Trends in Genetics

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REVIEWS

Review

Mouse Systems Genetics as a Prelude to Precision Medicine

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Mouse models have been instrumental in understanding human disease biology and proposing possible new treatments. The precise control of the environment and genetic composition of mice allows more rigorous observations, but limits the generalizability and translatability of the results into human applications. In the era of precision medicine, strategies using mouse models have to be revisited to effectively emulate human populations. Systems genetics is one promising paradigm that may promote the transition to novel precision medicine strategies. Here, we review the state-of-the-art resources and discuss how mouse systems genetics helps to understand human diseases and to advance the development of precision medicine, with an emphasis on the existing resources and strategies.

Highlights

The mouse is the premier model organism for human biomedical research.

So far, most of the research studies involving mouse models rely on a single or few genetic backgrounds and controlled external factors that limit the generalizability of the results.

Systems genetics improves the translational potential of mouse studies in human.

Systems genetics approaches in mouse panels could serve as the prototype and provide valuable insights for human precision medicine.

Promises and Problems with Precision Medicine

Most complex traits and diseases, such as height, longevity, and diabetes, are heritable and influenced by various genetic factors [1], while being modulated by environmental stimuli. Due every individual's unique genetic makeup, response to drugs [2], nutrition [3], and lifestyle [4] vary considerably from person to person. This uniqueness of every human being underpins the purpose of precision medicine, which posits that disease prediction, diagnosis, and treatment for each individual is based on personal genomic variations and external environments [5]. Precision medicine is an innovative approach that takes the variability in genetics, environment, and lifestyle of each individual into account in disease prevention and treatment, and provides better prediction of effective treatments, while concurrently minimizing the possibility of drug side effects [6]. Therefore, precision medicine requires a good understanding of the genetic bases of variation in phenotypes and their interaction with the environment in health and disease.

Despite the high expectations, there are several concerns with the implementation of precision medicine [7,8]. To date, the concept of precision medicine has been successful in the context of cancer, for example, the use of trastuzumab for breast cancer that is HER2 receptor positive [9], as well as for rare diseases, for example, the use of ivacaftor for cystic fibrosis patients with mutations in the *CFTR* gene [10,11]. One illustrative case for a precision medicine approach in the setting of rare disease is a female individual who was diagnosed with hereditary spastic paraplegia, but all the medical evaluations had been unsuccessful. Whole exome sequencing revealed a mutation in the *GCH1* gene, which was reported to be causal for a dopa-responsive dystonia. The mutation suggested that she might respond to levodopa and the patient noticed improvements after a few days of such treatment [12].

There are still doubts that precision medicine can achieve its full potential in complex diseases [13,14]. This could be partially explained by the fact that complex diseases are influenced by the combination of genetic variants and environmental factors, whereas most rare diseases are caused by a single genetic mutation. This complexity also contributes to the difficulty of generalizing findings from human groups to individuals. Some argue that the lack of group-to-individual generalizability of the statistical measures is a threat to human subject research [15,16].

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Several recent studies have proposed advanced statistical methods to accurately predict complex traits or disease risks (or polygenic risk scores) based on genetic variants [17–20], especially when traits show a high **heritability** (see Glossary) (e.g., height) [21]. However, confounding factors, including demographic, environmental, and other factors, such as sex and age, limit the portability of the prediction within and across human populations [22–24], emphasizing the importance of the external variables in such prediction.

For practical and ethical issues, model organisms have been used as simplified models for humans to study the genetic, molecular, and physiological basis of complex traits and to find therapeutic targets for human diseases. Mice have been the most studied animal models for many reasons, including their similarity in genetics, anatomy, and physiology to humans and the possibility of controlling the environmental factors (Box 1). In recent years, more and more **systems genetics** studies have been performed on mouse populations and have shown that mice from different genetic backgrounds exhibit distinct phenotypic responses, corroborating the principles that form the basis of precision medicine. Previously, many genetic determinants of complex traits have been identified using mouse populations and verified in human cohorts [25,26]. We review here the recent developments identified in mouse systems genetics studies on complex traits and diseases, summarize the existing resources and strategies, and discuss how they may help with the implementation of personalized and precision medicine approaches.

The Essentiality of Mouse Studies in Human Precision Medicine

For many decades, research studies using model organisms have been conducted to guide our understanding of biological processes, with the mouse being one of the most extensively used models. In 2011, 61% of all the animals used for experimental and other scientific purposes in the EU were mice (http://eara.eu/en/animal-research/animal-research-statistics-europe/#eu-statistical-report). A number of major breakthroughs in biomedical research and a large fraction of the current therapies were developed with the help of animal models, especially mice. In fact, 94 of the 106 Nobel Prizes in physiology or medicine were awarded to research using animal models (see the complete list at www.animalresearch.info/en/medical-advances/nobel-prizes/). One notable example is the discovery of cancer immunotherapy by James P. Allison and Tasuku Honjo, who received the Nobel Prize in 2018 [27]. Allison and Honjo's work would not have been possible without the extensive use of mouse tumor and immune deficiency models to uncover the immune cell mechanism of action [28,29]. Recently, however, there have been increasing doubts about the translational poten-

Box 1. Advantages of Using Mouse Systems Genetics to Study Human Diseases

1. Mice are similar to humans in many aspects, including genetics, anatomy, and physiology. The pathophysiology of disease in mice is also similar to that in humans.
2. The genomes of many commonly used mouse strains have been sequenced and there are developed tools to manipulate the mouse genome and record their phenotypes.
3. There are well-established mouse models for many diseases, as well as genetic reference panels for systems genetics studies.
4. Mice are cost effective due to their relatively short lifespan (2–3 years) and generation time and are easy to handle and breed.
5. The external environment of mouse models can be well controlled and monitored, which also facilitates the study of gene–environment interactions.
6. Studies using inbred mice allow resampling isogenic individuals to replicate the same experiment or perform multiple experiments to better estimate the influence of genetics and environment on phenotypes.
7. Researchers have access to all tissues in mice, especially those highly relevant in diseases, including deep tissues, which is impossible in most human studies because of ethical issues.
8. Mouse models can be used to capture the disease progression stages in longitudinal studies.
9. Mouse genetic populations are able to model the genetic diversity of human populations and require fewer individuals for genetic association analyses.
10. Unlike human genetic studies where data should always be kept highly confidential, data from mouse studies can be made publicly available to facilitate its reanalysis to the fullest extent.

Glossary

Forward genetics: a series of unbiased genetics approaches identifying the genes responsible for a phenotypic trait.
Genetic reference population (GRP): a panel of genetically diverse inbred strains with available genotype data that can be easily reproduced and extensively phenotyped.
Genome-wide association study (GWAS): a forward genetics approach to associate genetic variants with traits or diseases.
Heritability: the proportion of phenotypic variance in a population attributable to genetic variance.
High-impact variants: genetic variants that have strong impacts on the gene function, for example, missense, nonsense, and frameshift variants.
Hybrid mouse diversity panel (HMDP): a mouse panel consists of a set of inbred strains for the genetic diversity and mapping resolution, as well as many recombinant inbred strains for the mapping power.
Multi-omics: the integration of different omics datasets, for example, genomics, transcriptomics, proteomics, metabolomics, etc.
Quantitative trait locus (QTL): the genetic locus that correlates with a quantitative trait.
Reverse genetics: genetics approaches studying the function of a gene through either experimental or computational methods.
Systems genetics: an approach to understand the basis of complex traits and diseases through the integration of data from different omics layers.

tial of findings in mouse models [30,31]. In another review, we argued against this opinion and demonstrated, through evidence, the contributions of mouse studies in human drug discovery and in the general understanding of human biology [32] (Box 1).

Some of the successes of mouse studies for medical applications rely simply on access to intermediate phenotypes, as collected through transcriptomic, proteomic, and metabolomic studies of deep tissues. For instance, access to both serum and liver enabled a deep molecular dissection of lysine metabolism in the BXD mouse **genetic reference population (GRP)**, which, through mRNA expression quantitative trait locus (eQTL), protein quantitative trait locus (pQTL), and metabolite quantitative trait locus (mQTL) mapping, unequivocally established that *Dhtkd1* controls the levels of 2-aminoadipate (2-AA) and, as such, influences the onset of diabetes [33]. Earlier human studies established 2-AA as a biomarker of diabetes risk, but failed to causally link this observation to DHTKD1 itself in the absence of eQTL data [34].

In human studies, it is furthermore difficult and even impossible to assess critical environmental factors influencing disease development, therefore limiting the ability to study the underlying genetic determinants of complex traits and diseases, as well as the gene–environment interactions (GxE) [35]. By contrast, the environment of mice can be well controlled and modulated. Key examples are the identification of the complex risk factors contributing to the development of Alzheimer's disease (AD) and Parkinson's disease [36], or the GxE effects favoring the onset of asthma [37]. Another case in point is the influence of genetic and dietary effects on body weight; the heritability of body weight is over 70% in the BXD mouse GRP fed with either normal diet or a high-fat diet, but the heritability drops to around 30% when combining individuals fed with different diets, highlighting the importance of controlling external factors [38]. Such a dissection of GxE factors is nearly impossible for human studies, where confounders, such as diet and environment, cannot be standardized.

Another application of mouse models is to examine the influence of genome in the response to nutrients or drugs in so-called nutri- and pharmaco-genomic studies. One such example is the study of the beneficial effects of calorie restriction (CR) on lifespan using mouse individuals with different genetic backgrounds. Although CR has been considered as one of the most robust life-extending interventions, studies from mouse cohorts showed that CR was not universally beneficial for all individuals [39]. Indeed, the majority of mouse strains exhibited no lifespan extension after CR, while 1/4 of the strains even showed a reduced lifespan with CR [39]. The genes underlying the response to CR still remain to be identified. Acetaminophen overdose is the leading cause of acute liver injury in humans; however, the genetic basis of the interindividual differences in susceptibility to acetaminophen hepatotoxicity are not well understood [40]. A genome-wide association analysis, using a panel of 36 inbred mouse strains, identified CD44 as the candidate gene for acetaminophen-induced liver injury, which was later confirmed in humans [41]. Similar approaches have also been conducted to identify the genomic loci that influence hematotoxicity induced by chemotherapy drugs, such as doxorubicin, cyclophosphamide, or docetaxel [42]. Such translational mouse studies highlight the importance of genetics for precision medicine and suggest that it is worth taking the personal genetic background of patients into consideration.

One major criticism against mouse models is that results from mouse experiments do not always reflect human diseases. For example, there is no single model that recapitulates the pathophysiological and molecular aspects of nonalcoholic steatohepatitis [43], making expectations of the translatability of certain mouse studies unrealistic. However, the process of finding novel and refining existing mouse models is an ongoing iterative process [44–46]. For instance, new mouse models have been recently proposed for the most common form of heart failure in humans [47].

In addition, the emergence of new technologies such as CRISPR/Cas9 could unlock novel and more refined mouse models [48]. Furthermore, most mouse models are built on inbred strains with a fixed genome, while individuals with different genetic backgrounds may behave and progress differently under disease conditions [49,50]. Hence, we argue that many of the shortcomings of existing mouse models can be attributed to the extreme standardization of mouse experiments, where most research is performed in mice from one or a few genetic backgrounds, usually inbred strains. Laboratory mice housed in well-controlled and 'hygienic' cages do not experience the dynamic real-life environment as wild mice or humans [51]; laboratory mice, acquiring the natural microbes of wild mice, however, model well the immune responses in humans [51,52], highlighting the importance of microbial exposure and environmental challenges of laboratory animals in biomedical research.

Use the Correct Models in Mouse Studies

The choice of genetic background in biomedical research is a crucial but often neglected step. However, increasing evidence shows that individuals with different genetic backgrounds may behave and progress differently in disease conditions and can even react in opposite directions to external stimuli and treatments [49,50]. The response to morphine or cocaine [53], body weight gain upon high-fat diet [38], and lifespan changes after caloric restriction [39] are just a few examples. C57BL/6J is the most extensively used mouse strain in biomedical research [54]; however, many of the findings from C57BL/6J are not even generalizable to its substrains, like C57BL/6N, which has only 51 coding variants different from C57BL/6J [55,56]. From a genomic standpoint, C57BL/6J carries the minor alleles for 19% of the **high-impact variants** among the 30 sequenced inbred mouse strains [57,58] (Figure 1A), demonstrating that studies focusing on

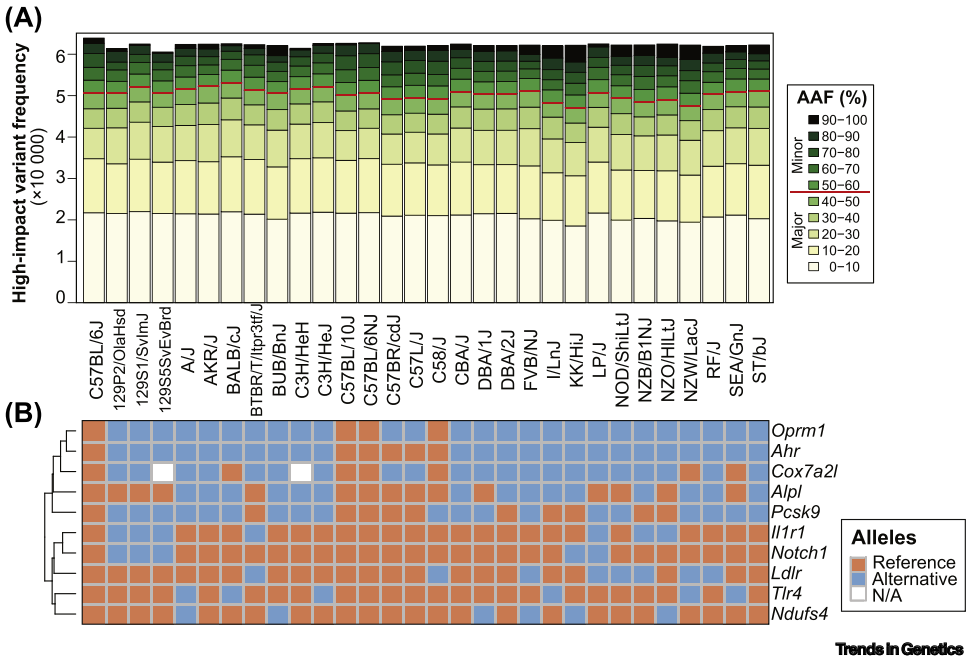

Figure 1. Genetic Difference across Inbred Mouse Strains. (A) The alternative allele frequency (AAF) of the high-impact variants across 30 inbred mouse strains whose genome was sequenced. Data were downloaded from the Mouse Genomes Project (www.sanger.ac.uk/sanger/Mouse_SnpViewer/). Wild-derived strains were removed from the analysis. The variation consequences were predicted with the Variant Effect Predictor. High-impact genetic variants were counted based on their AAF in these mouse strains and separated into ten bins. The proportions of the genetic variants possessing the minor alleles in respective strains were indicated by the red line. (B) The genetic diversity of high-impact variants for a set of genes that are crucial in physiology and diseases across 30 inbred strains. The alleles of C57BL/6J were used as the reference allele and other alleles were indicated as alternative alleles.

genes with these variants using C57BL/6J might not be well translated to most of the other strains. Furthermore, other mouse strains possess the minor allele in similar numbers of high-impact variants (Figure 1A), implying that choosing one strain over the others may lead to serious biases due to these naturally occurring variants. This would be the equivalent to studying diseases using a single human individual and then extrapolating results to the entire population.

In addition, high-impact genetic mutations in some strains lead to the disruption of genes crucial in certain biological processes (Figure 1B), therefore more attention should be paid when planning animal experiments. For example, C57BL/6J is known to carry a large deletion in the *Nnt* gene, which associates with impaired insulin secretion and glucose tolerance [56]. Some strains, including the widely used C57BL/6J and BALB/cJ strains, have a 6-bp deletion of *Cox7a2l* causing a two amino acid truncation and its inactivation, disrupting the formation of mitochondrial supercomplexes [38,59]. DBA/2J possesses coding and noncoding variants in *Oprm1*, a primary opioid receptor, and therefore exhibits weaker morphine preference and response compared with C57BL/6J [25,60]. Known mutations in genes that are relevant to the phenotype-of-interest must therefore be avoided in order to preclude unwanted biases. However, the genes crucial for traits and diseases are not always known. Therefore, mouse populations, instead of mice of a single genetic background, would serve as a natural starting point to find adequate models as well as to study the genetic basis of physiological traits and diseases. In this experimental setting, mouse strains with deleterious variants can serve as the counterpart model of human individuals with rare disease mutations to test their response to external challenges, for instance, relevant drugs.

Recently, several studies have been conducted to study the effects of disease-causing genetic mutations or environmental stimuli in different mouse strains and strong influence of genetic background on phenotypic responses was found (Figure 2). For instance, the phenotypic effects of *Cacna1c* and *Tcf7l2* mutations were evaluated in different genetic backgrounds by breeding heterozygous males to females from 30 inbred strains (Figure 2A) [49]. The phenotypic responses to these two mutations varied across different genetic backgrounds and in several cases there were even opposite effects [49], demonstrating that the genetic effects observed in animal models with a single genetic background are not generalizable to the whole population. A similar strategy was used to study the translatability of AD mouse models by crossing a heterozygous AD transgenic line with 28 genetically diverse BXD recombinant inbred strains (Figure 2A) [50]. Although most of the mice with transgenic alleles exhibited impaired cognitive function, the impact of transgene varied widely depending on the genetic background of the strains [50]. The translatability of AD mouse models was also tested by backcrossing AD animals to three wild-derived mouse strains; significant phenotypic variations in the neuropathological performance of the animals from different strains as well as genders was observed [61]. Similarly, over 100 inbred strains of mice from the **hybrid mouse diversity panel (HMDP)** were crossed with a strain with dyslipidemia-inducing mutations and the obtained F1 progeny were further exposed to a high-fat, high-cholesterol diet to promote atherosclerosis development [62]. Animals with different genetic backgrounds exhibited distinct susceptibility to atherosclerosis induced by hyperlipidemia, which is consistent with the results of human epidemiologic studies. Candidate genes underlying the atherosclerosis-related traits were then identified through association mapping and correlation analyses [62]. Likewise, a penetrant prostate cancer mouse model was crossed to the diversity outbred (DO) cohort and the obtained F1 males were used to study the effects of genetic variation on the susceptibility to prostate cancer [63]. Further integrative analyses identified several genes as aggressive prostate cancer modifiers, which were then validated in human [63]. The responses to four human-comparable mouse diets (American diet, Mediterranean, Japanese, and Maasai/ketogenic) were evaluated in four inbred mouse strains in an effort to find the best alternative to the American diet (Figure 2B) [64]. Of

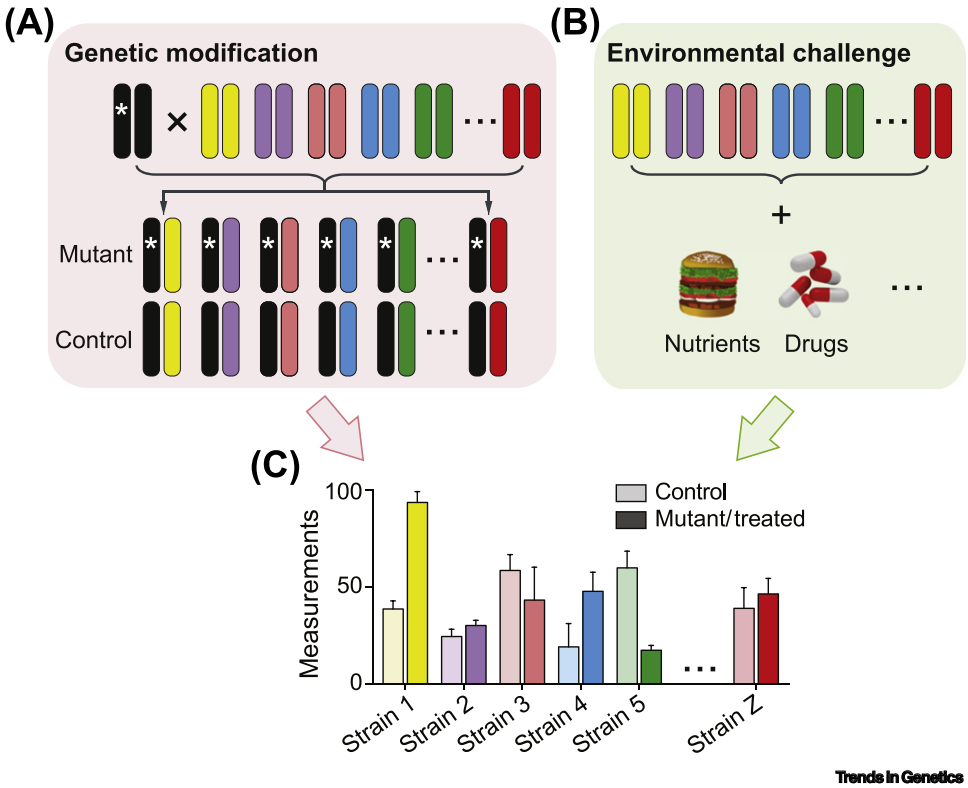

Figure 2. The Influence of Genetic Background on the Phenotypic Response to Genetic and Environmental Perturbations. (A) An inbred strain heterozygous for a disease-causing mutation is crossed to a panel of inbred strains to generate genetically diverse, but isogenic, F1 offspring. The progeny inheriting the mutation can be compared with their littermates to identify the influence of genetic background on disease pathogenesis. (B) The panel of inbred strains is challenged by either nutritional or pharmacological approaches to assess their respective response. (C) The response to genetic and environmental perturbations is highly affected by the genetic background of the strains.

note, the best diet was shown to be strain-dependent and it was proposed that health outcomes could be improved through a precision dietetics approach. Altogether, these studies highlight the importance of genetic diversity of animal models in biomedical research. Considering that most of the initial discoveries were made using mouse models of single genetic background, it is explicable that some findings from mouse studies were not well translated into humans [65].

Mouse studies have also played a critical role in drug target identification and validation, as well as in the preclinical *in vivo* evaluation of drug candidates. We summarized a drug research and development pipeline that highlights the use and essentiality of mouse studies in human precision medicine (Figure 3). In particular, we propose the use of mouse genetically diverse populations, instead of mice from single or limited genetic background, in the assessment of potential drugs. In this way, the toxicity and potency of these drug candidates can be fully evaluated on mouse individuals of different backgrounds. In addition, genes involved in the response or toxicity of the drugs can be identified using a pharmaco-genetics approach in mouse studies and in human clinical trials, which will allow the customization of drugs for patients based on their genetic makeups and environments (Figure 3).

Along with the use of animal models in identifying potential drug targets, a large number of animal experiments are performed to screen and evaluate the efficacy and safety of candidate drugs in preclinical animal studies. However, owing to the species difference in xenobiotic metabolism, the

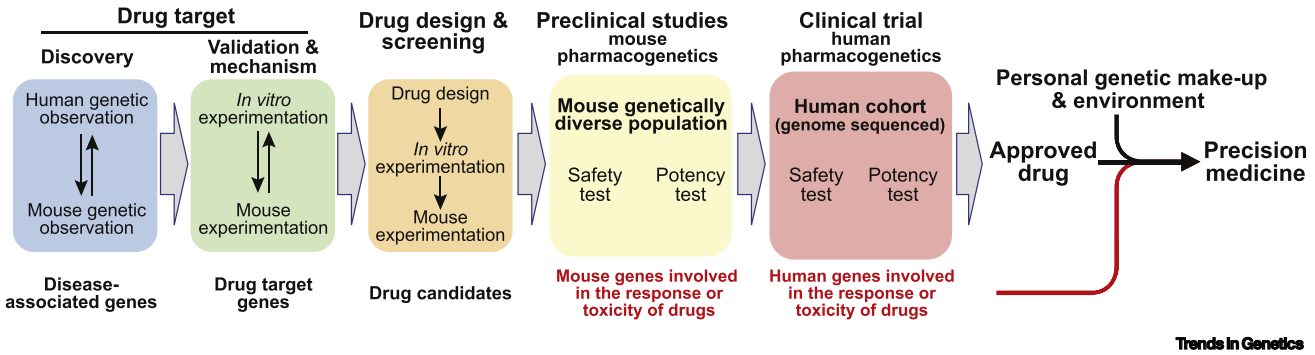

Figure 3. Drug Discovery and Development Pipeline with a Focus on the Reiteration between Genetics and Pharmacology and between Human and Mouse Studies as a Path towards Precision Medicine.

translation of pharmacokinetic and toxicological results from animals to human is not always straightforward. Genetically and chimeric humanized mouse models have now been developed to study the function of human drug-metabolizing enzymes and facilitate the assessment of the pharmacokinetic properties and toxicity to avoid the drug-induced liver injury in man [66,67].

Panels and Resources in Mouse Systems Genetics

For decades, research groups have generated various mouse GRPs, to study the genetic bases of phenotypic traits and diseases [68]. These mouse populations are often derived from several different parental strains that have distinct phenotypic performances. For example, the BXD cohort was derived from the C57BL/6J and DBA/2J strains that have different response to drugs and diet-induced obesity and thus this population is commonly used for neuropharmacological and metabolic research [69]; while the LXS cohort was generated from the inbred long-sleep (ILS) and inbred short-sleep (ISS) strains and is often used in neural and behavioral studies [70]. Unlike common cohorts originated from two inbred strains or individuals, the collaborative cross (CC) and DO cohorts more recently established advanced diversity panels that derived from eight parental strains through a community effort [71]. By including three wild-derived strains, the CC/DO founder strains capture nearly 90% (versus ~13% in BXDs) of the common genetic variations in *Mus musculus* strains [71,72], approximating human genetic diversity.

In general, mouse genetic panels can be divided into different categories depending on the breeding strategies, including inbred, F1 hybrids, F2 hybrids, outbred, heterogeneous, recombinant inbred, recombinant inbred intercross, recombinant inbred backcross, congenic, consomic, and conplastic strains (Figure 4 and Table 1). Different mouse cohorts have different genetic origins, availability, and usability [73], therefore attention should be paid when considering the cohort for specific experimental settings and research questions. Hybrid diversity panels, for example, the HMDP, rely on the available strains and combine inbred strains to increase mapping resolution and recombinant inbred strains for the mapping power [74,75].

Despite the advantages of mouse systems genetics, the cost and resources for such studies needed remain one of the major obstacles, especially for research labs with limited budget. However, researchers can benefit from existing data of previous systems genetics studies to generate and verify research hypotheses in their projects. With the development of high-throughput molecular technologies, the collection of omics data has become routine, especially in systems genetics studies using mouse genetic populations. Contributed by research groups around the world, large-scale omics data have been collected, ranging from epigenomics [76,77],

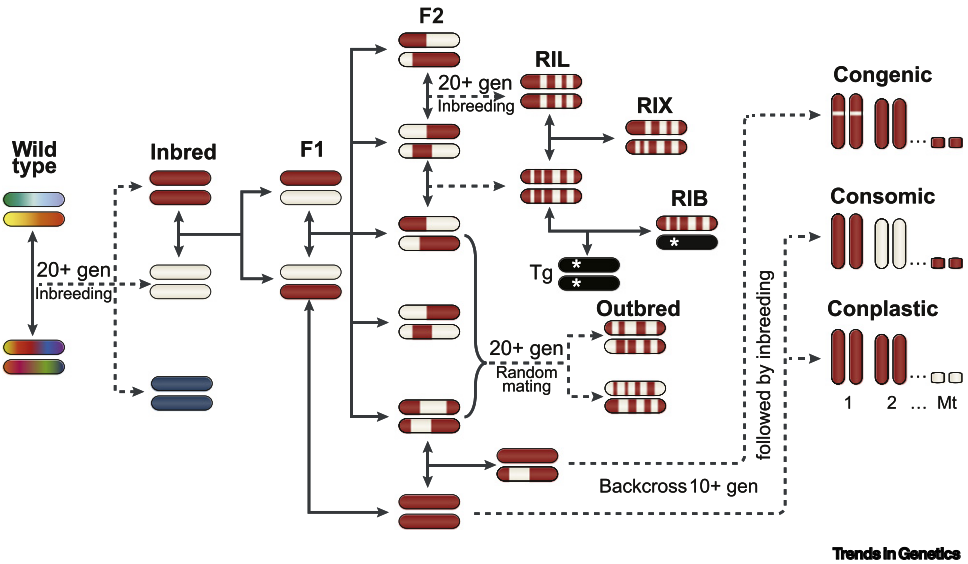

Figure 4. Breeding Scheme of Mouse Genetic Reference Populations. A brief breeding scheme summarizing the common mouse population types (text in bold). Different colors represent the genotypes of the chromosomes. The scheme mainly focuses on cohorts derived from two parental strains; multiparental populations employ similar but more complex breeding strategies [71]. Inbred strains are derived from at least 20 generations of brother–sister mating of wild type mice. Individuals of an inbred strain are considered as isogenic. F1 hybrids are generated by crossing mice of two different inbred strains, and F2 hybrids are produced by crossing F1 mice. Recombinant inbred lines (RILs) are derived from long-term inbreeding (usually over 20 generations) of F2 progenies. Recombinant inbred intercrosses (RIXs) are established by crossing mice from different RILs, while recombinant inbred backcrosses (RIBs) are produced by creating F1 hybrids from a transgenic strain (Tg) and RILs. Outbred mice can be generated through random mating of F2 progenies. The congenic strain is an inbred strain with a chromosomal segment substituted by the corresponding segment of another strain. Special types of congenic strains include consomic and conplastic strains, where a whole chromosome or the mitochondria are substituted by that of another strain. Consomic strains are also called chromosome substitution strains. Figure adapted from [68].

transcriptomics (data from the BXD and HMDP cohorts were partially summarized in [25,75]), proteomics [33,38,78], lipidomics [79–81], metabolomics [38,82], microbiome [83–85], as well as phenomics [25,26]. Mouse systems genetics studies often are unbiased towards the gene targets, therefore data from such studies can be reused to analyze any gene of interest for different research groups. There are various resources that provide access to the mouse systems genetics datasets (Table 1), as well as the systems approaches, including GeneNetwork (www.genenetwork.org), the Mouse Phenome Database (https://phenome.jax.org/), the Systems Genetics Resource (https://systems.genetics.ucla.edu/), the Attie Lab Diabetes Database (http://diabetes.wisc.edu/), the Diversity Outbred Database (www.jax.org/research-and-faculty/genetic-diversity-initiative/tools-data/diversity-outbred-database), the Swiss-BXD web interface (https://bxd.vital-it.ch), and Systems-Genetics.org (www.systems-genetics.org/). These systems genetics resources enable the possibility of reusing historically collected data to identify novel biological insights, such as those done previously [25,26,86,87].

Systems Genetics Approaches to Analyze Multi-Omics Data

The accumulating **multi-omics** datasets from mouse systems genetics studies provide valuable resources, which can form the foundation of systems genetics approaches to discover novel biological findings. Here, we summarized the commonly used, as well as newly described systems approaches analyzing these multi-omics datasets.

Table 1. The Commonly Used Mouse Genetic Cohorts and Data Sources[a]

Cohort type	Example cohort name	Parental strains	Data source	Refs
Inbred	–	–	https://phenome.jax.org/panels	[90]
Outbred	CFW	–	https://wp.cs.ucl.ac.uk/outbredmice/ https://doi.org/10.5061/dryad.2rs41	[108,109]
	DO	A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ	https://phenome.jax.org/panels/DO%20population www.jax.org/research-and-faculty/genetic-diversity-initiativetools-data/diversity-outbred-database	[78,88,99]
	AIL	LG/J x SM/J	https://palmerlab.org/protocols-data/	[110]
Heterogeneous	HS	A/J, AKR/J, BALBc/J, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J, LP/J	https://wp.cs.ucl.ac.uk/outbredmice/ heterogeneous-stock-mice/	[111]
	ITP	BALB/cByJ, C57BL/6J, C3H/HeJ, DBA/2J	https://phenome.jax.org/projects/ITP1	[107]
Recombinant inbred	BXD	C57BL/6J, DBA/2J	http://www.genenetwork.org	[33,38,86]
	LXS	ILS, ISS	http://www.genenetwork.org	[70]
	CC	A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ	https://phenome.jax.org/panels/CC	[71,72]
Hybrid diversity panel	HMDP	C57BL/6J, DBA/2J, A/J	https://systems.genetics.ucla.edu/data/hmdp	[75,81,87]
F1 hybrids	–	Two inbred strains	–	
F2 hybrids	B6BTBRF2	C57BL/6J, BTBR T^{+}tf/J	http://diabetes.wisc.edu/	[112,113]
	CASTB6F2	C57BL/6J, CAST/EiJ	https://systems.genetics.ucla.edu/data/B6_CAST	[114]
	BHF2	C57BL/6J, C3H/HeJ	https://systems.genetics.ucla.edu/data/C3H_B6	[114]
RIX	CC-RIX	A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ	–	[115,116]
RIB	AD-BXD	5XFAD, BXDs	–	[50]
	Ath-HMDP	CETP, ApoE3-Leiden, HMDP	https://systems.genetics.ucla.edu/data/ hmdp_apoe_leiden	[62]
Congenic	–	Two inbred strains	–	
Consomic	–	Two inbred strains	–	[117]
Conplastic	–	Two inbred strains	–	

[a]Abbreviations: AIL, Advanced intercross line; B6BTBRF2, F2 hybrids by crossing C57BL/6J (B6) with BTBR T^{+}tf/J (BTBR); BHF2, F2 hybrids by crossing C57BL/6J (B) with C3H/HeJ (H); BXD, recombinant inbred cohort by crossing C57BL/6J (B) with DBA/2J (D); CASTB6F2, F2 hybrids by crossing CAST/EiJ (CAST) with C57BL/6J (B6); CC, collaborative cross; CFW, Carworth Farms Swiss Webster; DO, diversity outbred; HMDP, the hybrid mouse diversity panel; HS, heterogeneous stock; ITP, interventions testing program; RIX, recombinant inbred intercross; RIB, recombinant inbred backcross.

Genetics approaches connecting genes with phenotypes can be generally separated into **forward genetics** and **reverse genetics** approaches (Figure 5A).

Common forward genetics analyses include **quantitative trait locus (QTL)** mapping (linkage studies applied on related individuals) and **genome-wide association study (GWAS)** (association studies using a large number of related or unrelated individuals), which are widely used to map the genetic loci that correlate with a particular trait, ranging from phenotypes, metabolites, proteins, transcripts, to epigenetic markers [76,77]. In recent years, studies have been performed to study the genes involved in the various diseases and complex traits, including insulin secretion [88], diabetes [37], hepatic steatosis or fibrosis [43,44], blood pressure [89], and bone density [90].

Epigenetics, such as DNA methylation and histone modifications, affects complex traits through regulating gene expression and activity. The DNA methylation levels were, for instance,

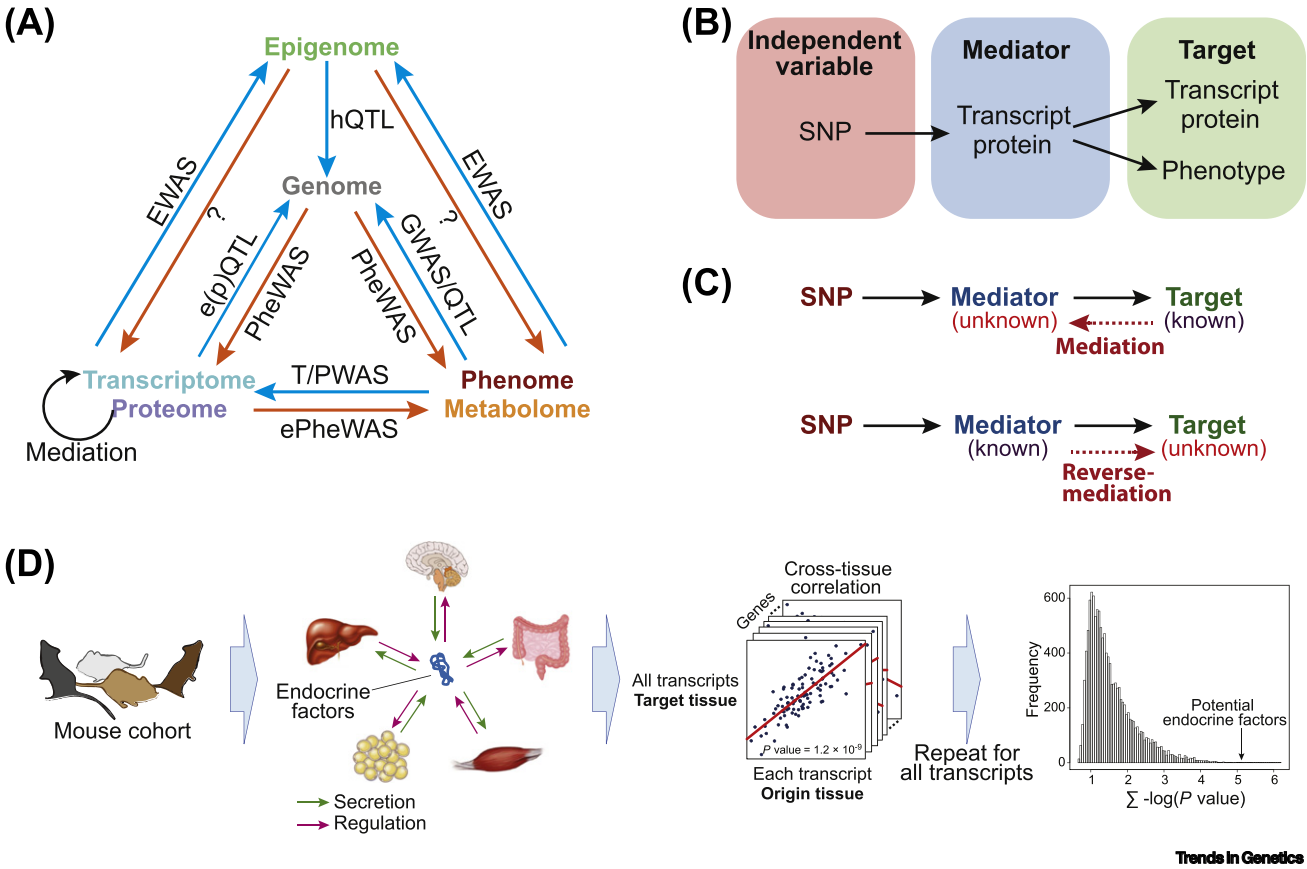

Figure 5. Systems Genetics Approaches. (A) A scheme of systems genetics approaches that can be applied to multi-omics data. Multi-omics datasets are divided into four categories: the genome, the epigenome, the transcriptome/proteome, and the phenome/metabolome. Arrows connecting different omics layers are colored blue if the respective approaches are forward genetics methods and orange if they are reverse-genetics methods. (B) Flow of the biological information. SNPs are independent variables that affect transcripts/protein or phenotypes (target) through influencing intermediate molecules (mediator), such as transcripts or proteins. (C) Mediation analysis starts with a known target and identifies the unknown mediator (upper), while reverse-mediation analysis starts from a known mediator to discover its downstream targets (lower). (D) Cross-tissue correlation to uncover endocrine factors. Expression datasets obtained from different tissues of the same mouse cohort can be used to identify the endocrine factors that regulate gene expression in other tissues. The *P* values of the correlation between the expression levels of each gene in the origin tissue and those of all genes in the target tissue are calculated and then aggregated after applying logarithm transformation. Genes with higher ∑ -log(*P* value) are potential endocrine factors. Abbreviations: ePheWAS, Expression-based PheWAS; eQTL, expression quantitative trait locus; EWAS, epigenome-wide association study; GWAS, genome-wide association study; hQTL, histone quantitative trait locus; PheWAS, phenome-wide association study; pQTL, protein quantitative trait locus; SNP, single nucleotide polymorphism; T/PWAS, transcriptome- or proteome-wide association study.

measured in the livers of 90 HMDP mouse strains, allowing epigenome-wide association studies to determine the association between variation in DNA methylation and complex phenotypic traits [77].

Phenome-wide association study (PheWAS), as a complementary approach to GWAS, examines the associations between one genetic variant and a large number of phenotypes (termed pleiotropy) [91], and could predict the possible side effects of targeting a specific gene or suggest potential candidate drugs for repositioning [92]. PheWAS was first used to analyze electronic health records in humans [93] and was later applied in mouse populations, particularly in the BXD cohort [25,26]. Genetic reference panels that are composed of inbred strains or recombinant inbred strains, which can be easily reproduced and extensively phenotyped, allow the accumulation of huge phenomic datasets and therefore are perfect sources for such reverse-genetics analyses.

Intermediate molecules, such as mRNA and protein, integrate the effects from genetic factors, including those poorly captured or hidden in common association studies [94], as well as effects from environmental factors. Several studies explored the use of these intermediate phenotypes and introduced the concept of transcriptome- or proteome-wide association study (T/PWAS), which suggest candidate genes by associating the phenotypic traits to the expression (transcript or protein) levels of the gene [95,96]. Conversely, a reverse approach [expression-based PheWAS (ePheWAS)] that identifies the associations between one gene and multiple phenotypic traits based on its gene expression has been proposed [25]. Different from common correlation analyses, T/PWAS and ePheWAS exploit mixed effect models to control for the population structure when exploring the connections between genes and phenotypes [97]. By applying the data from the BXDs, T/PWAS and ePheWAS uncovered a number of gene–phenotype associations, many of which were not recognized using genetic associations [25].

QTL mapping of traits in mouse cohorts often ends up with a genetic locus, composed of a list of candidate genes. Several studies proposed the use of mediation analysis to identify the causal gene (mediator) between the genetic variant (independent variable) and the trait of interest (dependent variable) (Figure 5B) [25,78,98,99]. Mediation analysis can be used either on gene expression levels to identify the regulatory mechanisms [25,78,99] or on phenotypic traits to discover the potential causal drivers contributing to the phenotypic variances [98] (Figure 5C upper). Contrary to mediation analysis, reverse-mediation analysis starts with the mediator (the gene with cis-QTL) and identifies its downstream targets [25] (Figure 5C lower).

Additional computational methods will surely emerge that exploit such huge datasets and a number of recent examples illustrate the power of such strategies. For instance, using transcriptome datasets obtained from different tissues of the HMDP cohort, a new strategy to identify important endocrine factors in the communication between tissues was developed (Figure 5D) [87]. Using expression datasets from large cohort studies, novel systems approaches, including the GeneBridge toolset (www.systems-genetics.org), have also been developed to identify the novel function of genes or new members of pathway modules [100]. A systems genetics approach, using expression data from mouse brain, identified CSF1R as a potential target for epilepsy and suggested CSF1R blockade as a novel therapeutic strategy [101]. Other studies applied gene network modeling algorithms to identify the potential regulators in complex diseases, for example cardiomyopathy [102], hepatic steatosis [103], as well as coronary artery disease [104].

Finally, there are many other integrative approaches available for the analysis of multi-omics data, but these have not yet been applied in mouse systems genetics studies. Examples include the transcriptome-wide association study (TWAS) that integrates GWAS with expression datasets from other independent cohorts to prioritize candidate gene for phenotypic traits. In addition, Mendelian randomization, which estimates the causal associations between a risk factor and diseases [105] or those between gene expression and complex traits [106], can be similarly applied in mouse genetic cohorts.

Outstanding Questions

What is the best approach to mimic human environments in mouse models?

How do we leverage multi-omics datasets to promote the understanding of the genetic architecture of diseases?

How do we translate the different responses to external stimuli, for example, drugs, of mice individuals from different genetic backgrounds to human?

How do we manage the data generated from mouse systems genetics studies in a traceable way to facilitate their reuse by the research community?

Concluding Remarks and Future Perspectives

Mouse models have long been used to study the basis of human diseases, to screen for potential drug targets, and to test the safety and efficiency of drugs in preclinical trials. We review here the recent advances applying systems genetics in mouse populations to understand the basis of complex traits and diseases. The resources of available archived datasets as well as the commonly used systems approaches are described. However, the infrastructures for the data

generation, storage, and integrative analyses in mouse systems genetics are not yet standardized and will require further work (see Outstanding Questions).

There are arguments that mouse models only poorly mimic human diseases and predict disease outcomes in human; we do not adhere to this opinion. It is clear that phenotypic traits as well as the response to disease-causing variants or environmental stimuli are strongly affected by the genetic background of the individuals. This exposes the disadvantages of the use of animals from a single genetic background and in a standardized environment in traditional animal studies. Accumulating evidence now argues for the use of genetically diverse mouse cohorts in assessing the effects of multiple external factors, such as done in the Interventions Testing Program studies, where the effects of various treatments on aging were tested in a large panel of genetically heterogeneous mice [107]. We hence propose here the concept of 'mouse precision medicine' and argue that it can serve as a better prototype for future mouse studies and as such provide valuable insights for human precision medicine.

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