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Genetically modified mice for research on human diseases: A triumph for Biotechnology or a work in progress?

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Abstract

Genetically modified mice are engineered as models for human diseases. These mouse models include inbred strains, mutants, gene knockouts, gene knockins, and 'humanized' mice. Each mouse model is engineered to mimic a specific disease based on a theory of the genetic basis of that disease. For example, to test the amyloid theory of Alzheimer's disease, mice with amyloid precursor protein genes are engineered, and to test the tau theory, mice with tau genes are engineered. This paper discusses the importance of mouse models in basic research, drug discovery, and translational research, and examines the question of how to define the "best" mouse model of a disease. The critiques of animal models and the caveats in translating the results from animal models to the treatment of human disease are discussed. Since many diseases are heritable, multigenic, age-related and experience-dependent, resulting from multiple gene-gene and gene-environment interactions, it will be essential to develop mouse models that reflect these genetic, epigenetic and environmental factors from a developmental perspective. Such models would provide further insight into disease emergence, progression and the ability to model two-hit and multi-hit theories of disease. The summary examines the biotechnology for creating genetically modified mice which reflect these factors and how they might be used to discover new treatments for complex human diseases such as cancers, neurodevelopmental and neurodegenerative diseases.

Keywords: Transgenic, mutant, gene knockout, gene knockin, humanized mice, mouse models, epigenetic, environmental, human diseases, two-hit theories, multi-hit theories

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Introduction

1. The problem of rare and incurable diseases

The aims of biomedical research are to discover the causes of diseases and to produce new treatments for these diseases. There are many diseases which are, at the moment, incurable. These include infectious and non-infectious diseases, neoplastic, autoimmune, genetic, and metabolic disorders. They also include rare diseases caused by inherited genetic abnormalities, terminal illnesses, and diseases that people live with all their lives, such as various types of cancer, auto-immune disorders, diabetes, some forms of heart disease, asthma, Alzheimer's and other forms of dementia, which cannot be "cured," but can be "managed" with the appropriate treatment. The list of currently incurable diseases (1) includes 100 different disorders, from allergic diseases (such as asthma) to Xeroderma pigmentosum (an inherited condition characterized by an extreme sensitivity to ultraviolet (UV) rays from sunlight). Rare diseases are those that affect fewer than 1 in 2000 people and the National Organization for Rare Disorders, Rare Disease Database [https://rarediseases.org] lists 1200 such diseases from Aarskog syndrome to Zollinger-Ellison Syndrome. One way to understand such diseases and find cures for them is through the study of animal models, many of which are genetically modified mice (2–8).

New mouse models for rare diseases are continuously being developed by the Jackson Laboratory Rare and Orphan Disease Centre (9,10) and the International Mouse Phenotyping Consortium (11). Mouse models have been developed for rare human skeletal

disorders (12), muscular dystrophies and congenital myopathies (13), rare metabolic, neuromuscular and opthalmological diseases (8), as well as rare neurodevelopmental (14), and neurodegenerative disorders (3,15). Mouse models are valuable for the study of the genetic mutations, molecular biology, and phenotypic expression of these rare human diseases (16,17).

Genetically modified mouse models of human diseases are bioengineered so that their genotype matches that of a human disease and their phenotype (morphology, biochemistry, physiology, or behaviour), is representative of the disease symptoms (see 18). The goal is to produce mouse models that are valid and reliable models of a human disease, with new mouse models continuously being created based on new knowledge about the genetic basis of each disease (11,19). The purpose of this paper is to examine the use of transgenic mouse models in the study of human diseases and the role of such models in discovering new treatments for these diseases. Some of the critiques of animal models are examined and examples given of new generations of animal models which have been developed to examine two-hit and multiple-hit theories of human diseases. While genetically modified mice have been a triumph for biotechnology, the new animal models of human diseases also incorporate epigenetic mechanisms that regulate heritable changes in gene expression that are not caused by changes in the DNA sequence, but in response to intrinsic cell signalling and extrinsic environmental factors.

2. The importance of animal models in the study of human diseases

A plethora of animal models have been developed to study human diseases, from fruit flies and zebrafish to mice, rats, dogs and pigs (20–25). These animal models are used in all branches of biomedical research, including studies on basic brain function (26); brain injury (27); cancer (5,28); immunology and inflammatory diseases such as multiple sclerosis (29,30); autoimmune disorders (31); and cardiovascular diseases such as hypertension and atherosclerosis (23,32). Animal models are also used to study neurodevelopmental disorders (33) including autism (34,35); ADHD (36,37); and schizophrenia (38), and for studying neurodegenerative disorders such as Alzheimer's disease (3,39–41); Huntington's disease (22,42–44); Amyotrophic lateral sclerosis (45), and Parkinson's disease (46,47). In addition, animal models are important for studies of pain mechanisms and disorders involving pain such as fibromyalgia (48,49). Finally, animal models are essential for understanding the developmental basis of disease and the environmental activation of diseases via epigenetic mechanisms (50–53).

The Covid-19 pandemic has focused the world on the need for animal models to study SARS-CoV-2 and related diseases (SARS-CoV and MERS-CoV) (54–57) as well as the need for animal models to study all rare and incurable diseases (58,59). The need for animal models for the study of Covid-19 infection is essential because Covid-19 affects many bodily systems, including the sensory systems: smell, taste and hearing (60–62); the brain and nervous system (63–65); the immune system (66,67); the endocrine system (68) and the interaction between the nervous, immune and endocrine systems (69). With such diverse effects and temporal variation between organs, whole-systems animal models, especially humanized mouse models, are required to study how Covid-19 causes such a wide range of symptoms (58,70–72).

3. Different types of mouse models of human disease

While all mice bred for research are derived from *Mus musculus*, each mouse model has a specific genotype which is reflected in the naming of each strain (73). This nomenclature was developed by the International Committee on Standardized Nomenclature for Mice and is listed on the Jax laboratory website (jax.org/nomenclature). Information on the genotypes of mouse models is provided by the Mouse Genome Database (MGD) and the mouse Gene Expression Database (GXD) from the JAX laboratory (http://www.informatics.jax.org; see 74–76). The Mouse Phenome Database (MPD; https://phenome.jax.org) maintains phenotypic data on mouse strains (77). This section describes some types of genetically modified mouse models. More complete information is given by the Guidelines for Nomenclature of Mouse and Rat Strains (Revised: September 2021) on the Mouse Genome Informatics (MGI) website http://www.informatics.jax.org/)

Outbred strains of mice are genetically variable mice that have been bred through mating unrelated individuals to maintain maximum heterozygosity. Some examples of outbred mouse strains are CD-1, HS4, ICR, NIH Swiss and SW mice (see 78). While many researchers prefer to use inbred mouse strains, some recommend the use of outbred strains (see 79–81) and others have developed heterogenous strains such as the Collaborative Cross and the Diversity Outbred mice by outbreeding eight strains of inbred mice (see 82).

Inbred strains of mice are derived from a single ancestral pair and mated brother to sister for 20 generations or more (DBA, C3H, A, C57BL,129, etc.). Genetic variability within an inbred strain is virtually nonexistent, and it is important to maintain the genetic stability of inbred strains from generation to generation as each inbred strain has a different genotype and phenotype (see 83,84). Substrains of an inbred strain are denoted with a slash (BALB/c, C57BL/6 or C57BL/10) and are mice that have been maintained separately from the parent colony for more than 20 generations of inbreeding or show genetic differences from the parent colony. For example, DBA/1J, DBA/1LacJ, and DBA/2J are substrains of the DBA inbred strain. The substrains of an inbred strain can differ greatly in their phenotypes, as has been demonstrated for the 129-mouse strain (85,86). For instance, although the C57BL/6 mouse is the most popular strain for research, there are many substrains of this mouse, each with its own phenotype, thus it is essential to know which substrain of C57BL/6 mice you are using, and which has been used as the background strain for genetically modified mice. For example, the C57BL/6N mice and their substrains all have the retinal degeneration 8 (rd8) mutation which affects their visual ability, while the C57BL/6J substrains do not have this mutation

(87). Conversely, C57BL/6J mice and their substrains all have a nicotinamide nucleotide transhydrogenase (Nnt) mutation which effects their β-cell mitochondrial glucose metabolism, while the C57BL/6N substrains do not have this mutation. Such background strain differences may confound the neurobiological and behavioural results of a single gene knockout mice model (88). The phenotypic characteristics of commonly used inbred mouse strains and substrains are discussed by Tam and Cheung (89).

Hybrid mice are derived from two inbred strains are labeled with the maternal strain first. Thus D2B6F1 are mice which are the first generation (F1) offspring of DBA/2 mothers and C57BL/6 fathers (DBA/2J x C57BL/6J)F1. B6129SF1/J are the F1 generation from C57BL/6J mothers and 129S fathers. Congenic strains are produced by repeated backcrosses to an inbred (background) strain, with selection for a specific marker from the donor strain. Thus a congenic strain is an inbred strain that contains a small genetic region (ideally a single gene) from another strain, but which is otherwise identical to the original inbred strain. For example, B6.AKR-H2k is a mouse strain with a C57BL/ 6J (B6) genetic background, which differs from that strain by the introduction of the H2k allele from the AKR/J strain (90). Recombinant inbred (RI) strains contain equal proportions of genes from two inbred strains which have been crossbred for 20 or more generations of brother-sister matings. For example, CxB mice are an RI strain derived from a cross of BALB/c and C57BL/6J inbred mice and BxD mice are an RI strain derived from cross breeding C57BL/6 x DBA/2 mice. When congenic strains are produced, embryonic stem cells derived from a 129 mouse strain are placed into a C57BL/6 or another recipient strain, resulting in a congenic strain with a section of 129 mouse genome in a C57BL/6 background. If the mutant mouse differs from the "wild-type" C57BL/6 mouse, the difference is attributed to the gene that was added or deleted. The difference could, however, also be due to "passenger genes" from the 129 embryonic stem cell donor that are transferred along with the gene of interest. A number of methods have been proposed for separating the gene of interest from the flanking region to eliminate the passenger genes (91–93).

Genetically engineered mice are those which have been genetically modified by (1) induced mutations, (2) conditional mutations, (3) genes added to the genome, making a transgenic or gene knock-in mouse; (4) mice with a gene deleted or gene "knock-out mice (94); and (5) 'humanized mice' (95). Mutant mice have an altered DNA sequence in some genes, resulting in a different genotype from the background strain. Mutations can arise spontaneously through DNA copying errors during cell division or can be induced by exposure to radiation or by chemical mutagens, such as *N*-ethyl-*N*-nitrosourea (ENU) (96). Mutations can be identified using high-throughput DNA sequencing technologies (97) and the phenotypes of these mutants characterized by comprehensive phenotyping assays (98–100) and by behavioural phenotyping assays (101–104). Mutant mice are designated with the name of the background strain, the affected gene and the mutant allele as well as the location where the strain was derived. For example, C57BL/6J-Apc^{Min}/J mice have a C57BL/6 background strain, with an Min mutation on the Apc (adenomatosis polyposis coli) gene and were developed at the JAX laboratory. The Coloboma mice, C3Sn.Cg-Cm/J, which have been used as a model of ADHD have a spontaneous mutation (Cm) on the congenic (C3H/HeH x 101/H)F1 strain. Conditional mutant mice are those that have a gene mutation that is only expressed when activated by exposure to a chemical stimulus. The two major systems used are the tetracycline-inducible system and the Cre/loxP recombinase system. To create a conditional mutant, two lines of transgenic mice are mated; one of which expresses the activator controlled by a chosen tissue-specific promoter, and the other carries the desired gene, its expression controlled by specific constructs. The mutation is activated by exposure to tetracycline (Tet-inducible system) or Cre recombinase (Cre/loxP recombinase system). Such conditional systems allow for the spatiotemporal control of the expression of gene mutations, which is essential when early expression of mutagenesis may be toxic or lethal to development of the animals, thus affecting phenotype analysis. Conditional mutant mice are called conditional knock-outs if activation causes the excision of a gene and conditional knock-ins if activation allows for the expression of a gene (105).

Transgenic mice which have had new genes introduced into their germ line are **gene "knock-in" mice**. The fertilized oocyte is injected with the new gene and implanted into a foster mother. When the transgenic mice are born, they are bred to create a transgenic mouse line. For example, the C57BL/6JTg(CD8)-1Jwg mouse is a C57BL/6 mouse from the Jackson Laboratory having the Tg(CD8)1Jwg transgene (106). In targeted transgenic knock-in mice the desired gene is inserted into a specific locus via CRISPR/Cas9* or homologous recombination (107). **Gene knockout mice** have a target gene that has been silenced or no longer expressed (108). A gene is "knocked-out" by introducing genetically altered embryonic stem cells into the germline to produce a chimeric mouse which can be bred to the wildtype background strain to produce +/- F1 mice, which when bred will produce +/+ (wt), +/-, -/+ (heterozygous) or -/- (homozygous) mice for the genes of interest. The -/+ (hemizygous) mouse is a male which has a transgene on the X chromosome (i.e., X-linked genes in some autism mouse models (109)). Because the offspring can be of different genotypes, each individual mouse in a litter must be genotyped using Real-time quantitative PCR (qPCR) or other methods, such as restriction fragment length polymorphism identification (RFLPI), random amplified polymorphic detection (RAPD), amplified fragment length polymorphism detection (AFLPD). Mass-sequencing technologies provide whole-genome genotyping and genome-wide association studies (GWAS) have led to the identification of single-nucleotide polymorphisms (SNPs) associated with certain traits (110–112).

Gene knockout mice can also be engineered via post-transcriptional gene silencing through RNA interference (RNAi) using small interfering RNAs (siRNA) which prevent the ex-

pression of the target gene (113,114). Most recently, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9)-mediated genome editing system has been used to engineer both gene knock-in and gene knock-out mice (115–118).

A humanized mouse model is one that has been engrafted with functional human tissues or one that expresses human transgenes, thus being engineered to express human gene products (95,119). Humanized mice which have been engrafted with functional human cells have been used to study the immune system and immunological disorders (71,119) (Figure 1). The use of such humanized mice, which can produce human T cells and B cells, improves the translational validity from the mouse model to human patients in the study of immune function. Humanized mice are also important animal models in cancer research (120) and in the study of neurodegenerative diseases (121). Genomically humanized mice are those in which entire human gene loci are transferred into the mouse genome (122). Human genes can be introduced into the mouse genome through several different genetic engineering methods, including homologous recombination and site-specific recombination-based technologies (see 71,122). Humanized mice have been developed to study neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease and Amyotrophic Lateral Sclerosis (15,121).

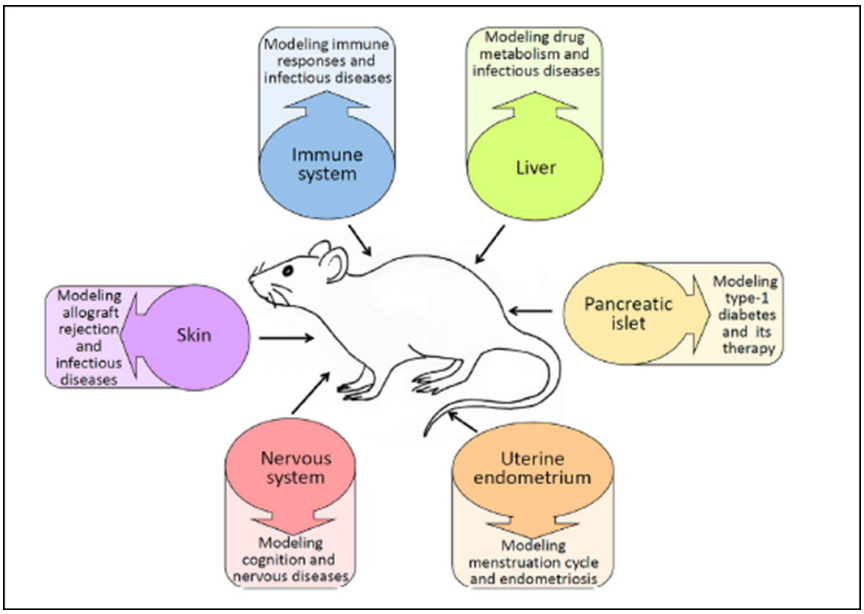


Figure 1. A schematic illustration of various categories of humanized mice. Representative human cells or tissues that have been engrafted to mice are shown with a description of the utility of the resulting humanized mice. Reproduced from Fujiwara S. 2018. Humanized mice: A brief overview on their diverse applications in biomedical research. J Cell Physiol. 233: 2889–2901.

4. Four research areas where animal models are essential for understanding human diseases

Animal models are essential in four general areas of research: (1) basic research, (2) developing animal models of diseases, (3) discovery of new treatments for human diseases, and (4) translational research.

4.1. Basic research drives all aspects of biomedical science. Its goal is to understand how fundamental biological, physiological, neural and behavioural processes function in "normal", heathy animals (123). To answer questions about development, for example, basic researchers use animal models to study systems development, including the brain and nervous system (124); the heart and cardiovascular system (125); and the immune system (126). Likewise animal models are important for understanding how bodily functions change during aging (127,128), as well as the process of aging in specific bodily systems such as the brain and nervous system (129), the immune system (130) and the cardiovascular system (131).

4.2. Animal models of human diseases are developed to understand disease mechanisms by characterizing the symptoms, and discovering the physiological, cellular and molecular causes of the disease. Such animal models can be spontaneous disease models or induced disease models. Spontaneous models are those that spontaneously develop a disease under natural conditions, such as dogs (beagles), degus (*Octodon degu*) and primates which spontaneously develop neurological disorders as they age (132,133) or companion animals such as dogs and cats which spontaneously develop cancers (134,135). A second type of spontaneous animal model for human diseases are mutant and transgenic animals. These genetically modified animals (usually mice) spontaneously develop human-like diseases, such as mutant mouse models which spontaneously develop cancerous tumors (136) and transgenic mouse models of Alzheimer's disease which develop amyloid plaques as they age (137). Induced animal models are generated using chemi-

cal or biological agents to activate disease processes (138). For example, intracerebral injections of Aβ peptides or other chemicals into the brains of rodents can be used to create animal models of Alzheimer's Disease (132) and tumors can be grafted onto mice to create models for cancer research (139). There are many differences in physiology and biochemistry between spontaneous and induced models of human diseases and both have their advantages and disadvantages, thus for any disease, it is important choose the most appropriate model for study (5,138,140).

4.3. Discovering new treatments for human diseases.

Once a reliable and valid animal model of a disease has been engineered, that model can be used to develop new treatments for the disease of interest. While drug discovery research is the most common use of animal models, other types of treatments being developed with animal models include gene therapy; dietary therapy; behaviour therapy and vaccine development.

4.3.1. Drug discovery

According to the Foundation for Biomedical Research (141), "animal research and testing were behind almost every prescription medicine available today", and their website lists the top 25 drugs discovered through animal research. Basic research plays a significant role in drug development (142) and mouse models are used for the discovery of new drugs to treat virtually every disease, including cancers (143,144), neurodevelopmental disorders (145–147), neuropsychiatric disorders (148,149), as well as Alzheimer's and other neurodegenerative disorders (41,150–152) and rare genetic disorders (16,153). As noted by Singh and Seed, (154 p.5) "Animal model-based scientific exploration remains a foundational pillar for the discovery and development of essential medicinals, designed and destined for the betterment of human health and condition".

Indeed, for clinical applications, the discovery of new drug treatments is the main reason for using animal models, and the title of the paper by Cacabelos et al (150) says it all: "What is the gold standard model for Alzheimer's disease drug discovery and development?" The same question can be asked of the (mouse) models of any disease. But here we come across a seemingly insurmountable stumbling block: complex diseases such as Alzheimer's disease have many subtypes and these may even be different diseases in themselves (155,156). The same may be true for Parkinson's disease (157,158), Autism (159,160), and other neurological disorders. Thus, different genetic mutations may underlie the different subtypes of each disease and different mouse models may be required to understand each subtype (161,162), not to mention the fact that different treatments may be required for each subtype (163–165). The logical conclusion is that each person with a neurodegenerative disease has their own subtype and requires personalized treatment (166–168). Therefore the plethora of mouse models for each disease may help to understand the subtypes of these diseases and provide the basis for developing different drug treatments for each subtype. Thus there can be no single gold standard model. Different models may be necessary to model different subtypes of these diseases.

4.3.2. Gene Therapy

Gene therapy techniques can replace a disease-causing mutated gene with a healthy copy of that gene or introduce a new gene to treat a disease using viral vectors. Targeted genome editing techniques such as CRISPR-Cas9 nuclease technology can be used to add genes, ablate genes or for "gene correction". Gene therapy techniques can be used to treat a number of human diseases, including dominant and recessive genetic conditions, cancers, cardiovascular disease, and neurodegenerative disorders (169–171). Animal models, particularly humanized mouse models, are essential for the development of gene therapies for HIV and cancer (172) and for neurological diseases (173,174). Gene therapies for Alzheimer's disease (175–177), Parkinson's disease (178), and Huntington's disease (179,180) have all been developed with mouse models. It is interesting that complex gene therapy may be able to treat multiple diseases. For example, Davidson et al. (181) showed that a combination gene therapy based on three genes (fibroblast growth factor 21, αKlotho, and mouse transforming growth factor-β receptor 2) delivered using adeno-associated viruses was able to treat four age-related-disease (obesity, type II diabetes, heart failure, and renal failure) in a mouse model.

4.3.3. Dietary therapy

Mouse models have been used to examine the effects of diet on neurodevelopmental disorders (182), including autism (183) and to test diet therapies for Alzheimer's disease (184,185) and cancer (186). For example, dietary restriction of the essential amino acid methionine reduces cancer cell growth in mouse models (186–188). A ketogenic diet has been shown to ameliorate cognitive impairment and neuroinflammation in the 5xFAD mouse model of Alzheimer's disease (189) and to reduce behavioural deficits in mouse models of Autism (190), suggesting that such a diet might be a 'metabolic treatment' for neurological disorders (191). Specific amino acid diets have been shown to reduce behavioural symptoms of autism in the BTBR mouse (192) and dietary choline reduced behavioural deficits in a mouse model of Rett syndrome by modulating synaptic plasticity (193). Dietary vitamin D has been proposed as a treatment for autism (194) and Rett syndrome (195) using mouse models. Finally, the spice Curcumin has been shown to reduce memory deficits in the APP/PS1 mouse model of Alzheimer's disease (196,197). Dietary treatments appear to have their effects by altering the gut microbiome (198,199) and, based on the results of mouse models, specific diets have been proposed as treatments for a number of neurodevelopmental and neurodegenerative disorders (200–202).

4.3.4. Behaviour therapy

Because many diseases have both environmental and genetic causes (see below), mouse models have been important in developing exercise and environmental enrichment as thera-

peutic approaches for cognitive and affective disorders (203). Exercise and environmental enrichment act as environmental stimulants to activate increased neurogenesis in the brain and thus may counteract neurodegeneration in neurological disorders (204–206). Exercise therapy has been beneficial in mouse models of Alzheimer's disease (207), Parkinson's disease (208), and multiple sclerosis (209). Environmental enrichment promotes recovery in mouse models of neonatal brain injury (210) and facilitates recovery from stroke (211). These behaviour therapies also reduce the neurobehavioural deficits in mouse models of schizophrenia (212) and cerebral amyloid angiopathy (213).

4.3.5. Vaccine development

Animal models are essential for the development of new vaccines (214,215) and the coronavirus pandemic has emphasized the importance of such models for the development of vaccines for such newly discovered viruses (216). Because viral diseases inhibit the activation of the immune system, new anti-viral treatments and the development of new vaccines against viral infections focus on the functioning of the immune system (217). For example, mouse models have been used to develop vaccines for hepatitis B (218), human immunodeficiency virus (HIV) and Dengue fever (219), as well as other infectious diseases (220). Humanized mice are especially important for the study of infectious diseases (71). Mouse models have also been used to develop experimental vaccines to treat Alzheimer's disease and other neurological disorders (221–224).

4.4. Translational research and critiques of the use of animal models

One of the main purposes of using animal models is to develop new treatments for human diseases that will translate into successful clinical trials. In translational research, the novel therapeutics developed from animal models are tested for disease treatment in humans. The problem is that many treatments developed with animal models are not successful in treating human disorders. There are many roadblocks that must be overcome in order to have successful translational research (225,226) (see Figure 2). The blame for the failure of translational research is often put on the animal models used and there have been regular pleas for "improved animal models" for biomedical research (227); regenerative medicine (228); neurodevelopmental disorders (226); and for brain disorders (229), particularly Parkinson's disease (46,230), and Alzheimer's disease (41,231–234).

The situation in the development of new drugs for Alzheimer's Disease has been eloquently expressed by Cacabelos et al. (150 page 1437), who state that:

"*Over the past 20 years, approximately 20,000 different sub-*

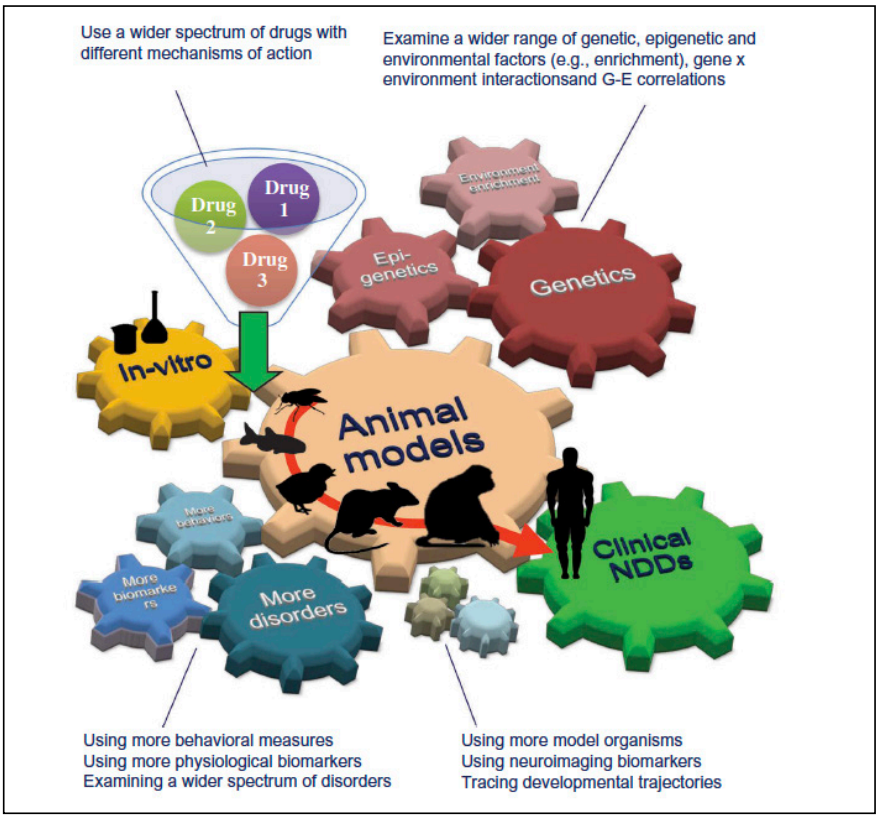


Figure 2. The central place of animal models in an integrated approach to improving pharmacotherapies of clinical neurodevelopmental and neurodegenerative disorders (NDD). Reproduced from Homberg JH, Kyzar EJ, Stewart AM, Nguyen M, Poudel MK, et al. 2016. Improving treatment of neurodevelopmental disorders: recommendations based on preclinical studies. Expert Opinion on Drug Discovery, 11: 11-25.

stances have been tested in 260 different animal models and only four AChEIs and one NMDA antagonist were fortunate to be approved by the FDA, EMA, Koseisho and other international regulatory agencies for the treatment of AD. The main reason for this historical failure may be attributed to four factors: (i) AD is a very complex disorder with a still undefined molecular pathogenesis, (ii) the animal models used for drug development are defective and insufficient, not faithfully reproducing the pathological substrate responsible for AD, (iii) the pharmaceutical properties of the bioactive principles in the drugs tested are inappropriate for AD; and (iv) the pharmacological strategies followed so far were erroneous due to pharmacokinetic, pharmacodynamic and pharmacogenetic reasons."

The problem is that the causes of many incurable diseases such as Alzheimer's disease are unknown, so each animal model is a stab in the dark: a test of a theory about the disease (i.e., a proof of principle). Thus, animal models are developed to mimic the human aspects of a specific disease. The result is that animal models of each disease are continuously "updated", as shown for humanized mice by Ye and Chen (71). Nadeau and Auwerx (235) refer to this as "the virtuous cycle", in which observations on human diseases lead to mouse models and the data from the mouse models leads to improved knowledge about the human disease (see Figure 3). One could extend this figure into a continuous feedback cycle: as more is learned about the human disease, new mouse models are engineered which lead to more new data for understanding the human disease, which leads to new mouse models, ad infinitum, or until a cure for the disease is discovered.

This process can be seen in the development of transgenic mouse models for AD in Table 1 of Mckean et al. (236) which shows the development from APP to 3xTgAD and TAPP mouse models. These authors suggest that the usefulness of mouse models may be at an end and new animal models of AD should be primates or large mammals such as farm animals (sheep and pigs) or companion animals (cats and dogs) which develop AD spontaneously. However, there are other alternatives. Cacabelos et al. (150 page 1437) point out that "the sophisticated transgenic mouse models are the most suitable for understanding AD pathogenesis and conceivably also for drug development, but they are still inadequate because the AD geno-phenotype is genuinely human and no animal model can fully reproduce it." They suggest that in addition to seeking better animal models, "the failure of preclinical to clinical translation in AD research and the poor results obtained in drug development based on transgenic mouse models associated with the amyloid-tau binomial dogma should make scientists and the pharmaceutical industry consider other more convincing pathogenic hypotheses". The same issues arise in research on cardiovascular disease. As pointed out by Zaragoza et al. (32 page 8):

"*Animal models of cardiovascular disease yield important insights into the genetic basis of human cardiovascular diseases and provide a test bed for pharmacological and treatments. Nonetheless, investigators need to carefully consider their*

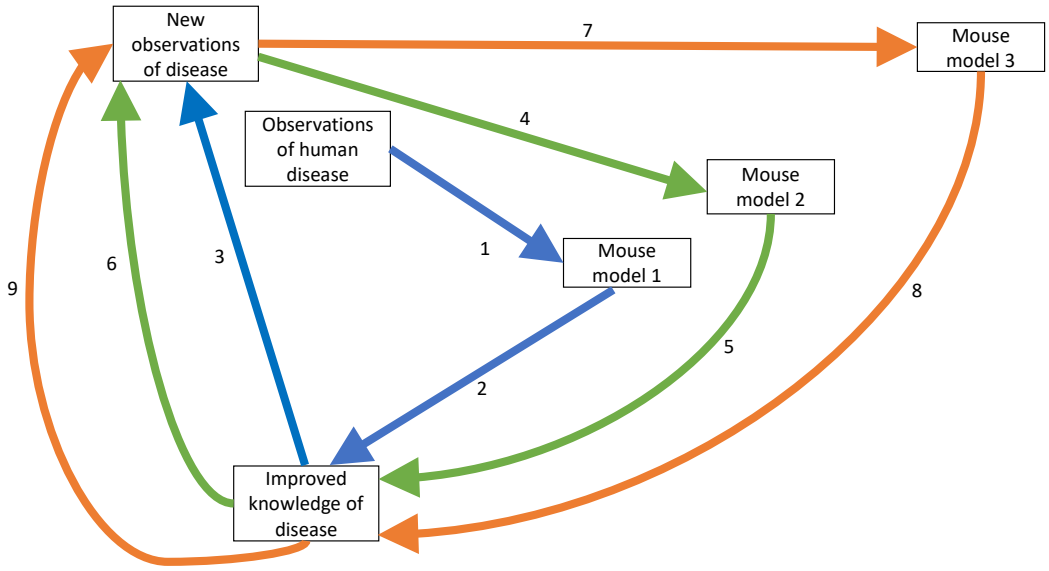


Figure 3. A transgenic mouse model (mouse model 1) is developed to test theories about the genotype of a disease and to have a phenotype that represents the human disease symptoms. Improved knowledge of the disease from this mouse model and new observations of people with the disease leads to the development of an improved Mouse model 2, and so on. As more is learned about the human disease, new mouse models are engineered which lead to a better understanding the human disease, which leads to new mouse models, ad infinitum, or until a cure for the disease is discovered.

choice of model: no single method perfectly recreates the human disease, and there are related considerations of cost, infrastructure and the requirement for specialized personnel. Taking these considerations into account, experimenters therefore need to select models that best reproduce the aspect of disease being investigated. In particular, when moving from bench to bedside it is essential to test procedures in relevant models that yield highly reproducible results, but despite these limitations, given the range of animal models available today it will always be possible to devise an appropriate strategy, and animal models remain the best tools for advancing the understanding of the mechanism of human cardiovascular disease."

5. What is the best animal model of disease X?

When a genetically modified mouse is engineered to be an animal model of a disease, one would expect it to be a reliable and valid model of that disease. However, there are many mouse models of each disease. Which is the best? In general, it depends on the theory of the underlying genetic causes of the disease (see 40) and the type of validity being considered. For example, to test the theory that ADHD is caused by abnormalities in dopamine levels, an animal model with spontaneous or induced dopamine disorders is developed (237). Likewise, to test the theory that Alzheimer's disease is caused by tau accumulation, a mouse model with tau accumulation is developed (238,239). To determine the reliability and validity of each mouse model requires the evaluation of both the genotype and phenotype of that model. For example, On the JAX website alone (www.jax.org),there are over 160 Mouse models of Alzheimer's disease, 190 mouse models of Parkinson's and 70 mouse models of Huntington's disease. These models are evaluated by neurobiological and behavioural phenotyping assays as described above, and there are many reviews on selecting the "best" models, which depend on the type of validity desired: construct validity, face validity or predictive validity (40). For example, for Alzheimer model mice, the goal is to "choose the model with closest construct validity to the human disease that recapitulates the phenotype under investigation" (240 page 7). This is, however, easier said than done as there are many variables to consider in choosing a mouse model (see Figure 4).

Since the goal is to match the mouse model to the disease of interest, there are several questions that must be answered in choosing the "best" mouse model (240). The points below refer primarily to mouse models of Alzheimer's disease, but similar questions can be asked about selecting the "best" mouse models of Parkinson's Disease (46,47,230), Huntington's Disease (43,241) and neurodevelopmental disorders such as Autism (242,243), and, more recently, Covid-19 (70).

Does the mouse model exhibit the full range of disease symptoms? For example, neurodegenerative diseases such as AD are known primarily for cognitive deficits, but patients also exhibit motor and neuropsychiatric symptoms, as well as weight loss, changes in metabolism and disruption of circadian rhythms. To what degree is this range of deficits shown in a mouse model (244)?

Does the model exhibit the neuropathology of the disease? This might include brain atrophy or synaptic pathology as measured in a neuropathology assay (245,246). For Alzheimer

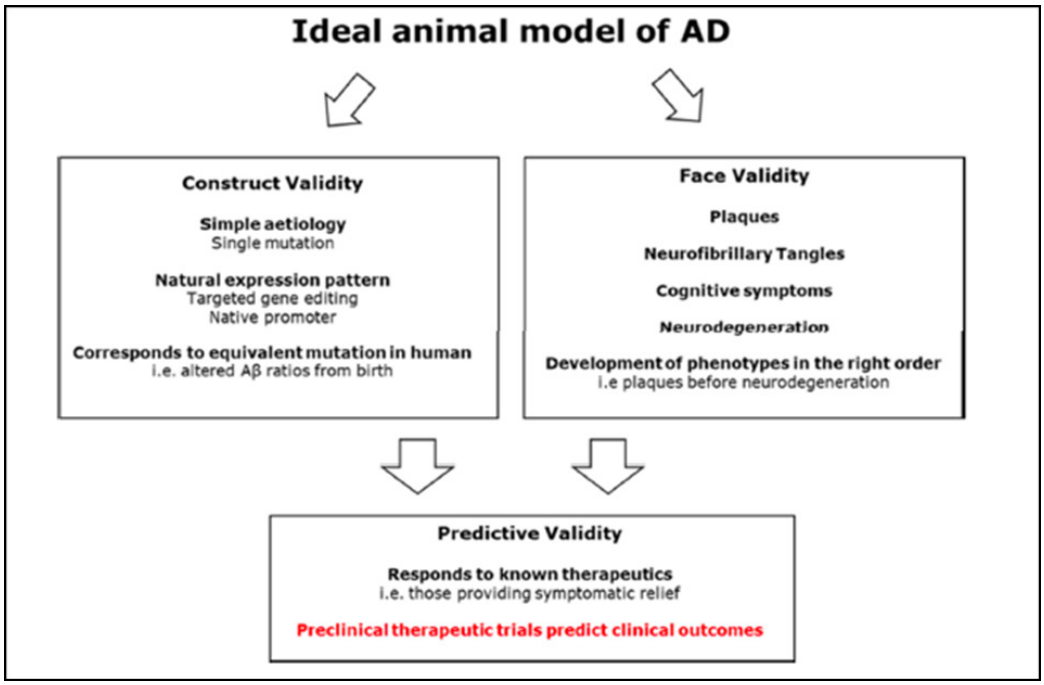


Figure 4. A diagram showing the desired qualities of an ideal animal model of Alzheimer's disease (AD) with high construct validity and face validity will have high predictive validity in testing new therapeutics, leading to higher translatability in clinical trials. Reproduced from Mckean NE, Handley RR, and Snell RG. 2021. A Review of the Current Mammalian Models of Alzheimer's Disease and Challenges That Need to Be Overcome. Int. J. Mol. Sci. 22: 13168. https://doi.org/ 10.3390/ijms222313168.

model mice, do they develop amyloid plaques, neurofibrillary tangles and/or and cerebral amyloid angiopathy (247)?

Do the mice show cognitive impairment or other behavioural abnormalities? This requires the use of extensive behavioural bioassays (248,249) and, if possible, the correlation between neuropathology and behavioural dysfunction (245,250).

Should a spontaneous model, an induced model, a transgenic mouse model, or a knock-in model be used? Although transgenic mouse models are some of the most frequently used, viral vectors have also been used to induce gene expression in rodent brains. This is not an easy decision as there are advantages and disadvantages to the use of each mouse model (see 240).

For transgenic mice, which gene transcript is used? For example, is the 695- isoform (APP695) used to induce APP in the brain of AD model mice or another transcript, such as the alternatively spliced isoforms, APP_{751}, or APP_{770} (251)?

For transgenic mice, which transgenic promotor is used? Is it the prion protein (Prnp), platelet-derived growth factor B chain (PDGFB), thymus cell surface antigen 1 (Thy1) or some other promotor (252)?

What is the source of the mice to be used? Which vendor should mice be purchased from: the Jackson Laboratory (Jax), Charles River, the Mutant Mouse Research and Resource Center (MMRRC), the RIKEN BioResource Center (BRC) or another breeder? The same mouse strain (eg. C57BL/6J) may differ in many phenotypic traits depending on the vendor, and C57BL/6J mice from different vendors may be considered substrains (87,253).

What background strains were used to construct the mouse model? Many transgenic mice were made using hybrid mouse strains such as C3B6, B6D2 or B6SJL but many have also been backcrossed to C57BL/6 backgrounds. The background strain is important as it defines the proper controls, but each background strain carries its own genetic baggage, with some carrying genes for cancer and other with mutations leading to retinal degeneration or other sensory-motor disabilities (88,254–257).

What is the expected lifespan of a mouse model? While a C57BL/6 mouse may live to 3 years of age (Over 1000 days), many transgenic mouse models have shorter lifespans and in some mice there are sex differences in lifespan (258).

At what age should the mouse model be tested? Models of neurodevelopmental disorders are generally tested from birth to 60 days of age, models of adult-onset disorders are tested from 60 to 270 days of age (3 to 9 months) while models of neurodegenerative disorders are tested much later in life, optimally after 12 months of age. For each animal model, the age at which the disease-associated phenotype first appears is the result of a complex interaction between the "strength of the transgene promoter, the aggressiveness of familial mutations included in the transgene, the number of transgene copies incorporated into the insertion site, the chromosomal location of transgene integration, and the genetic background on which the transgene is expressed" (240 page 9,also see 259–261).

Which sex of mouse should be tested? For some disorders (eg. Ovarian cancer) females are required and for other disorders (eg. Prostate cancer) males are required, but it is now customary (and increasingly mandatory) to test both sexes of mice in a disease model. In many transgenic mouse models of AD, females are more susceptible to the neuropathology of AD than males, but it is important to compare the rates of neural (262) and behavioural (259,261) deficits in both sexes over a number of ages in longitudinal or cross-sectional studies.

Is the mouse model comorbid for other factors that contribute to the disease? For example, AD has been associated with cardiovascular risk factors such as hypertension, type 2 diabetes, inflammation, stroke, stress and other factors (263). On the one hand, transgenic mouse models can be used to examine the nature of these comorbidities (264,265), but on the other hand, sensory and motor comorbidities may confound measures of cognitive disabilities (260,266).

Does the mouse model reflect non-neural factors that contribute to the disease? Non-neural cells such as astrocytes and microglia, cytokines from the immune system, vascular degeneration and hormonal factors may all be involved in disease onset. Are these factors present in the mouse model being used (267)?

Can the general effects of aging be separated from the effects of age-related diseases? In mouse models of neurodegenerative diseases, the general effects of aging must be separated from the specific effects of the disease. For example, in mouse models of cancer, aging effects must be separated from cancer effects (268). Likewise in mouse models of Alzheimer's disease, the neural and behavioural symptoms of the disease must be dissociated from those of normal aging (269,270). Finally, cognitive deficits must be dissociated from sensory and motor deficits as mice age (259,260,271).

Are there non-genetic (environmental) factors that contribute to the disease model? Genetic abnormalities have been the most important factor in the development of transgenic mouse models for AD and other diseases, but it is clear that environmental factors contribute to disease onset. For example, there are strain differences in mouse maternal behaviour and differential maternal treatment of offspring is a factor that can modulate neuro-behavioural development in disease models (272,273). How are these environmental factors represented in mouse models (274,275)?

6. Epigenetic mechanisms integrate genetic and environmental causes of disease

Gene mutations alone cannot account for all diseases. Environmental stimuli such as pollution, viral infections, the microbiome, maternal factors and social stress may contribute to the development of diseases by acting through epigenetic mechanisms, such as DNA methylation, histone acetylation, histone methylation and noncoding RNAs (50,276). In a rather circular fashion, the epigenetic machinery is encoded by the genome (277), and in turn regulates gene expression (278), thus affecting the development of neurodevelopmental disorders (279,280), cancers (281,282), immune responses to infec-

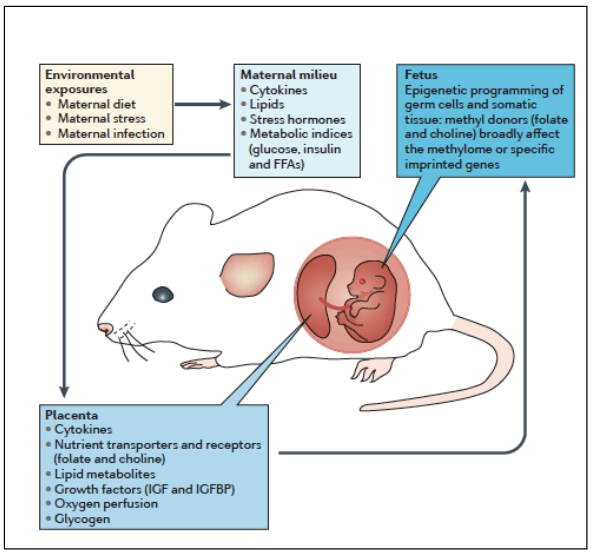


Figure 5. Examples of epigenetic programming during development. The figure shows the complex interactions between environmental stimuli, the maternal milieu, the placenta and fetal development. Maternal dietary deficiencies, chronic stress or infection can lead to endocrine disruptions in the maternal milieu, including increases in pro-inflammatory cytokines and stress hormones, and shifts in metabolic indices. These changes in signalling molecules can alter placental development and function and thus affect fetal growth and brain development by disrupting growth factors and methyl donor nutrients such as folate and choline, which can affect fetal somatic and germ cell epigenetic programming. FFAs, free fatty acids; IGF, insulin-like growth factor; IGFBP, IGF-binding protein. Reproduced from Bale T. 2015. Epigenetic and transgenerational reprogramming of brain development. Nature Reviews Neuroscience 16: 332-344.

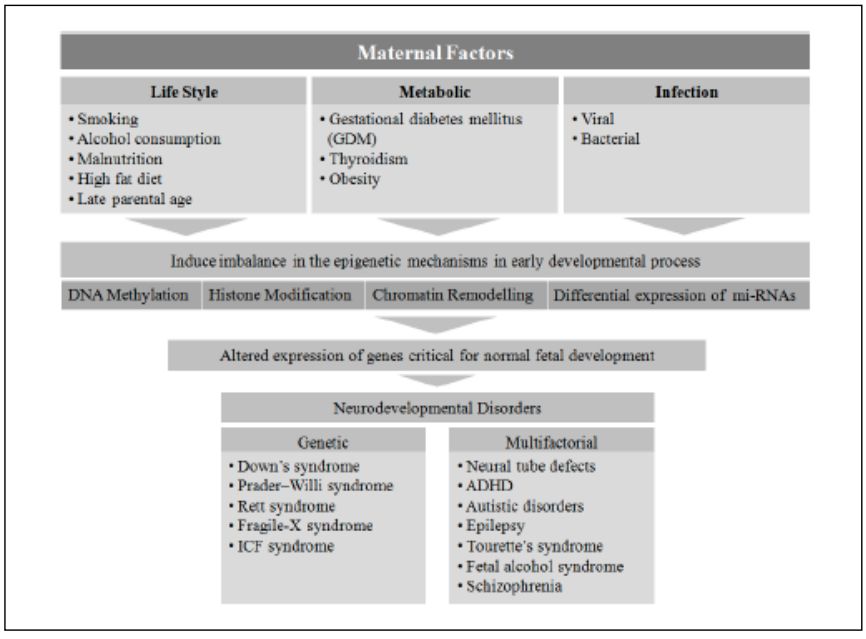


Figure 6. Some mechanisms through which maternal factors can lead to neurodevelopmental disorders via epigenetic programming. Maternal lifestyle-related factors, metabolic factors and infection lead to the activation of epigenetic mechanisms (DNA methylation, Histone modification, chromatin remodelling and differential expression of mi-RNAs) which lead to the altered expression of many genes associated with abnormal fetal development. This can result in a number of different neurodevelopmental disorders in the offspring and demonstrates how epigenetic programming provides the basis for the developmental origins of health and disease (DOHAD). Reproduced from Banik A, Kandilya D, Ramya S, Stünkel W, Chong YS, and Dheen ST. 2017. Maternal Factors that Induce Epigenetic Changes Contribute to Neurological Disorders in Offspring. Genes 8:150. doi:10.3390/genes8060150.

tions (283), and heart diseases (284), as well as human aging and diseases of aging (285), neurodegenerative diseases and brain injury (286). Gonadal hormones also act via epigenetic mechanisms to regulate the development of sex differences in the brain and other bodily systems such as the immune system, reproductive system, and cardiovascular system (287–289). Therefore, an important function of animal models of disease is to delineate the interactions between genetic and environmental factors in the development of diseases (290,291). Two ways that epigenetic processes can regulate gene expression are through epigenetic programming and epigenetic priming. Epigenetic programming occurs during early development while epigenetic priming can occur at any time during the lifespan.

Epigenetic programming is the result of exposure to specific environmental stimuli during critical periods of early development that modify developmental pathways (Figure 5), leading to stable and long-lasting alteration of phenotypic traits in adulthood (280,292). For example, several maternal factors, including infection, diet, and drug use during pregnancy, maternal behaviour during infancy, and exposure to stressors, or neurotoxins during critical periods of development can program mice for the development of diseases in adulthood (279,293). Epigenetic programming occurs during the perinatal and early postnatal life, which in humans comprises the first 1000 days (294), and in mice comprises the perinatal period up to 30 days of age (273,280,295,296). Epigenetic programming provides the basis for the DOHAD (Developmental origins of health and disease) model (Figure 6), which examines the effects of early environmental stimulation on the later development of diseases (52,297–299).

Epigenetic priming (or gene priming) is the modification of the epigenome of a cell such that specific chromatin domains are converted from a closed state ("heterochromatin"), in which gene transcription is inhibited, to an open state ("euchromatin") in which gene transcription is facilitated (300,301). This usually occurs in response to an external stimulus which activates an epigenetic signalling pathway that regulates DNA access by transcription factors or other gene regulating mechanisms. For example, endocrine disrupting chemicals in the environment can alter neuroendocrine pathways leading to abnormal steroid hormone levels which then act at nuclear hormone receptors (transcription factors) to alter gene expression which can cause neurodevelopmental disorders (302–304), abnormalities in sexual differentiation (305–307), or result in the

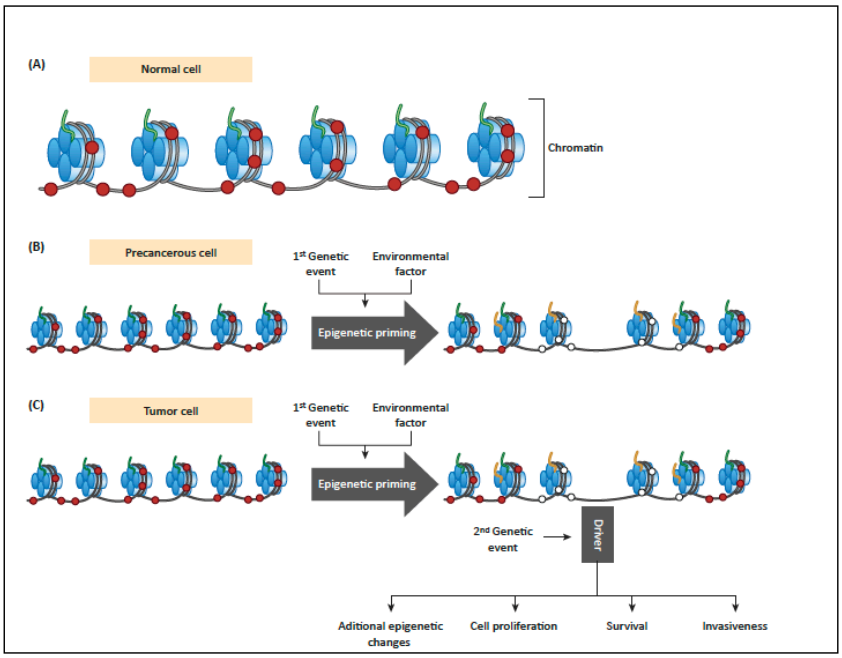


Figure 7. An example of epigenetic priming in the development of cancer. A normal cell (A), which exhibits normal chromatin modifications according to its identity and developmental stage, becomes a precancerous cell (B) by the action of a given oncogenic hit (first genetic hit) or by exposure to an environmental factor (tobacco smoke in lung adenocarcinoma); either of which can 'reset' the epigenetic and/or transcriptome status and reprogram the epigenome to give rise to a precancerous cell. (C) The malignant epigenetic priming will remain silent until specific second events trigger the development of a cancerous tumor cell. These second hits can occur randomly or be triggered by environmental exposures (such as infections). Blue cylinders represent nucleosomes, red and white circles symbolize changes in DNA methylation, and green and orange curved lines denote histone modifications. Reproduced from Vicente-Dueñas C, Hauer J, Cobaleda C, Borkhardt A, and Sánchez-García I. 2018. Epigenetic Priming in Cancer Initiation. Trends in Cancer, 4(6): 408-417.

development of cancers (308) or neurodegenerative disease in later life (309–311).

Thus, disease development may be a two-stage process: gene mutations interacting with epigenetic priming (Figure 7). Epigenetic priming is insidious as it can remain latent until it is triggered by external environmental stimuli or endogenous stimuli such as hormones (308). In the development of cancer, for example, gene mutations (oncogenes) can turn normal cells into precancerous cells, but an epigenetic primer may be required to convert these precancerous cells into full-blown tumor cells (308). This means that animal models of cancer should have both a genetic and an epigenetic component and that the development of new cancer treatments might focus on epigenetic mechanisms (312). If epigenetic priming is the driving force in tumor development, epigenetic treatments could prevent precancerous cells from developing into full-blown cancerous cells, because "epigenetic modifications, unlike genetic changes, can be erased, manipulated, and reinitiated even before a precancerous cell might evolve into cancer" (308 page 415).

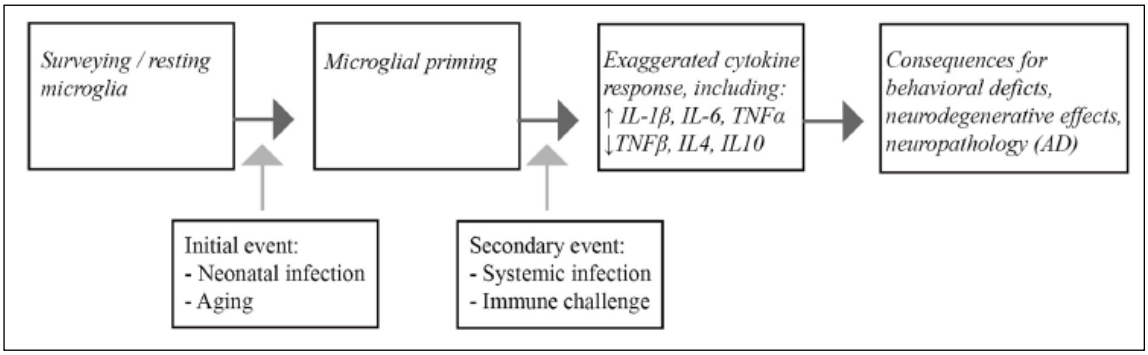


Figure 8. Microglial priming in neurodegenerative disorders. Microglia are present as surveying, quiescent cells in the brain. Neonatal infection or age-related damage can lead to the priming of the microglia. A second inflammatory event such as systemic infection or another type of immune challenge may result in an exaggerated microglial response of the primed cells, leading to increased release of pro-inflammatory cytokines. This imbalanced inflammatory response may affect the CNS, resulting in neuropathological changes and cognitive dysfunction associated with Alzheimer's Disease and other neurodegenerative disorders. Reprinted from: Hoeijmakers L, Heinen Y, van Dam AM, Lucassen PJ, Korosi A. 2016. Microglial Priming and Alzheimer's Disease: A Possible Role for (Early) Immune Challenges and Epigenetics? Front Hum Neurosci. 10:398.

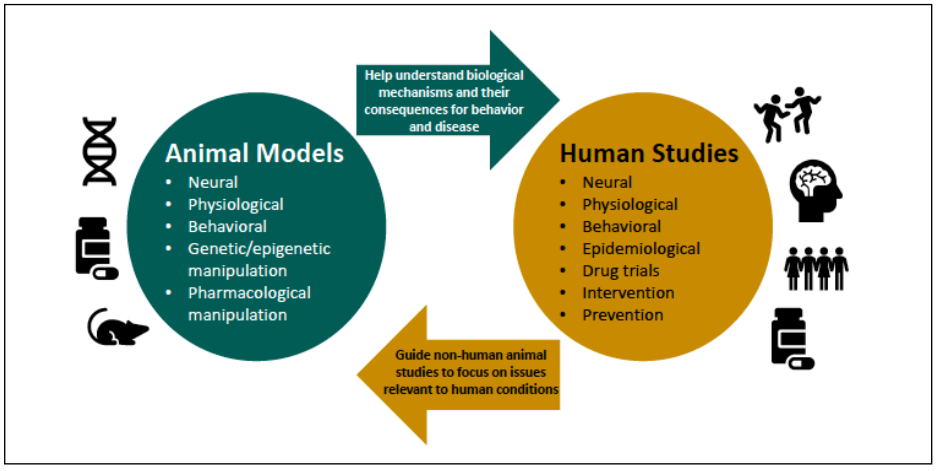


Figure 9. The relationship between animal models and human studies is reciprocal: both provide important insight into biology and behavior, guide the direction of research, and complement each other's findings. With animal models, however, we can utilize genetic, epigenetic, and pharmacological manipulations to help elucidate mechanisms and establish causality between environmental factors and behavior, health, and disease outcomes. Reproduced from Phillips NLH and Roth TL. 2019. Animal Models and Their Contribution to Our Understanding of the Relationship Between Environments, Epigenetic Modifications, and Behavior. Genes 10:47; doi:10.3390/genes10010047.

In the same way, epigenetic priming might underlie neurodevelopmental disorders (303,304), and neurodegenerative disorders (309,311). For example, the development of Alzheimer's disease may occur through the epigenetic programming of microglial cells (313–315). Epigenetic priming also appears to be involved in cognitive processes such as learning and memory in the brain (310) and in the immune system, as activated T cells are epigenetically primed to become memory T cells in response to specific antigens (302). It may turn out that epigenetic programming of gene activation is a ubiquitous mechanism for gene-environment interaction and that many diseases are the result of abnormalities in this interaction. For example, it is possible that epigenetic priming of microglial cells in the brain activates abeta and/or tau genes to initiate the onset of Alzheimer's disease (313) (Figure 8). This means that the next generation of mouse models for diseases must consider both genetic and epigenetic mechanisms.

Because many diseases have epigenetic as well as genetic causes, "epigenetic therapies" can be developed to target the epigenetic mechanisms underlying such diseases. Epigenetic processes such as DNA methylation, histone acetylation and histone methylation can be targeted by drugs or other therapies to reverse their control over gene expression (Figure 9). For example, DNA hypermethylation could be reversed to result in DNA hypomethylation. Non-coding RNA's could be accessed to regulate protein synthesis. Epigenetic drugs include DNA methyltransferase inhibitors, histone deacetylase inhibitors, histone methyltransferase inhibitors, and lysine demethylase inhibitors, all of which were developed using mouse models (316). For example, Rett syndrome is caused by mutations in the methyl CpG binding protein 2 (MeCP2) and mouse models of Rett syndrome, such as the Tsix/Mecp2 mouse model, are used to develop new epigenetic treatments for this disorder (317,318). Epigenetic treatments are being developed using mouse models for neurodevelopmental disorders such as autism (319), neurodegenerative disorders such as Alzheimer's disease (320,321), neuropsychiatric disorders (322) and for cancers (323,324).

6.1. Mouse models of gene x environment interactions

The question of "What is the best animal (mouse) model of disease X?" is difficult to answer because each disease has both genetic and non-genetic causal factors (50,278,282,289,303). Animal models created by genetic engineering (mutant, transgenic knock-in and gene knockout mice, humanized mice) may have the genetic components of a disease, but environmental stimuli may be required to activate the genetic mechanisms underlying the disease onset. Thus many "genetic" diseases may require epigenetic programming during early development or epigenetic priming to initiate the disease process (see 308). As mentioned above, an animal model may therefore require both the genetic mechanism and the epigenetic stimulation for disease development. For many diseases, the definition of the best mouse model has been couched in terms of genetics without consideration of environmental factors (see 40), however, in the future, epigenetic engineering may be required in order to develop new animal models of disease (325).

In theory, the optimal mouse model of a disease would show the genetic, epigenetic and environmental factors that contribute to the disease. This ideal has been approached with the development of epigenetic model mice, transgenic mice with epigenetic modifications, and mouse models with genetic, epigenetic and environmental manipulations. Mouse models with epigenetic modifications have been generated using different methods of gene editing, resulting in target-specific DNA methylation in the H9 differentially methylated region (326). This results in an imprinting disease model mouse that was generated by targeted demethylation of specific loci in the epigenome of fertilized eggs. Since hypermethylated DNA is associated with inactive chromatin and gene silencing and hypomethylated DNA is associated with open chromatin and the facilitation of gene expression, altering DNA methylation will alter gene expression profiles. DNA methylation, in turn, can be affected by environmental stimuli which lead to "epigenetic diseases" such as cancer, obesity, diabetes, and autism (326 page 1). Epigenetically modified transgenic mouse models have also been developed for cancer research (327). A second approach is to produce epigenetic modifications on transgenic mice, resulting in a mouse model with both genetic and epigenetic modifications. Such mouse models have been developed for Alzheimer's disease (320,328), Autism (329,330) and for some cancers (331).

As noted by Grayson and Guidotti (332), genetic predisposition and environmental factors interact in the neuropathology of complex neuropsychiatric disorders such as schizophrenia, bipolar disorder, and major depression. While genetic studies have identified several gene loci associated with such disorders, epigenetic factors, activated by environmental stimuli may act on these gene networks to initiate the neuropsychiatric disorders. As mentioned above these environmental factors can be the result of epigenetic programming or epigenetic priming. Transgenic mouse models of aging can include environmental factors by manipulating their diet (333), exercise (334) or postnatal stress (332).

The onset of many neuropsychiatric and other diseases may be explained by the two-hit or the multiple-hit hypotheses which involve the interaction of genetic, developmental, epigenetic, and environmental factors. For example, a genetic mutation may be regulated by environmental stimuli which activate epigenetic signalling pathways during a critical period of development (53,332,335–337). These may involve epigenetic programming and/or epigenetic priming. One way of looking at these interactions is the use of the four core genotypes mouse model which was developed to dissociate the roles of genes and hormones in sexual differentiation (338,339). Disorders of sexual differentiation can be the result of gene mutations or hormonal abnormalities and these hormonal abnormalities can be caused by environmental toxins acting as endocrine disruptors during critical periods of development (305,340). These endocrine disruptors can also disrupt brain development, leading

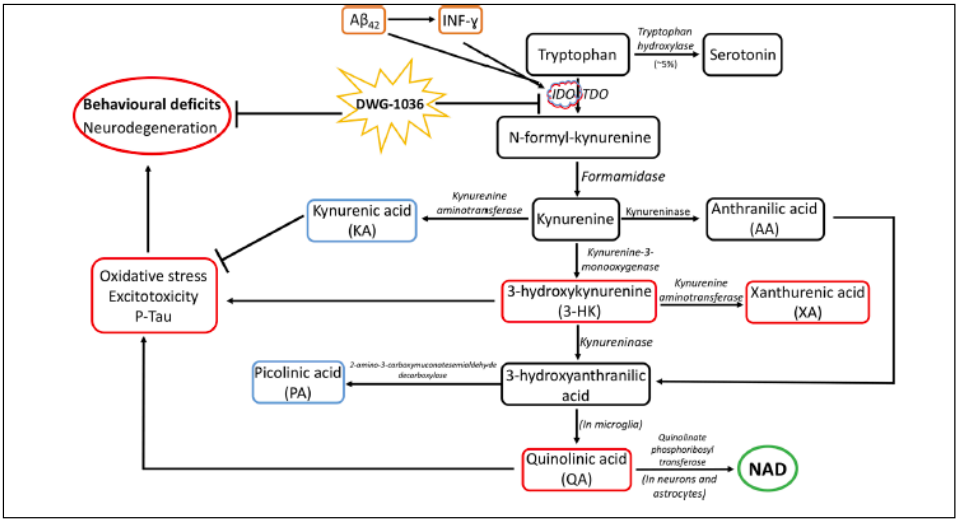


Figure 10. The kynurenine pathway (KP) and its suggested role in Alzheimer's disease (AD) pathogenesis. While some tryptophan is converted to serotonin by tryptophan hydroxylase, the majority gets metabolized by the KP. Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) are the rate-limiting enzymes of the KP, which metabolize tryptophan to N-formyl-kynurenine, which is further metabolized into kynurenine, 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid, quinolinic acid (QA), and eventually, nicotinamide adenine dinucleotide (NAD). Kynurenine is converted to anthranilic acid and kynurenic acid, which is neuroprotective. 3-Hydroxykynurenine is neurotoxic and can be converted to xanthurenic acid. 3-Hydroxyanthranilic acid is converted to picolinic acid, which is neuroprotective, whereas QA is neurotoxic. The involvement of the KP in AD pathogenesis is caused by the elevation of IDO by amyloid beta 42 ($A\beta_{42}$) and interferon gamma (INF-γ), resulting in neurodegeneration and the cognitive and behavioral deficits shown in AD. It is proposed that the IDO inhibitor DWG-1036 will act to reduce the neurobehavioral deficits seen in AD. RED = neurodegenerative processes; BLUE = neuroprotective processes. Reproduced from Fertan E, Stover KRJ, Brant MG, Stafford PM, Kelly B, Diez-Cecilia E, Wong AA, Weaver DF and Brown RE. 2019. Effects of the novel IDO inhibitor DWG-1036 on the behavior of male and female 3xTg-AD mice. Frontiers in Pharmacology 10:1044.

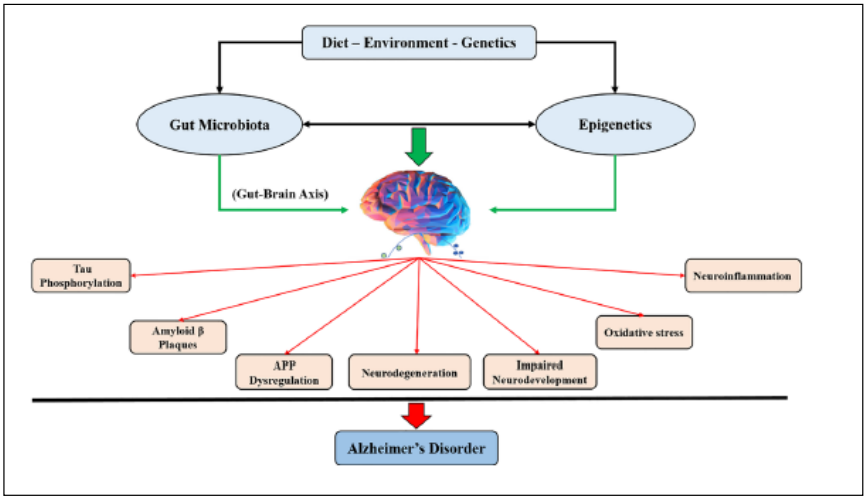


Figure 11. The impact of gut microbiota and epigenetics in the pathogenesis of Alzheimer's disease. Diet and environmental factors directly influence the composition of gut microbiota and epigenetic mechanisms throughout life. Gut microbiota and epigenetics are therefore dynamic processes that have shown bidirectional association amongst themselves. Gut microbiota and epigenetic factors are associated with a variety of mechanisms, including tau hyperphosphorylation, amyloid protein deposition, and oxidative stress, which contribute to the development and progression of AD. Reproduced from Nagu P, Parashar A, Behl T, and Mehta V. 2021. Gut Microbiota Composition and Epigenetic Molecular Changes Connected to the Pathogenesis of Alzheimer's Disease. Journal of Molecular Neuroscience 71:1436–1455. https://doi.org/10.1007/s12031-021-01829-3.

to neurodevelopmental (341) and neuropsychiatric disorders (342). Mouse models of endocrine disruption show long term neurodevelopmental abnormalities, which may affect both sexes or be specific to one sex (307,343).

7. The challenge for Biotechnology: How to develop the ideal mouse models for human diseases

It is clear that biotechnology plays an important role in the development of treatments for human diseases and that the development of these treatments will depend on the use of mouse models (19,71,344). Three approaches have been taken: (1) developing models based on new theories of the diseases; (2) going small, developing models based on one aspect of a disease; (3) going large, developing complex disease models. This discussion will focus on AD but will be relevant to all other incurable diseases.

(1) Developing models based on new theories of the disease. Armstrong (345,346) outlined the full range of possible causes of AD and Adams (347) has suggested new theories of AD involving lactic acid, ceramide, IL-1B, tumor necrosis factor a, folate, nicotinamide, kynurenine metabolites and microRNA. Given the recent attention to the kynurenine pathway (Figure 10) in AD (348–350), one might expect to see new mouse models of AD with genetic modifications to the kynurenine pathway (see 351). Breijyeh and Karaman (352) outline therapeutic approaches based on many of the theories of AD which have been developed with mouse models. For example, new findings for the role of the Txnip Gene (353,354) could lead to new mouse models and new treatments for AD (see also 40).

A more serious problem is that almost all mouse models are for early onset AD, which occurs in less than 5% of AD cases, while there are few models for late onset AD, which occurs in over 95% of AD cases (41). However, there are now some mouse models of late onset AD which may move the therapeutic field onto newer treatment strategies (355). Some of these new models show aspects of both neuropathological and neurobehavioural phenotypes of AD (356,357), while others have shown only the neuropathological phenotype (358), and will require an evaluation of the behavioural phenotype, which will lead to the next generation of transgenic mouse models.

(2) Going small: developing models based on one aspect of a disease

New mouse models might focus on only one aspect of a disease. Such models, for example, focus on Amyloid β oligomers ($A\beta_{1\text{-}42}$ protein) as in the Aβ-GFP transgenic mouse (359) or focusing on genes that are not obviously related to AD such

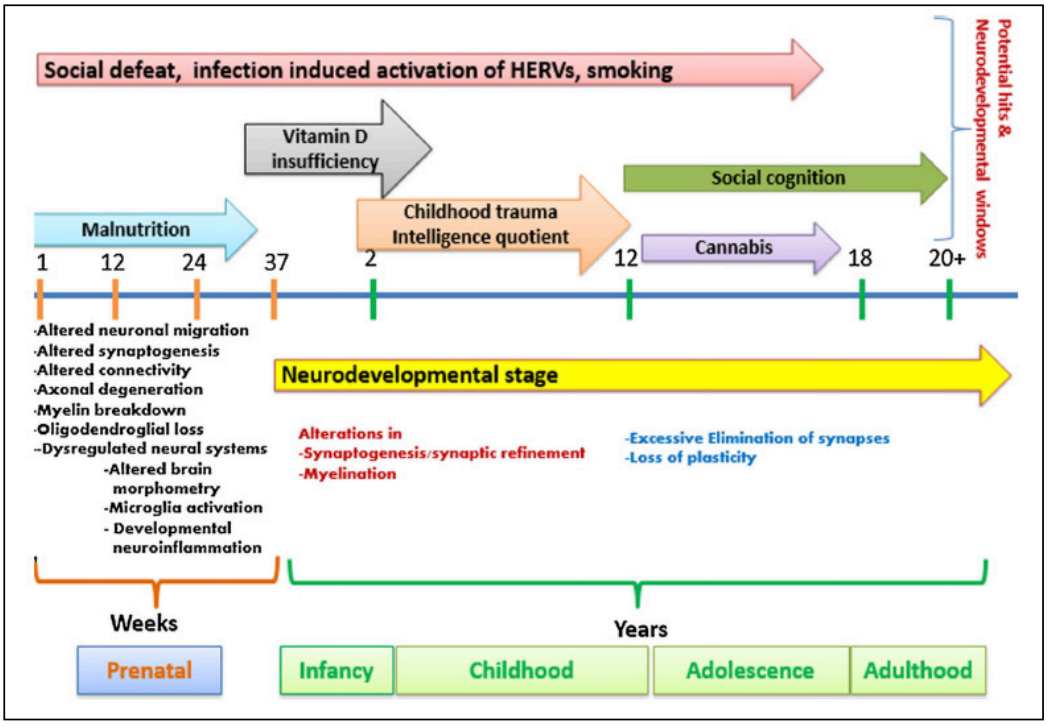


Figure 12. Neurodevelopmental windows showing a multiple-hit theory of schizophrenia. Schizophrenia may be conceptualized as a process which involves multiple vulnerability factors across numerous neurodevelopmental windows. Some hits such as under and over-nutrition are thought to act during early developmental periods, whereas others such as cannabis and social cognition act later with the neurodevelopmental period. Each environmental hit acts via epigenetic processes to alter gene expression. Reproduced from Davis J, Eyre H, Jacka FN, Dodd S, Dean O, et al. 2016. A review of vulnerability and risks for schizophrenia: Beyond the two-hit hypothesis. Neurosci Biobehav Rev. 65:185-94. doi: 10.1016/j.neubiorev.2016.03.017.

as TDP-43 (TAR DNA-binding protein 43), which never-theless may be related to a number of neurodegenerative disorders (360,361). Likewise, mutations in the Phospholipase D3 (PLD3) gene suggest that this enzyme may be involved in the neural and behavioural dysfunctions in AD (362,363).

(3) Going big: developing complex disease models

The interactions between environmental and genetic mechanisms in neurodevelopmental and neurodegenerative diseases and the development of multiple hit hypotheses of specific diseases have led to the development of complex mouse models which involve multiple causal factors (53). Such models have been created by taking a transgenic mouse model of AD, then adding a mutation of a gene regulating epigenetic mechanism and then providing environmental stimuli such as prenatal stress, exposure to neurotoxins, a high fat diet, or neonatal social stressors to examine the effects of these multiple hits on brain and behaviour of the resulting mouse model (see Figure 9).

Some such complex mouse models have been constructed. Griñán-Ferre, et al (275) showed that environmental enrichment improved cognitive performance in 5xFAD mouse models, reduced neuropathology, reduced DNA-methylation levels and increased histone H3 and H4 acetylation levels. They claim that environmental enrichment modulated epigenetic processes which then improved neural and behavioural function. Thus this study combined a transgenic mouse model of AD, environmental enrichment, and epigenetic mechanisms. Gut bacteria can also act as an environmental signal to activate epigenetic processes underlying Alzheimer's disease symptoms in animal models. For example, Nagu et al. (364) suggest that diet interacts with gut microflora to activate epigenetic processes which can affect the onset of AD, and Figure 11 shows how different diets given to transgenic mouse models of AD can produce an interaction of diet, gut microflora and AD-related genes. Shen et al. (365) found that antenatal hypoxia exacerbated the neural and behavioural symptoms of AD in the 5xFAD mouse model, showing that early life stress can accelerate the onset of AD-related neurobehavioural pathology. Wang et al. (366) showed that if the 3xTg-AD mouse model consumed a high-fat diet, they showed increased cerebrovascular disorders which exacerbated their neural and behavioural symptoms of AD. Prolonged social isolation accelerates the onset of neuropathological and behavioural AD symptoms in 5xFAD mouse models and these were not improved by environmental enrichment (367). Liang et al. (368) showed that social housing facilitated cognitive improvement but did not reduce amyloid plaque distribution in the APP/PS1 mouse model of AD. However, the synaptic function, dendritic spine density, and complexity of neuronal networks were increased by social housing and additional protein and mRNA analyses of isolated astrocyte and microglia revealed that several glial cell genes related to anti-inflammatory progression were significantly upregulated, while pro-inflammatory markers were decreased.

In each of these examples, environmental variables and epigenetic mechanisms have been investigated in transgenic mouse models to further understand the interaction between the environment and the genes in the development of neurodegenerative disorders (162). The interaction of two or more systems is a way that mouse models can be used to study the mechanisms underlying **the two-hit (or multiple-hit)** hypotheses of diseases as diverse as Alzheimer's disease (369), Parkinson's disease (370) and Schizophrenia (371), to fatty liver disease (372) and Covid-19 (373). New generations of transgenic mouse models can be engineered to study these multiple hit theories of disease (374,375). For example, the three-hit mouse model of schizophrenia involves genetic engineering, early life stress and later life exposure to environmental toxins or drugs (376) (see Figure 12).

Summary

The future of genetically modified mouse models of human diseases

Although there are animal models of almost every human disease, there as many critiques of these models as there are models. It is true that no animal model completely replicates the human disease. However, the relationship between the human disease and the animal models of that disease are continuously evolving: as more is known about each disease, new theories develop and new generations of animal models are developed which help to further understand the human disease, and this improved understanding results in new theories and new animal models, as indicated in Figure 3.

Already new generations of humanized mouse models are being used to understand the devastating effects of Covid 19 (SARS-CoV-2) on the body (377). The ACE2 transgenic mice were the first animal model developed to study SARS (378) and, based on the knowledge gained from these mice and the study of Covid 19 patients, a new mouse model, the MISTR6 mice has been developed (379). This new humanized mouse model has human hematopoietic stem cells and progenitor cells, giving it a human-like immune system, with humanized monocytes and macrophages. Following Sars-CoV-2 infection, these mice develop weight loss, and lung pathology that mimics that of human patients, and were not seen in the ACE2 mice. Sefik et al then showed that treatment of these mice with human monoclonal recombinant antibodies was most effective in the early stages of the disease while treatment with dexamethasone was only effective in later stages. Thus, the two stages of the disease, infectious viral clearance and immunopathology, required different treatments. The next generation of Covid-19 mouse models may provide more information about the progression and treatment of the disease.

In this paper, I have examined some of the biotechnology behind the development of animal models of incurable human diseases, with an emphasis on transgenic mouse models of neurodegenerative disorders such as Alzheimer's disease. I have outlined the various types of mouse models, the research areas in which they are utilized and the criticisms of these models. Finally, I have looked at the evolution of animal models and the

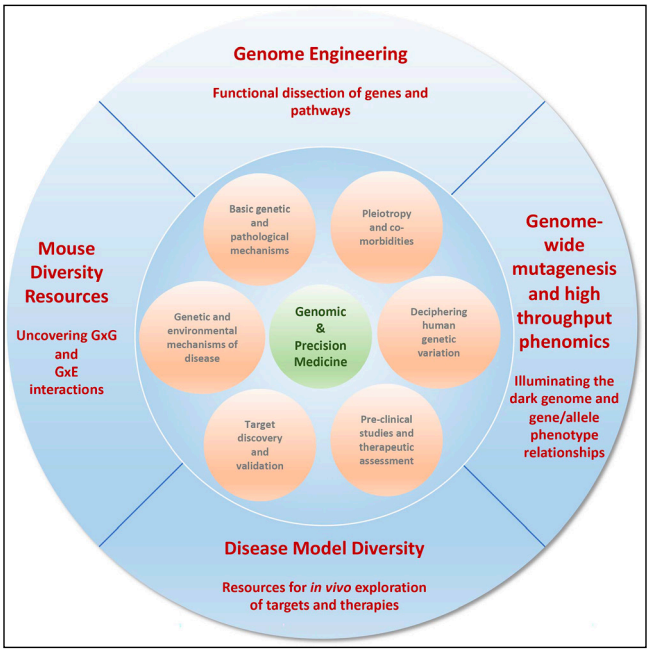


Figure 13. A diagram showing how genetically modified mouse models and genomics approaches (outer circle) contribute to the landscape of mechanistic, systems, pathobiology, disease genetics, pre-clinical and therapeutic studies (inner circle) that will be critical to address the challenges of genomic and precision medicine. Reproduced from Brown SDM. 2021. Advances in mouse genetics for the study of human disease. Hum Mol Genet. 30(R2):R274-R284. doi: 10.1093/hmg/ddab153.

feedback mechanisms between knowledge of human diseases, theories about the origins of the disease and then the development and testing of these theories using genetically modified mice and other animal models. The future use of transgenic mouse models of diseases will be to test two-hit and multiple -hit hypotheses of diseases, in which the genetic, environmental and developmental components of each disease are examined in complex experimental designs. In each generation of animal models, more is found out about each disease and new theories developed. One should not be discouraged at the number of generations of animal models required to discover the causes and treatments of each incurable disease as advances in mouse models continue to develop (19,71) (Figure 13).

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References

1. List of Currently Incurable Diseases. *Disabled World* Available from: https://www.disabled-world.com/definitions/lists/incurable.php. 2020. Accessed February 28, 2022.
2. Beckers J, Wurst W, de Angelis MH. Towards better mouse models: enhanced genotypes, systemic phenotyping and envirotype modelling. Nat Rev Genet 2009;10:371–380.
3. Dawson TM, Golde TE, Lagier-Tourenne C. Animal models of neurodegenerative diseases. Nat Neurosci 2018;21:1370–1379.
4. Gitler AD, Dhillon P, Shorter J. Neurodegenerative disease: models, mechanisms, and a new hope. Dis Model Mech 2017;10:499–502.
5. Onaciu A, Munteanu R, Munteanu VC, et al. Spontaneous and Induced Animal Models for Cancer Research. Diagn Basel Switz 2020;10:E660.
6. Riehle C, Bauersachs J. Small animal models of heart failure. Cardiovasc Res 2019;115:1838–1849.
7. Tsuneyama K, Nishitsuji K, Matsumoto M, et al. Animal models for analyzing metabolic syndrome-associated liver diseases. Pathol Int 2017;67:539–546.
8. Vaquer G, Rivière F, Mavris M, et al. Animal models for metabolic, neuromuscular and ophthalmological rare diseases. Nat Rev Drug Discov 2013;12:287–305.
9. Flora A. The Versatile Mouse Model for Rare Disease Research. The Jackson Laboratory Available from: https://www.jax.org/news-and-insights/jax-blog/2019/may/the-versatile-mouse-model-for-rare-disease-research. Ac-

cessed April 3, 2022.
10. Moore M. A new world of opportunity in rare diseases. The Jackson Laboratory Available from: https://www.jax.org/news-and-insights/2018/october/a-new-world-of-opportunity-in-rare-diseases. Accessed April 3, 2022.
11. Cacheiro P, Haendel MA, Smedley D, et al. New models for human disease from the International Mouse Phenotyping Consortium. Mamm Genome Off J Int Mamm Genome Soc 2019;30:143–150.
12. Brommage R, Ohlsson C. High Fidelity of Mouse Models Mimicking Human Genetic Skeletal Disorders. Front Endocrinol;10 Available from: https://www.frontiersin.org/article/10.3389/fendo.2019.00934. 2020. Accessed April 3, 2022.
13. Sztretye M, Szabó L, Dobrosi N, et al. From Mice to Humans: An Overview of the Potentials and Limitations of Current Transgenic Mouse Models of Major Muscular Dystrophies and Congenital Myopathies. Int J Mol Sci 2020;21:8935.
14. Deshpande O, Lara RZ, Zhang OR, et al. ZNF423 patient variants, truncations, and in-frame deletions in mice define an allele-dependent range of midline brain abnormalities. PLOS Genet 2020;16:e1009017.
15. Nair RR, Corrochano S, Gasco S, et al. Uses for humanised mouse models in precision medicine for neurodegenerative disease. Mamm Genome Off J Int Mamm Genome Soc 2019;30:173–191.
16. Murillo-Cuesta S, Artuch R, Asensio F, et al. The Value of Mouse Models of Rare Diseases: A Spanish Experience. Front Genet 2020;11:583932.
17. Zárybnický T, Heikkinen A, Kangas SM, et al. Modeling Rare Human Disorders in Mice: The Finnish Disease Heritage. Cells 2021;10:3158.
18. Wojczynski MK, Tiwari HK. Definition of phenotype. Adv Genet 2008;60:75–105.
19. Brown SDM. Advances in mouse genetics for the study of human disease. Hum Mol Genet 2021;30:R274–R284.
20. Adhikary PP, Ul Ain Q, Hocke AC, et al. COVID-19 highlights the model dilemma in biomedical research. Nat Rev Mater 2021;6:374–376.
21. Flores-Santin J, Burggren WW. Beyond the Chicken: Alternative Avian Models for Developmental Physiological Research. Front Physiol 2021;12:712633.
22. Howland D, Ellederova Z, Aronin N, et al. Large Animal Models of Huntington's Disease: What We Have Learned and Where We Need to Go Next. J Huntingt Dis 2020;9:201–216.
23. Leong X-F, Ng C-Y, Jaarin K. Animal Models in Cardiovascular Research: Hypertension and Atherosclerosis. BioMed Res Int 2015;2015:528757.
24. Obeid M, Khabbaz RC, Garcia KD, et al. Translational Animal Models for Liver Cancer. Am J Interv Radiol;2 . Epub ahead of print February 24, 2018. DOI: 10.25259/AJIR-11-2017.
25. Vandamme TF. Use of rodents as models of human diseases. J Pharm Bioallied Sci 2014;6:2–9.
26. Homberg JR, Adan RAH, Alenina N, et al. The continued need for animals to advance brain research. Neuron 2021;109:2374–2379.
27. Ma X, Aravind A, Pfister BJ, et al. Animal Models of Traumatic Brain Injury and Assessment of Injury Severity. Mol Neurobiol 2019;56:5332–5345.
28. Yee NS, Ignatenko N, Finnberg N, et al. Animal Models of Cancer Biology. Cancer Growth Metastasis 2015;8s1:CGM.S37907.
29. Procaccini C, De Rosa V, Pucino V, et al. Animal models of Multiple Sclerosis. Eur J Pharmacol 2015;759:182–191.
30. Wagar LE, DiFazio RM, Davis MM. Advanced model systems and tools for basic and translational human immunology. Genome Med 2018;10:73.
31. Yu X, Petersen F. A methodological review of induced animal models of autoimmune diseases. Autoimmun Rev 2018;17:473–479.
32. Zaragoza C, Gomez-Guerrero C, Martin-Ventura JL, et al. Animal models of cardiovascular diseases. J Biomed Biotechnol 2011;2011:497841.
33. Harris JC. Animal models of neurodevelopmental disorders with behavioral phenotypes. Curr Opin Psychiatry 2021;34:87–93.
34. Chadman KK. Animal models for autism in 2017 and the consequential implications to drug discovery. Expert Opin Drug Discov 2017;12:1187–1194.
35. Varghese M, Keshav N, Jacot-Descombes S, et al. Autism spectrum disorder: neuropathology and animal models. Acta Neuropathol (Berl) 2017;134:537–566.
36. Sontag TA, Tucha O, Walitza S, et al. Animal models of attention deficit/hyperactivity disorder (ADHD): a critical review. Atten Deficit Hyperact Disord 2010;2:1–20.
37. de la Peña JB, Dela Peña IJ, Custodio RJ, et al. Exploring the Validity of Proposed Transgenic Animal Models of Attention-Deficit Hyperactivity Disorder (ADHD). Mol Neurobiol 2018;55:3739–3754.
38. Winship IR, Dursun SM, Baker GB, et al. An Overview of Animal Models Related to Schizophrenia. Can J Psychiatry Rev Can Psychiatr 2019;64:5–17.
39. Götz J, Bodea L-G, Goedert M. Rodent models for Alzheimer disease. Nat Rev Neurosci 2018;19:583–598.
40. Mullane K, Williams M. Preclinical Models of Alzheimer's Disease: Relevance and Translational Validity. Curr Protoc Pharmacol 2019;84:e57.
41. Scearce-Levie K, Sanchez PE, Lewcock JW. Leveraging preclinical models for the development of Alzheimer disease therapeutics. Nat Rev Drug Discov 2020;19:447–462.
42. Barker RA, Björklund A. Animal Models of Parkinson's Disease: Are They Useful or Not? J Park Dis 2020;10:1335–1342.
43. Farshim PP, Bates GP. Mouse Models of Huntington's Disease. In: Precious SV, Rosser AE, Dunnett SB (eds) Huntington's Disease. New York, NY: Springer:97–120.
44. Menalled L, Brunner D. Animal models of Huntington's

disease for translation to the clinic: best practices. Mov Disord Off J Mov Disord Soc 2014;29:1375–1390.
45. Lutz C. Mouse models of ALS: Past, present and future. Brain Res 2018;1693:1–10.
46. Kin K, Yasuhara T, Kameda M, et al. Animal Models for Parkinson's Disease Research: Trends in the 2000s. Int J Mol Sci 2019;20:E5402.
47. Pingale T, Gupta GL. Classic and evolving animal models in Parkinson's disease. Pharmacol Biochem Behav 2020;199:173060.
48. Abboud C, Duveau A, Bouali-Benazzouz R, et al. Animal models of pain: Diversity and benefits. J Neurosci Methods 2021;348:108997.
49. Brum ES, Becker G, Fialho MFP, et al. Animal models of fibromyalgia: What is the best choice? Pharmacol Ther 2022;230:107959.
50. Brookes E, Shi Y. Diverse epigenetic mechanisms of human disease. Annu Rev Genet 2014;48:237–268.
51. Heindel JJ. The developmental basis of disease: Update on environmental exposures and animal models. Basic Clin Pharmacol Toxicol 2019;125 Suppl 3:5–13.
52. Ong M-L, Lin X, Holbrook JD. Measuring epigenetics as the mediator of gene/environment interactions in DOHaD. J Dev Orig Health Dis 2015;6:10–16.
53. Phillips NLH, Roth TL. Animal Models and Their Contribution to Our Understanding of the Relationship Between Environments, Epigenetic Modifications, and Behavior. Genes 2019;10:E47.
54. Heegaard PMH, Sturek M, Alloosh M, et al. Animal Models for COVID-19: More to the Picture Than ACE2, Rodents, Ferrets, and Non-human Primates. A Case for Porcine Respiratory Coronavirus and the Obese Ossabaw Pig. Front Microbiol 2020;11:573756.
55. Muñoz-Fontela C, Dowling WE, Funnell SGP, et al. Animal models for COVID-19. Nature 2020;586:509–515.
56. Pandey K, Acharya A, Mohan M, et al. Animal models for SARS-CoV-2 research: A comprehensive literature review. Transbound Emerg Dis 2021;68:1868–1885.
57. Singh A, Singh RS, Sarma P, et al. A Comprehensive Review of Animal Models for Coronaviruses: SARS-CoV-2, SARS-CoV, and MERS-CoV. Virol Sin 2020;35:290–304.
58. Genzel L, Adan R, Berns A, et al. How the COVID-19 pandemic highlights the necessity of animal research. Curr Biol CB 2020;30:R1014–R1018.
59. Pechanova O. Why We Still Need Reliable Animal Models. Pathophysiology 2020;27:44–45.
60. Walker A, Pottinger G, Scott A, et al. Anosmia and loss of smell in the era of covid-19. BMJ 2020;370:m2808.
61. Paolo G. Does COVID-19 cause permanent damage to olfactory and gustatory function? Med Hypotheses 2020;143:110086.
62. Jafari Z, Kolb BE, Mohajerani MH. Hearing Loss, Tinnitus, and Dizziness in COVID-19: A Systematic Review and Meta-Analysis. Can J Neurol Sci 2022; 49(2): 184–195.
63. Wu Y, Xu X, Chen Z, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18–22.
64. Fotuhi M, Mian A, Meysami S, et al. Neurobiology of COVID-19. J Alzheimers Dis JAD 2020;76:3–19.
65. Iadecola C, Anrather J, Kamel H. Effects of COVID-19 on the Nervous System. Cell 2020;183:16-27.e1.
66. Ghazavi A, Ganji A, Keshavarzian N, et al. Cytokine profile and disease severity in patients with COVID-19. Cytokine 2021;137:155323.
67. Schultze JL, Aschenbrenner AC. COVID-19 and the human innate immune system. Cell 2021;184:1671–1692.
68. Somasundaram NP, Ranathunga I, Ratnasamy V, et al. The Impact of SARS-Cov-2 Virus Infection on the Endocrine System. J Endocr Soc 2020;4:bvaa082.
69. Raony Í, de Figueiredo CS, Pandolfo P, et al. Psycho-Neuroendocrine-Immune Interactions in COVID-19: Potential Impacts on Mental Health. Front Immunol 2020;11:1170.
70. Gurumurthy CB, Quadros RM, Richardson GP, et al. Genetically modified mouse models to help fight COVID-19. Nat Protoc 2020;15:3777–3787.
71. Ye W, Chen Q. Potential Applications and Perspectives of Humanized Mouse Models. Annu Rev Anim Biosci 2022;10:395–417.
72. Veenhuis RT, Zeiss CJ. Animal Models of COVID-19 II. Comparative Immunology. ILAR J 2021;ilab010.
73. Taft RA, Davisson M, Wiles MV. Know thy mouse. Trends Genet TIG 2006;22:649–653.
74. Blake JA, Baldarelli R, Kadin JA, et al. Mouse Genome Database (MGD): Knowledgebase for mouse–human comparative biology. Nucleic Acids Res 2021;49:D981–D987.
75. Bult CJ, Blake JA, Smith CL, et al. Mouse Genome Database (MGD) 2019. Nucleic Acids Res 2019;47:D801–D806.
76. Ringwald M, Richardson JE, Baldarelli RM, et al. Mouse Genome Informatics (MGI): latest news from MGD and GXD. Mamm Genome 2022;33:4–18.
77. Bogue MA, Philip VM, Walton DO, et al. Mouse Phenome Database: a data repository and analysis suite for curated primary mouse phenotype data. Nucleic Acids Res 2020;48:D716–D723.
78. Chia R, Achilli F, Festing MFW, et al. The origins and uses of mouse outbred stocks. Nat Genet 2005;37:1181–1186.
79. Tuttle AH, Philip VM, Chesler EJ, et al. Comparing phenotypic variation between inbred and outbred mice. Nat Methods 2018;15:994–996.
80. Tuttle AH, Philip VM, Chesler EJ, et al. Author Correction: Comparing phenotypic variation between inbred and outbred mice. Nat Methods 2019;16:206.
81. Tuttle AH, Philip VM, Chesler EJ, et al. Author Correction: Comparing phenotypic variation between inbred and outbred mice. Nat Methods 2020;17:947.
82. Saul MC, Philip VM, Reinholdt LG, et al. High-Diversity Mouse Populations for Complex Traits. Trends Genet 2019;35:501–514.
83. Beck JA, Lloyd S, Hafezparast M, et al. Genealogies of mouse inbred strains. Nat Genet 2000;24:23–25.

84. Casellas J. Inbred mouse strains and genetic stability: a review. Anim Int J Anim Biosci 2011;5:1–7.
85. Simpson EM, Linder CC, Sargent EE, et al. Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. Nat Genet 1997;16:19–27.
86. Threadgill DW, Yee D, Matin A, et al. Genealogy of the 129 inbred strains: 129/SvJ is a contaminated inbred strain. Mamm Genome Off J Int Mamm Genome Soc 1997;8:390–393.
87. Mekada K, Yoshiki A. Substrains matter in phenotyping of C57BL/6 mice. Exp Anim 2021;70:145–160.
88. Fertan E, Wong AA, Purdon MK, et al. The effect of background strain on the behavioral phenotypes of the MDGA2+/- mouse model of autism spectrum disorder. Genes Brain Behav 2021;20:e12696.
89. Tam WY, Cheung K-K. Phenotypic characteristics of commonly used inbred mouse strains. J Mol Med 2020;98:1215–1234.
90. Flaherty L, Bolivar V. Congenic and Consomic Strains. In: Byron C. Jones and Pierre Mormede (editors). Neurobehavioral Genetics: Methods and Applications, second edition. Boca Raton: CRC Press: 2006; 115-128.
91. Farkas C, Fuentes-Villalobos F, Rebolledo-Jaramillo B, et al. Streamlined computational pipeline for genetic background characterization of genetically engineered mice based on next generation sequencing data. BMC Genomics 2019;20:131.
92. Schellinck HM, Cyr DP, Brown RE. Chapter 7 - How Many Ways Can Mouse Behavioral Experiments Go Wrong? Confounding Variables in Mouse Models of Neurodegenerative Diseases and How to Control Them. In: Brockmann HJ, Roper TJ, Naguib M, et al. (eds) Advances in the Study of Behavior. Academic Press: 2010;41: 255–366.
93. Vanden Berghe T, Hulpiau P, Martens L, et al. Passenger Mutations Confound Interpretation of All Genetically Modified Congenic Mice. Immunity 2015;43:200–209.
94. Gurumurthy CB, Lloyd KCK. Generating mouse models for biomedical research: technological advances. Dis Model Mech 2019;12:dmm029462.
95. Fujiwara S. Humanized mice: A brief overview on their diverse applications in biomedical research. J Cell Physiol 2018;233:2889–2901.
96. Acevedo-Arozena A, Wells S, Potter P, et al. ENU mutagenesis, a way forward to understand gene function. Annu Rev Genomics Hum Genet 2008;9:49–69.
97. Simon MM, Moresco EMY, Bull KR, et al. Current strategies for mutation detection in phenotype-driven screens utilising next generation sequencing. Mamm Genome Off J Int Mamm Genome Soc 2015;26:486–500.
98. Fuchs H, Gailus-Durner V, Adler T, et al. Mouse phenotyping. Methods 2011;53:120–135.
99. Lalonde R, Filali M, Strazielle C. SHIRPA as a Neurological Screening Battery in Mice. Curr Protoc 2021;1:e135.
100. Rogers DC, Fisher EM, Brown SD, et al. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mamm Genome Off J Int Mamm Genome Soc 1997;8:711–713.
101. Crawley JN. What's wrong with my mouse?: behavioral phenotyping of transgenic and knockout mice. Hoboken, N.J.: Wiley-Interscience Available from: http://www.123library.org/book_details/?id=15036. 2007. Accessed March 8, 2022.
102. Garcia-Gomes MSA, Zanatto DA, Yamamoto PK, et al. A Simple and Fast Battery Test for Phenotypic Characterization of Mice. Bio-Protoc 2020;10:e3568.
103. van der Staay FJ, Steckler T. Behavioural phenotyping of mouse mutants. Behav Brain Res 2001;125:3–12.
104. Wahlsten D. Mouse behavioral testing: how to use mice in behavioral neuroscience. 1st ed. London ; Burlington, VT: Academic, 2011.
105. Jaisser F. Inducible gene expression and gene modification in transgenic mice. J Am Soc Nephrol JASN 2000;11 Suppl 16:S95–S100.
106. Si-Hoe SL, Wells S, Murphy D. Production of transgenic rodents by the microinjection of cloned DNA into fertilized one-cell eggs. Mol Biotechnol 2001;17:151–182.
107. Doyle A, McGarry MP, Lee NA, et al. The construction of transgenic and gene knockout/knockin mouse models of human disease. Transgenic Res 2012;21:327–349.
108. Hall B, Limaye A, Kulkarni AB. Overview: Generation of Gene Knockout Mice. Curr Protoc Cell Biol 2009;44:19.12.1-19.12.17.
109. Hamilton SM, Spencer CM, Harrison WR, et al. Multiple autism-like behaviors in a novel transgenic mouse model. Behav Brain Res 2011;218:29–41.
110. Jacquot S, Chartoire N, Piguet F, et al. Optimizing PCR for Mouse Genotyping: Recommendations for Reliable, Rapid, Cost Effective, Robust and Adaptable to High-Throughput Genotyping Protocol for Any Type of Mutation. Curr Protoc Mouse Biol 2019;9:e65.
111. Panneer SK, Arindkar SK, Nagarajan P. Mouse Genetics and Breeding. In: Nagarajan P, Gudde R, Srinivasan R (eds) Essentials of Laboratory Animal Science: Principles and Practices. Singapore: Springer: 2021;343–371.
112. Vaisman BL. Genotyping of Transgenic Animals by Real-Time Quantitative PCR with TaqMan Probes. In: Freeman LA (ed) Lipoproteins and Cardiovascular Disease: Methods and Protocols. Totowa, NJ: Humana Press:233–251.
113. Delic S, Streif S, Deussing JM, et al. Genetic mouse models for behavioral analysis through transgenic RNAi technology. Genes Brain Behav 2008;7:821–830.
114. Mittal V. Improving the efficiency of RNA interference in mammals. Nat Rev Genet 2004;5:355–365.
115. Du P, Lou C, Zhao X, et al. CRISPR-Based Genetic Switches and Other Complex Circuits: Research and Application. Life 2021;11:1255.
116. Horii T, Arai Y, Yamazaki M, et al. Validation of microinjection methods for generating knockout mice by CRISPR/

Cas-mediated genome engineering. Sci Rep 2014;4:4513.
117. Lanigan TM, Kopera HC, Saunders TL. Principles of Genetic Engineering. Genes 2020;11:291.
118. Nakajima K, Kazuno A-A, Kelsoe J, et al. Exome sequencing in the knockin mice generated using the CRISPR/Cas system. Sci Rep 2016;6:34703.
119. Brehm MA, Shultz LD, Greiner DL. Humanized mouse models to study human diseases. Curr Opin Endocrinol Diabetes Obes 2010;17:120–125.
120. Tian H, Lyu Y, Yang Y-G, et al. Humanized Rodent Models for Cancer Research. Front Oncol 2020;10:1696.
121. Dash PK, Gorantla S, Poluektova L, et al. Humanized Mice for Infectious and Neurodegenerative disorders. Retrovirology 2021;18:13.
122. Devoy A, Bunton-Stasyshyn RKA, Tybulewicz VLJ, et al. Genomically humanized mice: technologies and promises. Nat Rev Genet 2012;13:14–20.
123. Buffalo EA, Movshon JA, Wurtz RH. From basic brain research to treating human brain disorders. Proc Natl Acad Sci U S A 2019;201919895.
124. Molnár Z, Clowry G. Cerebral cortical development in rodents and primates. Prog Brain Res 2012;195:45–70.
125. Savolainen SM, Foley JF, Elmore SA. Histology atlas of the developing mouse heart with emphasis on E11.5 to E18.5. Toxicol Pathol 2009;37:395–414.
126. Gensollen T, Iyer SS, Kasper DL, et al. How colonization by microbiota in early life shapes the immune system. Science 2016;352:539–544.
127. Ackert-Bicknell CL, Anderson LC, Sheehan S, et al. Aging Research Using Mouse Models. Curr Protoc Mouse Biol 2015;5:95–133.
128. Vanhooren V, Libert C. The mouse as a model organism in aging research: usefulness, pitfalls and possibilities. Ageing Res Rev 2013;12:8–21.
129. Ximerakis M, Lipnick SL, Innes BT, et al. Single-cell transcriptomic profiling of the aging mouse brain. Nat Neurosci 2019;22:1696–1708.
130. Shimada A, Hasegawa-Ishii S. Senescence-accelerated Mice (SAMs) as a Model for Brain Aging and Immunosenescence. Aging Dis 2011;2:414–435.
131. Shirakabe A, Ikeda Y, Sciarretta S, et al. Aging and Autophagy in the Heart. Circ Res 2016;118:1563–1576.
132. Kaushal A, Wani WY, Anand R, et al. Spontaneous and induced nontransgenic animal models of AD: modeling AD using combinatorial approach. Am J Alzheimers Dis Other Demen 2013;28:318–326.
133. Partridge B, Rossmeisl JH. Companion animal models of neurological disease. J Neurosci Methods 2020;331:108484.
134. Cekanova M, Rathore K. Animal models and therapeutic molecular targets of cancer: utility and limitations. Drug Des Devel Ther 2014;8:1911–1921.
135. Vail DM, MacEwen EG. Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest 2000;18:781–792.
136. Jackson JG, Lozano G. The mutant p53 mouse as a pre-clinical model. Oncogene 2013;32:4325–4330.
137. Lampreht Tratar U, Horvat S, Cemazar M. Transgenic Mouse Models in Cancer Research. Front Oncol;2018;8.
138. Reza Khorramizadeh M, Saadat F. Chapter 8 - Animal models for human disease. In: Verma AS, Singh A (eds) Animal Biotechnology (Second Edition). Boston: Academic Press:2020;153–171.
139. Ruggeri BA, Camp F, Miknyoczki S. Animal models of disease: pre-clinical animal models of cancer and their applications and utility in drug discovery. Biochem Pharmacol 2014;87:150–161.
140. Rapoport B, Banuelos B, Aliesky HA, et al. Critical Differences between Induced and Spontaneous Mouse Models of Graves' Disease with Implications for Antigen-Specific Immunotherapy in Humans. J Immunol 2016; 197(12): 4560–4568.
141. Animals Behind Top Drugs. Foundation for Biomedical Research Available from: https://fbresearch.org/medical-advances/animal-research-achievements/animal-research-top-drugs/. 2021. Accessed February 28, 2022.
142. Mohs RC, Greig NH. Drug discovery and development: Role of basic biological research. Alzheimers Dement Transl Res Clin Interv 2017;3:651–657.
143. Ireson CR, Alavijeh MS, Palmer AM, et al. The role of mouse tumour models in the discovery and development of anticancer drugs. Br J Cancer 2019;121:101–108.
144. Wege AK. Humanized Mouse Models for the Preclinical Assessment of Cancer Immunotherapy. BioDrugs Clin Immunother Biopharm Gene Ther 2018;32:245–266.
145. Berry-Kravis EM, Lindemann L, Jønch AE, et al. Drug development for neurodevelopmental disorders: lessons learned from fragile X syndrome. Nat Rev Drug Discov 2018;17:280–299.
146. Chadman KK, Fernandes S, DiLiberto E, et al. Do animal models hold value in Autism spectrum disorder (ASD) drug discovery? Expert Opin Drug Discov 2019;14:727–734.
147. Díaz-Caneja CM, State MW, Hagerman RJ, et al. A white paper on a neurodevelopmental framework for drug discovery in autism and other neurodevelopmental disorders. Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol 2021;48:49–88.
148. Howe JR, Bear MF, Golshani P, et al. The mouse as a model for neuropsychiatric drug development. Curr Biol CB 2018;28:R909–R914.
149. Tricklebank MD, Robbins TW, Simmons C, et al. Time to re-engage psychiatric drug discovery by strengthening confidence in preclinical psychopharmacology. Psychopharmacology (Berl) 2021;238:1417–1436.
150. Cacabelos R, Carrera I, Martínez-Iglesias O, et al. What is the gold standard model for Alzheimer's disease drug discovery and development? Expert Opin Drug Discov 2021;16:1415–1440.
151. Koprich JB, Kalia LV, Brotchie JM. Animal models of α-synucleinopathy for Parkinson disease drug development.

Nat Rev Neurosci 2017;18:515–529.
152. Vitek MP, Araujo JA, Fossel M, et al. Translational animal models for Alzheimer's disease: An Alzheimer's Association Business Consortium Think Tank. Alzheimers Dement N Y N 2020;6:e12114.
153. Sun W, Zheng W, Simeonov A. Drug discovery and development for rare genetic disorders. Am J Med Genet A 2017;173:2307–2322.
154. Singh VK, Seed TM. How necessary are animal models for modern drug discovery? Expert Opin Drug Discov 2021;16:1391–1397.
155. Jellinger KA. Towards a Biological Definition of Alzheimer Disease. Int J Neurol Neurother 2020; 7(1): 095.
156. Jellinger KA. Recent update on the heterogeneity of the Alzheimer's disease spectrum. J Neural Transm 2022;129:1–24.
157. Fereshtehnejad S-M, Postuma RB. Subtypes of Parkinson's Disease: What Do They Tell Us About Disease Progression? Curr Neurol Neurosci Rep 2017;17:34.
158. Qian E, Huang Y. Subtyping of Parkinson's Disease - Where Are We Up To? Aging Dis 2019;10:1130.
159. Agelink van Rentergem JA, Deserno MK, Geurts HM. Validation strategies for subtypes in psychiatry: A systematic review of research on autism spectrum disorder. Clin Psychol Rev 2021;87:102033.
160. Easson AK, Fatima Z, McIntosh AR. Functional connectivity-based subtypes of individuals with and without autism spectrum disorder. Netw Neurosci 2019;3:344–362.
161. Drummond E, Wisniewski T. Alzheimer's disease: experimental models and reality. Acta Neuropathol (Berl) 2017;133:155–175.
162. Gurdon B, Kaczorowski C. Pursuit of precision medicine: Systems biology approaches in Alzheimer's disease mouse models. Neurobiol Dis 2021;161:105558.
163. Devi G, Scheltens P. Heterogeneity of Alzheimer's disease: consequence for drug trials? Alzheimers Res Ther 2018;10:122.
164. Marras C, Chaudhuri KR, Titova N, et al. Therapy of Parkinson's Disease Subtypes. Neurotherapeutics 2020;17:1366–1377.
165. Ringman JM, Goate A, Masters CL, et al. Genetic heterogeneity in Alzheimer disease and implications for treatment strategies. Curr Neurol Neurosci Rep 2014;14:499.
166. Forloni G. Alzheimer's disease: from basic science to precision medicine approach. BMJ Neurol Open 2020;2:e000079.
167. Galvin JE. Advancing personalized treatment of Alzheimer's disease: a call for the N-of-1 trial design. Future Neurol 2018;13:151–160.
168. Ryden LE, Lewis SJG. Parkinson's Disease in the Era of Personalised Medicine: One Size Does Not Fit All. Drugs Aging 2019;36:103–113.
169. Bhardwaj S, Kesari KK, Rachamalla M, et al. CRISPR/Cas9 gene editing: New hope for Alzheimer's disease therapeutics. J Adv Res. 2021.
170. Cring MR, Sheffield VC. Gene therapy and gene correction: targets, progress, and challenges for treating human diseases. Gene Ther 2022;29:3–12.
171. Dunbar CE, High KA, Joung JK, et al. Gene therapy comes of age. Science 2018;359:eaan4672.
172. Carrillo MA, Zhen A, Kitchen SG. The Use of the Humanized Mouse Model in Gene Therapy and Immunotherapy for HIV and Cancer. Front Immunol 2018;9:746.
173. Gopinath C, Nathar TJ, Ghosh A, et al. Contemporary Animal Models For Human Gene Therapy Applications. Curr Gene Ther 2015;15:531–540.
174. Ingusci S, Verlengia G, Soukupova M, et al. Gene Therapy Tools for Brain Diseases. Front Pharmacol 2019;10:724.
175. Park H, Oh J, Shim G, et al. In vivo neuronal gene editing via CRISPR–Cas9 amphiphilic nanocomplexes alleviates deficits in mouse models of Alzheimer's disease. Nat Neurosci 2019;22:524–528.
176. Stepanichev M. Gene Editing and Alzheimer's Disease: Is There Light at the End of the Tunnel? Front Genome Ed 2020;2:4.
177. Yoo TJ. Anti-Inflammatory Gene Therapy Improves Spatial Memory Performance in a Mouse Model of Alzheimer's Disease. J Alzheimers Dis 2022; 85(3): 1001–1008.
178. Van Laar AD, Van Laar VS, San Sebastian W, et al. An Update on Gene Therapy Approaches for Parkinson's Disease: Restoration of Dopaminergic Function. J Park Dis 2021;11:S173–S182.
179. Wu Z, Parry M, Hou X-Y, et al. Gene therapy conversion of striatal astrocytes into GABAergic neurons in mouse models of Huntington's disease. Nat Commun 2020;11:1105.
180. Yang S, Chang R, Yang H, et al. CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. J Clin Invest 2017;127:2719–2724.
181. Davidsohn N, Pezone M, Vernet A, et al. A single combination gene therapy treats multiple age-related diseases. Proc Natl Acad Sci U S A 2019;116:23505–23511.
182. Blusztajn JK, Slack BE, Mellott TJ. Neuroprotective Actions of Dietary Choline. Nutrients 2017;9:E815.
183. Zilkha N, Kuperman Y, Kimchi T. High-fat diet exacerbates cognitive rigidity and social deficiency in the BTBR mouse model of autism. Neuroscience 2017;345:142–154.
184. Lilamand M, Porte B, Cognat E, et al. Are ketogenic diets promising for Alzheimer's disease? A translational review. Alzheimers Res Ther 2020;12:42.
185. Lin K-H, Chiu C-H, Kuo W-W, et al. The preventive effects of edible folic acid on cardiomyocyte apoptosis and survival in early onset triple-transgenic Alzheimer's disease model mice. Environ Toxicol 2018;33:83–92.
186. Gao X, Sanderson SM, Dai Z, et al. Dietary methionine influences therapy in mouse cancer models and alters human metabolism. Nature 2019;572:397–401.
187. Abid MA, Abid MB. Commentary: Dietary methionine influences therapy in mouse cancer models and alters human metabolism. Front Oncol 2020; 10. 2020. Accessed April 3, 2022.

188. Wanders D, Hobson K, Ji X. Methionine Restriction and Cancer Biology. Nutrients 2020;12:684.
189. Xu Y, Jiang C, Wu J, et al. Ketogenic diet ameliorates cognitive impairment and neuroinflammation in a mouse model of Alzheimer's disease. CNS Neurosci Ther 2022;28:580–592.
190. Brady M, Beltramini A, Vaughan G, et al. Benefits of a ketogenic diet on repetitive motor behavior in mice. Behav Brain Res 2022;422:113748.
191. Norwitz NG, Dalai SS, Palmer CM. Ketogenic diet as a metabolic treatment for mental illness. Curr Opin Endocrinol Diabetes Obes 2020;27:269–274.
192. Wu J, de Theije CGM, da Silva SL, et al. Dietary interventions that reduce mTOR activity rescue autistic-like behavioral deficits in mice. Brain Behav Immun 2017;59:273–287.
193. Chin EWM, Lim WM, Ma D, et al. Choline Rescues Behavioural Deficits in a Mouse Model of Rett Syndrome by Modulating Neuronal Plasticity. Mol Neurobiol 2019;56:3882–3896.
194. Vuillermot S, Luan W, Meyer U, et al. Vitamin D treatment during pregnancy prevents autism-related phenotypes in a mouse model of maternal immune activation. Mol Autism 2017;8:9.
195. Ribeiro MC, MacDonald JL. Vitamin D modulates cortical transcriptome and behavioral phenotypes in an Mecp2 heterozygous Rett syndrome mouse model. Neurobiol Dis 2022;165:105636.
196. Lu W-T, Sun S-Q, Li Y, et al. Curcumin Ameliorates Memory Deficits by Enhancing Lactate Content and MCT2 Expression in APP/PS1 Transgenic Mouse Model of Alzheimer's Disease. Anat Rec 2019;302:332–338.
197. Reddy PH, Manczak M, Yin X, et al. Protective Effects of Indian Spice Curcumin Against Amyloid-β in Alzheimer's Disease. J Alzheimers Dis 2018;61:843–866.
198. De Filippis F, Vitaglione P, Cuomo R, et al. Dietary Interventions to Modulate the Gut Microbiome—How Far Away Are We From Precision Medicine. Inflamm Bowel Dis 2018;24:2142–2154.
199. Newell C, Bomhof MR, Reimer RA, et al. Ketogenic diet modifies the gut microbiota in a murine model of autism spectrum disorder. Mol Autism 2016;7:37.
200. Cryan JF, O'Riordan KJ, Sandhu K, et al. The gut microbiome in neurological disorders. Lancet Neurol 2020;19:179–194.
201. Kraeuter A-K, Phillips R, Sarnyai Z. Ketogenic therapy in neurodegenerative and psychiatric disorders: From mice to men. Prog Neuropsychopharmacol Biol Psychiatry 2020;101:109913.
202. Zhang C, Franklin CL, Ericsson AC. Consideration of Gut Microbiome in Murine Models of Diseases. Microorganisms 2021;9:1062.
203. Rogers J, Renoir T, Hannan AJ. Gene-environment interactions informing therapeutic approaches to cognitive and affective disorders. Neuropharmacology 2019;145:37–48.
204. Eisinger BE, Zhao X. Identifying molecular mediators of environmentally enhanced neurogenesis. Cell Tissue Res 2018;371:7–21.
205. Garthe A, Roeder I, Kempermann G. Mice in an enriched environment learn more flexibly because of adult hippocampal neurogenesis. Hippocampus 2016;26:261–271.
206. Grońska-Pęski M, Gonçalves JT, Hébert JM. Enriched Environment Promotes Adult Hippocampal Neurogenesis through FGFRs. J Neurosci Off J Soc Neurosci 2021;41:2899–2910.
207. De Sousa RAL, Rodrigues CM, Mendes BF, et al. Physical exercise protocols in animal models of Alzheimer's disease: a systematic review. Metab Brain Dis 2021;36:85–95.
208. da Silva WAB, Ferreira Oliveira K, Caroline Vitorino L, et al. Physical exercise increases the production of tyrosine hydroxylase and CDNF in the spinal cord of a Parkinson's disease mouse model. Neurosci Lett 2021;760:136089.
209. Houdebine L, Gallelli CA, Rastelli M, et al. Effect of physical exercise on brain and lipid metabolism in mouse models of multiple sclerosis. Chem Phys Lipids 2017;207:127–134.
210. Forbes TA, Goldstein EZ, Dupree JL, et al. Environmental enrichment ameliorates perinatal brain injury and promotes functional white matter recovery. Nat Commun 2020;11:964.
211. Livingston-Thomas J, Nelson P, Karthikeyan S, et al. Exercise and Environmental Enrichment as Enablers of Task-Specific Neuroplasticity and Stroke Recovery. Neurother J Am Soc Exp Neurother 2016;13:395–402.
212. Huang Y, Jiang H, Zheng Q, et al. Environmental enrichment or selective activation of parvalbumin-expressing interneurons ameliorates synaptic and behavioral deficits in animal models with schizophrenia-like behaviors during adolescence. Mol Psychiatry 2021;26:2533–2552.
213. Robison LS, Francis N, Popescu DL, et al. Environmental Enrichment: Disentangling the Influence of Novelty, Social, and Physical Activity on Cerebral Amyloid Angiopathy in a Transgenic Mouse Model. Int J Mol Sci 2020;21:E843.
214. Gerdts V, Littel-van den Hurk S van D, Griebel PJ, et al. Use of animal models in the development of human vaccines. Future Microbiol 2007;2:667–675.
215. Kiros TG, Levast B, Auray G, et al. The Importance of Animal Models in the Development of Vaccines. Innov Vaccinol 2012;251–264.
216. Atlante S, Mongelli A, Barbi V, et al. The epigenetic implication in coronavirus infection and therapy. Clin Epigenetics 2020;12:156.
217. Nehme Z, Pasquereau S, Herbein G. Control of viral infections by epigenetic-targeted therapy. Clin Epigenetics 2019;11:55.
218. Hwang J-R, Park S-G. Mouse models for hepatitis B virus research. Lab Anim Res 2018;34:85–91.
219. Krishnakumar V, Durairajan SSK, Alagarasu K, et al. Recent Updates on Mouse Models for Human Immunode-

ficiency, Influenza, and Dengue Viral Infections. Viruses 2019;11:252.
220. Sarkar S, Heise MT. Mouse Models as Resources for Studying Infectious Diseases. Clin Ther 2019;41:1912–1922.
221. Ji W, Gong B, Jin H, et al. Recent Progress Towards Vaccines and Antibody-based Therapies Against Alzheimer's Disease. Mini-Rev Med Chem 2021; 21(19): 3062–3072.
222. Carrera I, Etcheverría I, Fernández-Novoa L, et al. Vaccine Development to Treat Alzheimer's Disease Neuropathology in APP/PS1 Transgenic Mice. Int J Alzheimers Dis 2012; 2012e376138.
223. Cossu D, Ruberto S, Yokoyama K, et al. Efficacy of BCG vaccine in animal models of neurological disorders. Vaccine 2022;40:432–436.
224. Herline K, Drummond E, Wisniewski T. Recent advancements toward therapeutic vaccines against Alzheimer's disease. Expert Rev Vaccines 2018;17:707–721.
225. Banik A, Brown RE, Bamburg J, et al. Translation of Pre-Clinical Studies into Successful Clinical Trials for Alzheimer's Disease: What are the Roadblocks and How Can They Be Overcome? J Alzheimers Dis 2015; 47(4): 815–843.
226. Homberg JR, Kyzar EJ, Stewart AM, et al. Improving treatment of neurodevelopmental disorders: recommendations based on preclinical studies. Expert Opin Drug Discov 2016;11:11–25.
227. Bockamp E, Maringer M, Spangenberg C, et al. Of mice and models: improved animal models for biomedical research. Physiol Genomics 2002;11:115–132.
228. Cibelli J, Emborg ME, Prockop DJ, et al. Strategies for improving animal models for regenerative medicine. Cell Stem Cell 2013;12:271–274.
229. Stewart AM, Kalueff AV. Developing better and more valid animal models of brain disorders. Behav Brain Res 2015;276:28–31.
230. Lama J, Buhidma Y, Fletcher EJR, et al. Animal models of Parkinson's disease: a guide to selecting the optimal model for your research. Neuronal Signal 2021;5:NS20210026.
231. Reardon S. Frustrated Alzheimer's researchers seek better lab mice. Nature 2018;563:611–612.
232. Li C, Briner A, Götz J. The search for improved animal models of Alzheimer's disease and novel strategies for therapeutic intervention. Future Med Chem 2019;11:1853–1857.
233. Veening-Griffioen DH, Ferreira GS, van Meer PJK, et al. Are some animal models more equal than others? A case study on the translational value of animal models of efficacy for Alzheimer's disease. Eur J Pharmacol 2019;859:172524.
234. Tai LM, Maldonado Weng J, LaDu MJ, et al. Chapter One - Relevance of transgenic mouse models for Alzheimer's disease. In: Teplow DB (ed) Progress in Molecular Biology and Translational Science. 2021;177: 1–48.
235. Nadeau JH, Auwerx J. The virtuous cycle of human genetics and mouse models in drug discovery. Nat Rev Drug Discov 2019;18:255–272.
236. Mckean NE, Handley RR, Snell RG. A Review of the Current Mammalian Models of Alzheimer's Disease and Challenges That Need to Be Overcome. Int J Mol Sci 2021;22:13168.
237. Rahi V, Kumar P. Animal models of attention-deficit hyperactivity disorder (ADHD). Int J Dev Neurosci Off J Int Soc Dev Neurosci 2021;81:107–124.
238. Espíndola SL, Damianich A, Alvarez RJ, et al. Modulation of Tau Isoforms Imbalance Precludes Tau Pathology and Cognitive Decline in a Mouse Model of Tauopathy. Cell Rep 2018;23:709–715.
239. Fung CW, Guo J, Fu H, et al. Atrophy associated with tau pathology precedes overt cell death in a mouse model of progressive tauopathy. Sci Adv 2020;6:eabc8098.
240. Jankowsky JL, Zheng H. Practical considerations for choosing a mouse model of Alzheimer's disease. Mol Neurodegener 2017;12:89.
241. Kaye J, Reisine T, Finkbeiner S. Huntington's disease mouse models: unraveling the pathology caused by CAG repeat expansion. Fac Rev 2021; 10(77).
242. Gunn RK, Huentelman MJ, Brown RE. Are Sema5a mutant mice a good model of autism? A behavioral analysis of sensory systems, emotionality and cognition. Behav Brain Res 2011;225:142–150.
243. Verma V, Paul A, Amrapali Vishwanath A, et al. Understanding intellectual disability and autism spectrum disorders from common mouse models: synapses to behaviour. Open Biol 2019;9:180265.
244. Cortés N, Andrade V, Maccioni RB. Behavioral and Neuropsychiatric Disorders in Alzheimer's Disease. J Alzheimers Dis JAD 2018;63:899–910.
245. Locci A, Orellana H, Rodriguez G, et al. Comparison of memory, affective behavior, and neuropathology in APPNLGF knock-in mice to 5xFAD and APP/PS1 mice. Behav Brain Res 2021;404:113192.
246. Seo N-Y, Kim GH, Noh JE, et al. Selective Regional Loss of Cortical Synapses Lacking Presynaptic Mitochondria in the 5xFAD Mouse Model. Front Neuroanat 2021;15:690168.
247. Whitesell JD, Buckley AR, Knox JE, et al. Whole brain imaging reveals distinct spatial patterns of amyloid beta deposition in three mouse models of Alzheimer's disease. J Comp Neurol 2019;527:2122–2145.
248. Brown RE, Bolivar S. The importance of behavioural bioassays in neuroscience. J Neurosci Methods 2018;300:68–76.
249. Puzzo D, Lee L, Palmeri A, et al. Behavioral assays with mouse models of Alzheimer's disease: practical considerations and guidelines. Biochem Pharmacol 2014;88:450–467.
250. Ameen-Ali KE, Wharton SB, Simpson JE, et al. Review: Neuropathology and behavioural features of transgenic murine models of Alzheimer's disease. Neuropathol Appl Neurobiol 2017;43:553–570.
251. Belyaev ND, Kellett KAB, Beckett C, et al. The transcriptionally active amyloid precursor protein (APP) intracellular domain is preferentially produced from the 695 iso-

form of APP in a {beta}-secretase-dependent pathway. J Biol Chem 2010;285:41443–41454.
252. Andrä K, Abramowski D, Duke M, et al. Expression of APP in transgenic mice: a comparison of neuron-specific promoters. Neurobiol Aging 1996;17:183–190.
253. Fontaine DA, Davis DB. Attention to Background Strain Is Essential for Metabolic Research: C57BL/6 and the International Knockout Mouse Consortium. Diabetes 2016;65:25–33.
254. Bryant CD, Zhang NN, Sokoloff G, et al. Behavioral differences among C57BL/6 substrains: implications for transgenic and knockout studies. J Neurogenet 2008;22:315–331.
255. Doetschman T. Influence of genetic background on genetically engineered mouse phenotypes. Methods Mol Biol Clifton NJ 2009;530:423–433.
256. Reilly KM. The Effects of Genetic Background of Mouse Models of Cancer: Friend or Foe? Cold Spring Harb Protoc 2016;2016:pdb.top076273.
257. Wong AA, Brown RE. Visual detection, pattern discrimination and visual acuity in 14 strains of mice. Genes Brain Behav 2006;5:389–403.
258. Rae EA, Brown RE. The problem of genotype and sex differences in life expectancy in transgenic AD mice. Neurosci Biobehav Rev 2015;57:238–251.
259. O'Leary TP, Mantolino HM, Stover KR, et al. Age-related deterioration of motor function in male and female 5xFAD mice from 3 to 16 months of age. Genes Brain Behav 2020;19:e12538.
260. O'Leary TP, Brown RE. Visuo-spatial learning and memory impairments in the 5xFAD mouse model of Alzheimer's disease: Effects of age, sex, albinism, and motor impairments. Genes Brain Behav 2022;e12794.
261. Stevens LM, Brown RE. Reference and working memory deficits in the 3xTg-AD mouse between 2 and 15-months of age: a cross-sectional study. Behav Brain Res 2015;278:496–505.
262. Lau JC, Lerch JP, Sled JG, et al. Longitudinal neuroanatomical changes determined by deformation-based morphometry in a mouse model of Alzheimer's disease. NeuroImage 2008;42:19–27.
263. Foidl BM, Humpel C. Can mouse models mimic sporadic Alzheimer's disease? Neural Regen Res 2020;15:401–406.
264. Maciejewska K, Czarnecka K, Szymański P. A review of the mechanisms underlying selected comorbidities in Alzheimer's disease. Pharmacol Rep PR 2021;73:1565–1581.
265. Martini AC, Forner S, Trujillo-Estrada L, et al. Past to Future: What Animal Models Have Taught Us About Alzheimer's Disease. J Alzheimers Dis 2018; 64(s1): S365–S378.
266. Wong AA, Brown RE. Prevention of vision loss protects against age-related impairment in learning and memory performance in DBA/2J mice. Front Aging Neurosci 2013;5:52.
267. Fakhoury M. Microglia and Astrocytes in Alzheimer's Disease: Implications for Therapy. Curr Neuropharmacol 2018;16:508–518.
268. Li H, Wei C, Zhou R, et al. Mouse models in modeling aging and cancer. Exp Gerontol 2019;120:88–94.
269. Bilkei-Gorzo A. Genetic mouse models of brain ageing and Alzheimer's disease. Pharmacol Ther 2014;142:244–257.
270. Boche D, Gordon MN. Diversity of transcriptomic microglial phenotypes in aging and Alzheimer's disease. Alzheimers Dement 2022;18:360–376.
271. O'Leary TimothyP, Shin S, Fertan E, et al. Reduced acoustic startle response and peripheral hearing loss in the 5xFAD mouse model of Alzheimer's disease. Genes Brain Behav 2017;16:554–563.
272. Blaney CE, Gunn RK, Stover KR, et al. Maternal genotype influences behavioral development of 3×Tg-AD mouse pups. Behav Brain Res 2013;252:40–48.
273. Weaver ICG, Cervoni N, Champagne FA, et al. Epigenetic programming by maternal behavior. Nat Neurosci 2004;7:847–854.
274. Agarwal D, Kumari R, Ilyas A, et al. Crosstalk between epigenetics and mTOR as a gateway to new insights in pathophysiology and treatment of Alzheimer's disease. Int J Biol Macromol 2021;192:895–903.
275. Griñán-Ferré C, Izquierdo V, Otero E, et al. Environmental Enrichment Improves Cognitive Deficits, AD Hallmarks and Epigenetic Alterations Presented in 5xFAD Mouse Model. Front Cell Neurosci 2018;12:224.
276. Zhang X, Hong R, Chen W, et al. The role of long noncoding RNA in major human disease. Bioorganic Chem 2019;92:103214.
277. Jakovcevski M, Akbarian S. Epigenetic mechanisms in neurological disease. Nat Med 2012;18:1194–1204.
278. Cholewa-Waclaw J, Bird A, Schimmelmann M von, et al. The Role of Epigenetic Mechanisms in the Regulation of Gene Expression in the Nervous System. J Neurosci 2016;36:11427–11434.
279. Banik A, Kandilya D, Ramya S, et al. Maternal Factors that Induce Epigenetic Changes Contribute to Neurological Disorders in Offspring. Genes 2017;8:E150.
280. Bale TL. Epigenetic and transgenerational reprogramming of brain development. Nat Rev Neurosci 2015;16:332–344.
281. Darwiche N. Epigenetic mechanisms and the hallmarks of cancer: an intimate affair. Am J Cancer Res 2020;10:1954–1978.
282. Herceg Z, Vaissière T. Epigenetic mechanisms and cancer: an interface between the environment and the genome. Epigenetics 2011;6:804–819.
283. Zhang Q, Cao X. Epigenetic regulation of the innate immune response to infection. Nat Rev Immunol 2019;19:417–432.
284. Lim TB, Foo SYR, Chen CK. The Role of Epigenetics in Congenital Heart Disease. Genes 2021;12:390.
285. Saul D, Kosinsky RL. Epigenetics of Aging and Aging-Associated Diseases. Int J Mol Sci 2021;22:401.
286. Bertogliat MJ, Morris-Blanco KC, Vemuganti R. Epigenetic mechanisms of neurodegenerative diseases and acute

brain injury. Neurochem Int 2020;133:104642.
287. Deegan DF, Nigam P, Engel N. Sexual Dimorphism of the Heart: Genetics, Epigenetics, and Development. Front Cardiovasc Med 2021;8:668252.
288. McCarthy MM, Nugent BM. Epigenetic Contributions to Hormonally-Mediated Sexual Differentiation of the Brain. J Neuroendocrinol 2013;25:1133–1140.
289. Yu YE, Xing Z, Do C, et al. Chapter 1 - Genetic and epigenetic pathways in Down syndrome: Insights to the brain and immune system from humans and mouse models. In: Dierssen M (ed) Progress in Brain Research. 2020;251:1–28.
290. Berdasco M, Esteller M. Genetic syndromes caused by mutations in epigenetic genes. Hum Genet 2013;132:359–383.
291. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33 Suppl:245–254.
292. Zapata-Martín Del Campo CM, Martínez-Rosas M, Guarner-Lans V. Epigenetic Programming of Synthesis, Release, and/or Receptor Expression of Common Mediators Participating in the Risk/Resilience for Comorbid Stress-Related Disorders and Coronary Artery Disease. Int J Mol Sci 2018;19:E1224.
293. Zhang T-Y, Meaney MJ. Epigenetics and the Environmental Regulation of the Genome and Its Function. Annu Rev Psychol 2010;61:439–466.
294. Linnér A, Almgren M. Epigenetic programming-The important first 1000 days. Acta Paediatr Oslo Nor 1992 2020;109:443–452.
295. Blewitt M, Whitelaw E. The Use of Mouse Models to Study Epigenetics. Cold Spring Harb Perspect Biol 2013;5:a017939.
296. Seki Y, Williams L, Vuguin PM, et al. Minireview: Epigenetic programming of diabetes and obesity: animal models. Endocrinology 2012;153:1031–1038.
297. Bianco-Miotto T, Craig JM, Gasser YP, et al. Epigenetics and DOHaD: from basics to birth and beyond. J Dev Orig Health Dis 2017;8:513–519.
298. Kubota T, Miyake K, Hariya N, et al. Understanding the epigenetics of neurodevelopmental disorders and DOHaD. J Dev Orig Health Dis 2015;6:96–104.
299. Simeoni U, Armengaud J-B, Siddeek B, et al. Perinatal Origins of Adult Disease. Neonatology 2018;113:393–399.
300. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell 2007;128:669–681.
301. Borrelli E, Nestler EJ, Allis CD, et al. Decoding the epigenetic language of neuronal plasticity. Neuron 2008;60:961–974.
302. Bonifer C, Cockerill PN. Chromatin priming of genes in development: Concepts, mechanisms and consequences. Exp Hematol 2017;49:1–8.
303. Ernst C, Jefri M. Epigenetic priming in neurodevelopmental disorders. Trends Mol Med 2021;27:1106–1114.
304. Mastrototaro G, Zaghi M, Sessa A. Epigenetic Mistakes in Neurodevelopmental Disorders. J Mol Neurosci MN 2017;61:590–602.
305. Gore AC, Krishnan K, Reilly MP. Endocrine-disrupting chemicals: Effects on neuroendocrine systems and the neurobiology of social behavior. Horm Behav 2019;111:7–22.
306. León-Olea M, Martyniuk CJ, Orlando EF, et al. Current concepts in neuroendocrine disruption. Gen Comp Endocrinol 2014;203:158–173.
307. Patisaul HB, Fenton SE, Aylor D. Animal models of endocrine disruption. Best Pract Res Clin Endocrinol Metab 2018;32:283–297.
308. Vicente-Dueñas C, Hauer J, Cobaleda C, et al. Epigenetic Priming in Cancer Initiation. Trends Cancer 2018;4:408–417.
309. Berson A, Nativio R, Berger SL, et al. Epigenetic Regulation in Neurodegenerative Diseases. Trends Neurosci 2018;41:587–598.
310. Burns AM, Gräff J. Cognitive epigenetic priming: leveraging histone acetylation for memory amelioration. Curr Opin Neurobiol 2021;67:75–84.
311. Qureshi IA, Mehler MF. Chapter 5 - Epigenetic mechanisms underlying nervous system diseases. In: Geschwind DH, Paulson HL, Klein C (eds) Handbook of Clinical Neurology. Elsevier:2018;43–58.
312. Takeshima H, Ushijima T. Accumulation of genetic and epigenetic alterations in normal cells and cancer risk. Npj Precis Oncol 2019;3:1–8.
313. Hoeijmakers L, Heinen Y, van Dam A-M, et al. Microglial Priming and Alzheimer's Disease: A Possible Role for (Early) Immune Challenges and Epigenetics? Front Hum Neurosci 2016;10:398.
314. Martins-Ferreira R, Leal B, Costa PP, et al. Microglial innate memory and epigenetic reprogramming in neurological disorders. Prog Neurobiol 2021;200:101971.
315. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. Nat Rev Neurol 2014;10:217–224.
316. Ganesan A, Arimondo PB, Rots MG, et al. The timeline of epigenetic drug discovery: from reality to dreams. Clin Epigenetics 2019;11:174.
317. Good KV, Vincent JB, Ausió J. MeCP2: The Genetic Driver of Rett Syndrome Epigenetics. Front Genet 2021;12:620859.
318. Vashi N, Justice MJ. Treating Rett syndrome: from mouse models to human therapies. Mamm Genome Off J Int Mamm Genome Soc 2019;30:90–110.
319. Mossink B, Negwer M, Schubert D, et al. The emerging role of chromatin remodelers in neurodevelopmental disorders: a developmental perspective. Cell Mol Life Sci CMLS 2021;78:2517–2563.
320. Bustos FJ, Ampuero E, Jury N, et al. Epigenetic editing of the Dlg4/PSD95 gene improves cognition in aged and Alzheimer's disease mice. Brain J Neurol 2017;140:3252–3268.
321. Coppedè F. Epigenetic regulation in Alzheimer's disease: is it a potential therapeutic target? Expert Opin Ther Targets 2021;25:283–298.
322. Ricq EL, Hooker JM, Haggarty SJ. Toward development

of epigenetic drugs for central nervous system disorders: Modulating neuroplasticity via H3K4 methylation. Psychiatry Clin Neurosci 2016;70:536–550.
323. Hogg SJ, Beavis PA, Dawson MA, et al. Targeting the epigenetic regulation of antitumour immunity. Nat Rev Drug Discov 2020;19:776–800.
324. Rugo HS, Jacobs I, Sharma S, et al. The Promise for Histone Methyltransferase Inhibitors for Epigenetic Therapy in Clinical Oncology: A Narrative Review. Adv Ther 2020;37:3059–3082.
325. Chen Z, Li S, Subramaniam S, et al. Epigenetic Regulation: A New Frontier for Biomedical Engineers. Annu Rev Biomed Eng 2017;19:195–219.
326. Horii T, Morita S, Hino S, et al. Successful generation of epigenetic disease model mice by targeted demethylation of the epigenome. Genome Biol 2020;21:77.
327. Pajovic S, Siddaway R, Bridge T, et al. Epigenetic activation of a RAS/MYC axis in H3.3K27M-driven cancer. Nat Commun 2020;11:6216.
328. Lardenoije R, van den Hove DLA, Havermans M, et al. Age-related epigenetic changes in hippocampal subregions of four animal models of Alzheimer's disease. Mol Cell Neurosci 2018;86:1–15.
329. Momoi T, Fujita E, Senoo H, et al. Genetic factors and epigenetic factors for autism: endoplasmic reticulum stress and impaired synaptic function. Cell Biol Int 2009;34:13–19.
330. Schepici G, Cavalli E, Bramanti P, et al. Autism Spectrum Disorder and miRNA: An Overview of Experimental Models. Brain Sci 2019;9:E265.
331. Schmitz RL, Weissbach J, Kleilein J, et al. Targeting HDACs in Pancreatic Neuroendocrine Tumor Models. Cells 2021;10:1408.
332. Grayson DR, Guidotti A. DNA Methylation in Animal Models of Psychosis. Prog Mol Biol Transl Sci 2018;157:105–132.
333. Monacelli F, Acquarone E, Giannotti C, et al. Vitamin C, Aging and Alzheimer's Disease. Nutrients 2017;9:E670.
334. Horsburgh S, Robson-Ansley P, Adams R, et al. Exercise and inflammation-related epigenetic modifications: focus on DNA methylation. Exerc Immunol Rev 2015;21:26–41.
335. Cabezudo D, Baekelandt V, Lobbestael E. Multiple-Hit Hypothesis in Parkinson's Disease: LRRK2 and Inflammation. Front Neurosci 2020;14:376.
336. Patrick KL, Bell SL, Weindel CG, et al. Exploring the "Multiple-Hit Hypothesis" of Neurodegenerative Disease: Bacterial Infection Comes Up to Bat. Front Cell Infect Microbiol 2019;9:138.
337. Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. Trends Neurosci 2006;29:349–358.
338. Arnold AP, Chen X. What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? Front Neuroendocrinol 2009;30:1–9.
339. Arnold AP, McCarthy MM. Sexual Differentiation of the Brain and Behavior: A Primer. In: Pfaff DW, Volkow ND (eds) Neuroscience in the 21st Century: From Basic to Clinical. New York, NY: Springer:2016;2139–2168.
340. Walker DM, Gore AC. Epigenetic impacts of endocrine disruptors in the brain. Front Neuroendocrinol 2017;44:1–26.
341. Schug TT, Blawas AM, Gray K, et al. Elucidating the Links Between Endocrine Disruptors and Neurodevelopment. Endocrinology 2015;156:1941–1951.
342. Kuehner JN, Bruggeman EC, Wen Z, et al. Epigenetic Regulations in Neuropsychiatric Disorders. Front Genet 2019;10:268.
343. Repouskou A, Papadopoulou A-K, Panagiotidou E, et al. Long term transcriptional and behavioral effects in mice developmentally exposed to a mixture of endocrine disruptors associated with delayed human neurodevelopment. Sci Rep 2020;10:9367.
344. Tan Q, Zoghbi HY. Mouse models as a tool for discovering new neurological diseases. Neurobiol Learn Mem 2019;165:106902.
345. Armstrong RA. What causes alzheimer's disease? Folia Neuropathol 2013;51:169–188.
346. Armstrong RA. Risk factors for Alzheimer's disease. Folia Neuropathol 2019;57:87–105.
347. Adams JD. Probable Causes of Alzheimer's Disease. Sci 2021;3:16.
348. Fertan E, Stover KRJ, Brant MG, et al. Effects of the Novel IDO Inhibitor DWG-1036 on the Behavior of Male and Female 3xTg-AD Mice. Front Pharmacol 2019;10:1044.
349. Sorgdrager FJH, Vermeiren Y, Van Faassen M, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. J Neurochem 2019;151:656–668.
350. Willette AA, Pappas C, Hoth N, et al. Inflammation, negative affect, and amyloid burden in Alzheimer's disease: Insights from the kynurenine pathway. Brain Behav Immun 2021;95:216–225.
351. Sharma VK, Singh TG, Prabhakar NK, et al. Kynurenine Metabolism and Alzheimer's Disease: The Potential Targets and Approaches. Neurochem Res . Epub ahead of print February 8, 2022. DOI: 10.1007/s11064-022-03546-8.
352. Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. Mol Basel Switz 2020;25:E5789.
353. Fertan E, Rodrigues GJ, Wheeler RV, et al. Cognitive Decline, Cerebral-Spleen Tryptophan Metabolism, Oxidative Stress, Cytokine Production, and Regulation of the Txnip Gene in a Triple Transgenic Mouse Model of Alzheimer Disease. Am J Pathol 2019;189:1435–1450.
354. Tsubaki H, Tooyama I, Walker DG. Thioredoxin-Interacting Protein (TXNIP) with Focus on Brain and Neurodegenerative Diseases. Int J Mol Sci 2020;21:E9357.
355. Oblak AL, Forner S, Territo PR, et al. Model organism development and evaluation for late-onset Alzhei-

mer's disease: MODEL-AD. Alzheimers Dement N Y N 2020;6:e12110.
356. Baglietto-Vargas D, Forner S, Cai L, et al. Generation of a humanized Aβ expressing mouse demonstrating aspects of Alzheimer's disease-like pathology. Nat Commun 2021;12:2421.
357. Kotredes KP, Oblak A, Pandey RS, et al. APOEe4. Trem2*R47H Mice Show Changes in Alzheimer's Disease-Relevant Processes in the Absence of Amyloid Plaques. 2021; DOI:10.21203/rs.3.rs-580913/v1.
358. Mehder RH, Bennett BM, Andrew RD. Morphometric Analysis of Hippocampal and Neocortical Pyramidal Neurons in a Mouse Model of Late Onset Alzheimer's Disease. J Alzheimers Dis JAD 2020;74:1069–1083.
359. Ochiishi T, Kaku M, Kiyosue K, et al. New Alzheimer's disease model mouse specialized for analyzing the function and toxicity of intraneuronal Amyloid β oligomers. Sci Rep 2019;9:17368.
360. Wong P, Ho WY, Yen Y-C, et al. The vulnerability of motor and frontal cortex-dependent behaviors in mice expressing ALS-linked mutation in TDP-43. Neurobiol Aging 2020;92:43–60.
361. Murava AL, Meadows S, Palaguachi F, et al. Dementia-linked TDP-43 dysregulation in astrocytes impairs memory, antiviral signaling, and chemokine-mediated astrocytic-neuronal interactions. Alzheimers Dement 2021;17:e058562.
362. Nackenoff AG, Hohman TJ, Neuner SM, et al. PLD3 is a neuronal lysosomal phospholipase D associated with β-amyloid plaques and cognitive function in Alzheimer's disease. PLoS Genet 2021;17:e1009406.
363. Rosene MJ, Hsu S, Martinez R, et al. Defining the role of PLD3 in Alzheimer's disease pathology. Alzheimers Dement 2021;17:e058730.
364. Nagu P, Parashar A, Behl T, et al. Gut Microbiota Composition and Epigenetic Molecular Changes Connected to the Pathogenesis of Alzheimer's Disease. J Mol Neurosci 2021;71:1436–1455.
365. Shen G, Hu S, Zhao Z, et al. Antenatal Hypoxia Accelerates the Onset of Alzheimer's Disease Pathology in 5xFAD Mouse Model. Front Aging Neurosci 2020;12:251.
366. Wang M, Lv J, Huang X, et al. High-fat diet-induced atherosclerosis promotes neurodegeneration in the triple transgenic (3 × Tg) mouse model of Alzheimer's disease associated with chronic platelet activation. Alzheimers Res Ther 2021;13:144.
367. Peterman JL, White JD, Calcagno A, et al. Prolonged isolation stress accelerates the onset of Alzheimer's disease-related pathology in 5xFAD mice despite running wheels and environmental enrichment. Behav Brain Res 2020;379:112366.
368. Liang F, Yang S, Zhang Y, et al. Social housing promotes cognitive function through enhancing synaptic plasticity in APP/PS1 mice. Behav Brain Res 2019;368:111910.
369. Zhu X, Lee H, Perry G, et al. Alzheimer disease, the two-hit hypothesis: an update. Biochim Biophys Acta 2007;1772:494–502.
370. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. Neuropathol Appl Neurobiol 2007;33:599–614.
371. Feigenson KA, Kusnecov AW, Silverstein SM. Inflammation and the two-hit hypothesis of schizophrenia. Neurosci Biobehav Rev 2014;38:72–93.
372. Fang Y-L, Chen H, Wang C-L, et al. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From "two hit theory" to "multiple hit model." World J Gastroenterol 2018;24:2974–2983.
373. Bouayed J, Bohn T. The link between microglia and the severity of COVID-19: The "two-hit" hypothesis. J Med Virol 2021;93:4111–4113.
374. Baranov SV, Jauhari A, Carlisle DL, et al. Two hit mitochondrial-driven model of synapse loss in neurodegeneration. Neurobiol Dis 2021;158:105451.
375. Möller M, Swanepoel T, Harvey BH. Neurodevelopmental Animal Models Reveal the Convergent Role of Neurotransmitter Systems, Inflammation, and Oxidative Stress as Biomarkers of Schizophrenia: Implications for Novel Drug Development. ACS Chem Neurosci 2015;6:987–1016.
376. Bouet V, Percelay S, Leroux E, et al. A new 3-hit mouse model of schizophrenia built on genetic, early and late factors. Schizophr Res 2021;228:519–528.
377. Hansen J, Baum A, Pascal KE, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science 2020;369:1010–1014.
378. Netland J, Meyerholz DK, Moore S, et al. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol 2008;82:7264–7275.
379. Sefik E, Israelow B, Mirza H, et al. A humanized mouse model of chronic COVID-19. Nat Biotechnol 2021;1–15.