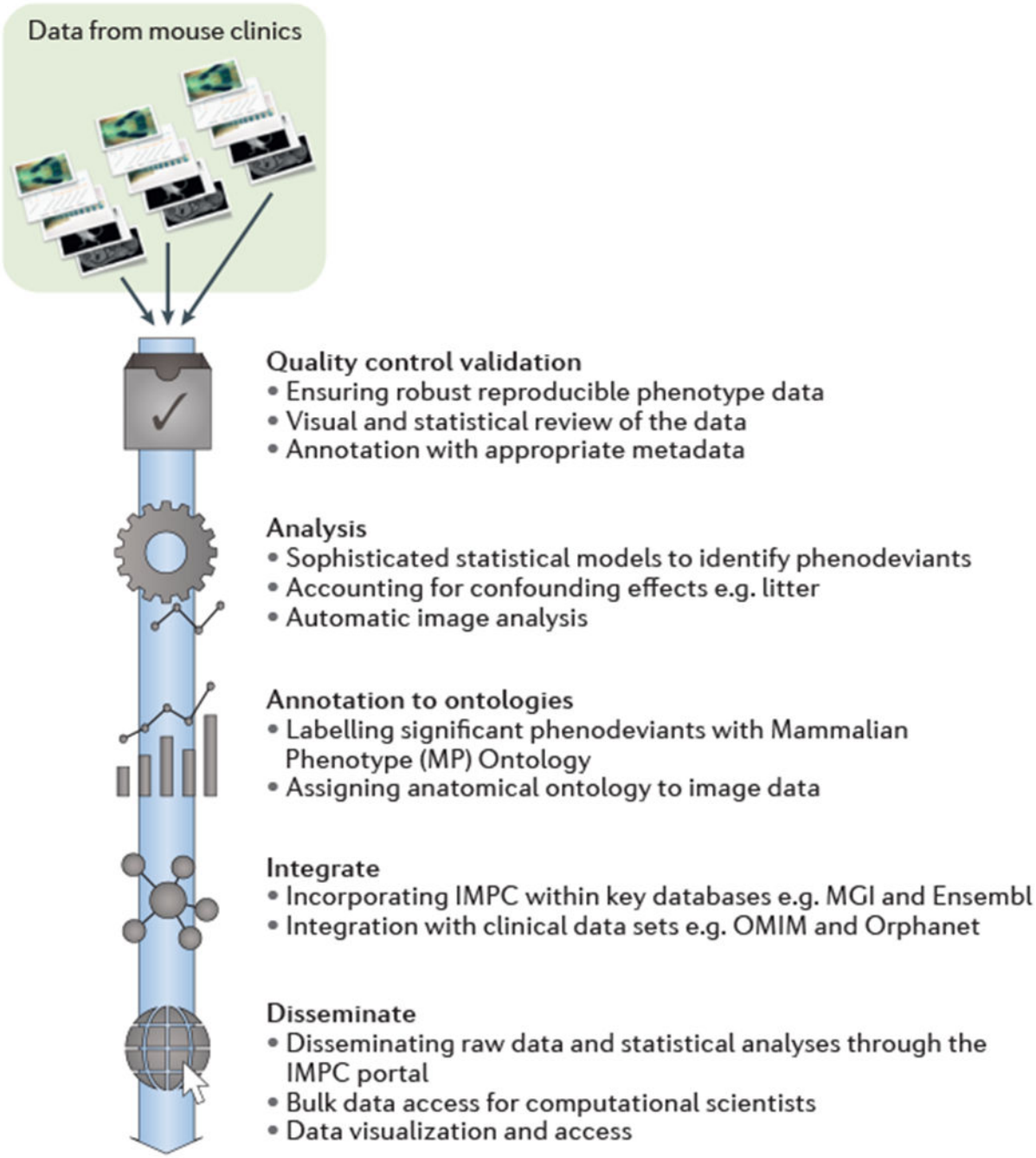

Fig. 5. Overview of data flow for large-scale, broad-based mouse phenotyping programmes.

Mouse clinics acquire diverse multi-dimensional datasets, including categorical and continuous data alongside a variety of image data. Data is uploaded routinely to a data coordination centre where it undergoes processing through a standardized pipeline including data validation, robust quality control, statistical analysis, annotation and data integration followed by dissemination to the scientific community.

MGI, Mouse Genome Informatics

OMIM, Online Mendelian Inheritance in Man

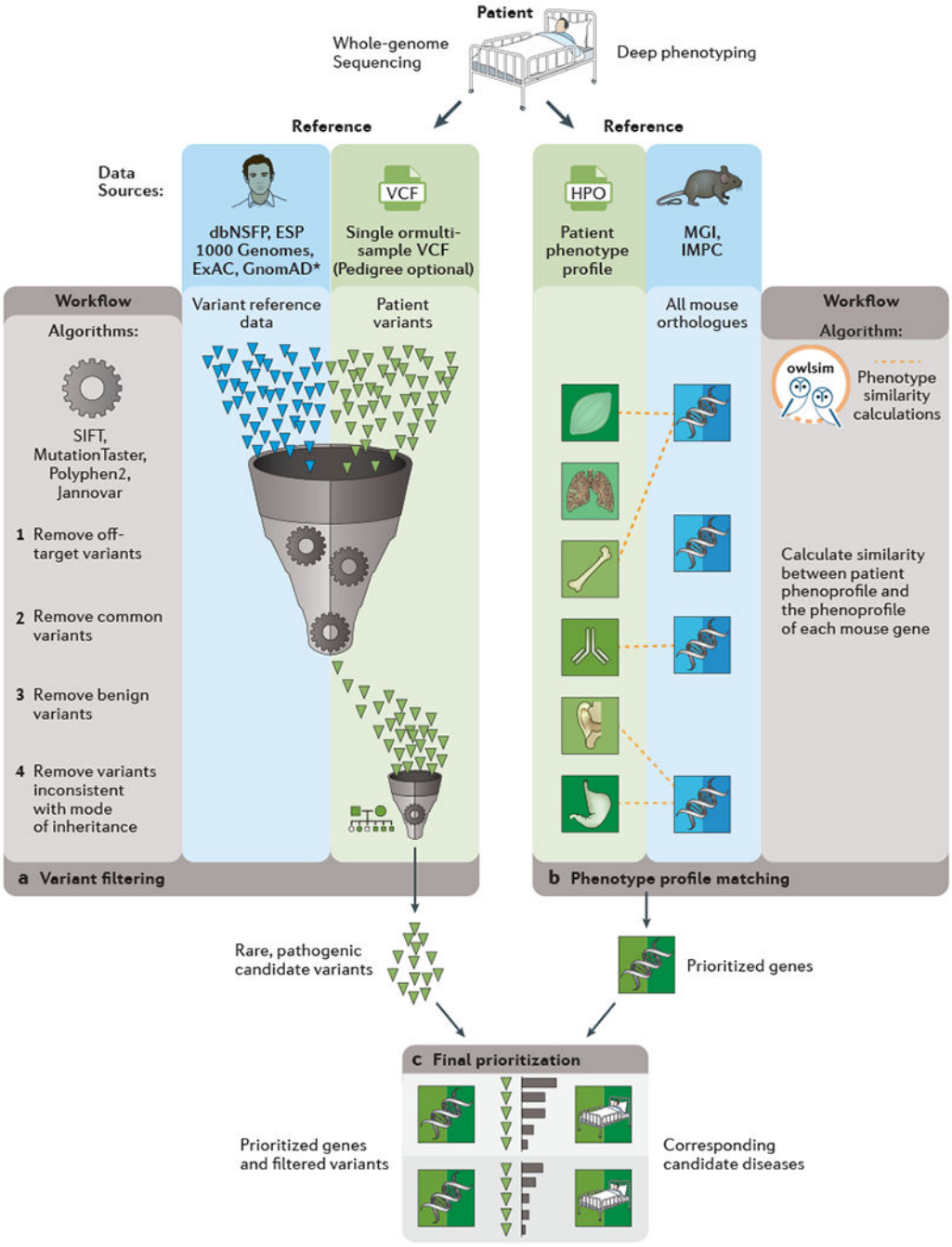

Fig. 6. Integration of human and mouse data for rare disease genetics.

The figure exemplifies the data sources and algorithms available from the Monarch Initiative portal and variant prioritization software suite (Exomiser). Candidate, rare, pathogenic variants from patient genomes are identified by comparison against reference variant sources such as the Exome Aggregation Consortium (ExAC) to determine the population frequency of variants, and the use of algorithms such as Jannovar and Polyphen2 for predicting which variants are likely to have deleterious, potentially pathogenic, effects. Candidate genes are identified by semantic comparisons of the patient's phenotypic profile against reference

genotype-to-phenotype datasets for human disease as well as model organisms as produced by phenomics programmes, such as the International Mouse Phenotyping Consortium (IMPC). The final set of prioritized, rare pathogenic variants in genes with functional evidence from the phenotype comparisons are presented back to the clinician for a final diagnostic decision

dbNSFP, database of nonsynonymous SNPs and their functional predictions

ESP, Exome Sequencing Project;

GnomAD, Genome Aggregation Database;

1000g, 1000 Genomes Project;

HPO, Human Phenotype Ontology;

MGI, Mouse Genome Informatics;

SIFT, Sorts Intolerant From Tolerant database;

VCF, variant call format.

Table 1.

Major high-throughput knockout mutagenesis and phenotyping programmes and consortia.

Programme	Date	Aims	Mutant allele	Refs
International Knockout Mouse Consortium (IKMC)[a]	2003–2015	Generate mutations in embryonic stem cells for every mouse gene	1. Gene trap alleles 2. Knock-out first, conditional ready tm1a[b] alleles 3. KOMP Regeneron alleles[c]	56,57
European Union Mouse Genetics Research for Public Health and Industrial Applications (EUMORPHIA)	2002–2006	Develop and test a robust set of phenotyping SOPs, reproducible across multiple centres (EMPReSS)	Employed a panel of standard inbred strains to demonstrate between-centre reproducibility	54
European Mouse Disease Clinic (EUMODIC)	2007–2012	Multi-centre programme generating and analysing hundreds of mouse mutant lines employing standardized EUMORPHIA procedures	Used tm1a[b] alleles available from IKMC	11
Mouse Genetics Programme, Sanger Institute (MGP)	2007–present	Analysed hundreds of mutant lines employing standardized EUMORPHIA procedures and other SOPs	Used both tm1a[b], tm1b[b] alleles and KOMP Regeneron[c] alleles	10
International Mouse Phenotyping Consortium (IMPC)	2011–present	Analysed thousands of mutant lines employing standardized IMPReSS procedures	Used tm1b[b], KOMP Regeneron[c] alleles as well as CRISPR–Cas9 critical exon deletion alleles	12

[a]The IKMC incorporated a number of programmes including European Conditional Mouse Mutagenesis Program (EUCOMM), the Knockout Mouse Project (KOMP), the North American Conditional Mouse Mutagenesis Project (NorCOMM) and the Texas A&M Institute for Genomic Medicine (TIGM).

[b]The IKMC most commonly produced targeted mutation is the tm1a knock-out first, conditional ready allele used by several programmes. The tm1a allele can be converted by Cre excision into a tm1b null mutant allele. Both the tm1a and tm1b alleles incorporate a lacZ reporter that enables determination of expression patterns of the disrupted gene. However, for the current CRISPR–Cas9 critical exon deletion alleles generated by IMPC a lacZ reporter is not incorporated.

[c]As part of the IKMC programme a number of targeted alleles were produced using large bacterial artificial chromosome (BAC) targeting vectors leading to the ablation of the entire gene, so-called KOMP Regeneron alleles. These were used in various programmes, but were usually a small minority of alleles. EMPReSS, European Mouse Phenotyping Resource of Standardised Screens[54]; IMPReSS, International Mouse Phenotyping Resource of Standardised Screens (http://www.mousephenotype.org/impress). SOPs, standard operating procedures.