MODEL ANIMALS USED IN BIOMEDICAL RESEARCH

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Abstract. Given the complexity and huge variety of human diseases and areas of medicine aimed at reducing or eliminating the negative consequences of various disorders in the normal functioning of complex systems, it is important to study these complex processes in model organisms. This article provides a short overview of human diseases and some applied areas of medicine in which some progress has been made through the study of model animals. In the future, new knowledge obtained on various animal models can be used to elucidate the etiology of disorders, with subsequent implementation in clinical medicine.

Keywords: model animal, animal model, biomedicine, biomedical research.

List of Abbreviations

AM – Animal model
AC – Arrhythmogenic cardiomyopathy
DDD – Degenerative disc disease
ND – Neurodegenerative disease
AD – Alzheimer's disease
ALS – Amyotrophic lateral sclerosis
PD – Parkinson's disease
HD – Huntington's disease
FGID – Functional gastrointestinal disorder
FD – Functional dyspepsia
IBS – Irritable bowel syndrome
WS – Wolfram syndrome
FHM – Familial hemiplegic migraine
FASPS – Familial advanced sleep phase syndrome
CDB – Cortical depression brain
MA – Migraine aura
ASD – Autism spectrum disorder
FLS – Fragile X Syndrome
DIO – Diet-induced obesity
AMD – age-related macular degeneration

Introduction

Animal model (AM) is a non-human species used in biomedical research because it can mimic aspects of a biological process or disease found in humans. AM (e.g., mice, rats, zebrafish, and others) are similar to humans in their anatomy, physiology, or response to a pathogen that researchers can extrapolate research findings to the AM to better understand human physiology and disease. Using model animals, researchers can perform experiments that would be impractical or ethically prohibited with humans (https://www.genome.gov/genetics-glossary/Animal-Model).

Thus, laboratory, model animals are indispensable assistants to scientists in solving such basic problems of modern medicine as the prevention and treatment of various diseases (Gajdaj & Gajdaj, 2019).

Animals used in various studies are related to the evolution of human history. There is evidence that Aristotle in ancient Greece successfully used animals to understand the human body. The main breakthrough in animal modeling occurred in the 18th-19th centuries owing to such research scientists as Jean Baptiste Van Helmont, Francesco Redi, John Needham, Lazaro Spallanzani, Antoine Laurent Lavoisier and Louis Pasteur, who studied the origin of life on the AM (Mukherjee *et al.*, 2022). At the same time, human physiology, anatomy, pathology, and pharmacology were also studied in model animals. With the advances made in drug development, biomedicine and preclinical trials, the importance of their research has increased many times over the past decades, since the therapeutic outcome and drug safety are the most important criteria for selecting drugs and medical devices for use in humans (Mukherjee

et al., 2022, Pehlivanovic *et al.*, 2019). The scientific application of AM in the field of biological research and drug development is a centuries-old practice due to the marked similarities in physiology and anatomy between humans and animals, especially mammals (Mukherjee *et al.*, 2022). It must be taken into account that the physiological processes of humans, as well as mammals, are complex in terms of circulatory factors, hormones, cellular structures and tissue systems. The process of selecting an AM for biomedical research is a very difficult task, as not all models are acceptable due to various limitations. There are many factors to consider when selecting the ideal model animal for biomedical testing. The most important criteria are the correct selection of models in terms of similarities between animal species and humans in terms of physiological and/or pathophysiological aspects.

Many animal species such as Drosophila, Danio rerio, Caenorhabditis elegans, Xenopus and mammals (mice, rabbits, rats, cats, dogs, pigs and monkeys) have been recognized worldwide for their phylogenetic similarity to humans (Mukherjee *et al.*, 2022). The choice of an appropriate animal model is in most cases a tedious job and sometimes depends on the assumptions and convenience of the study and investigators without regard to whether the model will be suitable or not. The irrational selection of an inappropriate animal model for scientific research will lead to incorrect results, as well as to the misuse of resources and lives. Moreover, it leads to erroneous, duplicative and inappropriate experiments (Mukherjee *et al.*, 2022). To minimize these issues, scientists have recently expanded their research to create animal models that are very specific to the study in question. One such option is the creation of transgenic animals by introducing genetic information directly into the embryo, either by injecting foreign DNA or using retroviral vectors (Mukherjee *et al.*, 2022; Simmons, 2008). Through the introduction of human cells into recipient animals, researchers can study the action of pathogens in the same way as it happens in the human body (Ernst, 2016; Mukherjee *et al.*, 2022). The correct choice of animal models is mainly related to the nature of the investigational medicinal product/medical device. In many cases, one animal model cannot indicate a human disease by itself; in this case, a combination of several models is used (Dam & Deyn, 2011; Mukherjee *et al.*, 2022). Recently, the need for non-human primates has continued to grow in several areas of human disease research (AIDS, Parkinson's disease, hepatitis, dentistry, orthopedic surgery, cardiovascular surgery, psychological disorders, toxicology research, drug development, toxicology research, and vaccine development (Bailey, 2019). The discovery of vaccines and diagnostic methods for the AM not only benefits people, but also increases the lifespan of animals and prevents many zoonotic diseases, owing to the production of many vaccines, drugs such as rabies, tetanus, parvovirus, feline leukemia... (Mukherjee *et al.*, 2022).

Thus, the widespread use of model animals has become possible and is due to the use of various technologies, approaches, such as methods for inducing human diseases in other organisms, large-scale mutation screening, transgenesis, knockout technologies, chromosomal rearrangements, genetic modifications, editing ... (Bailey, 2019; Gerull, 2020; Simmons, 2008).

The following is a brief overview of some of the diseases and medicine area for which animal models have been successfully and extensively used.

Arrhythmogenic cardiomyopathy (AC)

Arrhythmogenic cardiomyopathy (AC) has been clinically identified since the 1980s and causes right-sided or biventricular cardiomyopathy associated with ventricular arrhythmia. Although it is a rare heart disease, it is responsible for a significant proportion of sudden cardiac deaths, especially in athletes. Most AC patients carry one or more genetic variants in desmosomal genes. In the 1990s, several knockout mouse models of genes encoding desmosomal proteins involved in intercellular adhesion were the first to detect embryonic mortality due to heart defects. Increased interest in human cardiovascular genetics has led to the discovery of

mutations, first in desmosomal genes and then in more than 25 different genes. It should be noted that even in the clinic, routine genetic diagnosis is important for risk prediction in patients and their relatives with AC. Based on advances in animal genetic engineering, various transgenic, knockout, or cardiospecific knockout animal models for desmosomal and non-desmosomal proteins have been created, leading to important discoveries in this field.

Based on this knowledge obtained at the level of animals, organs, tissues, cells and molecules, it is possible to develop effective personalized, targeted treatments for arrhythmogenic cardiomyopathy. Of the recent advances in genetic technology, the emergence of the CRISPR/Cas9 genome editing approach has simplified the creation of knockout and knockout models and will become the technology of choice for studying human gene mutations in the future (Gerull & Brodehl, 2020).

Degenerative disc disease (DDD)

Degenerative disc disease (DDD) is a painful, chronic and progressive disease characterized by inflammation, structural and biological deterioration of intervertebral disc tissues. DDD is defined as a cell, age, and genetically dependent degenerative process that can be accelerated by environmental factors. It is one of the main causes of chronic back pain and disability that affects millions of people around the world. Current treatment options, such as physical rehabilitation, pain management, and surgery, can only provide temporary pain relief. Although no animal models (mouse, rat, rabbit, pig, cattle, sheep, goat, dog, and primates) could accurately reproduce human clinical conditions, these animal models have played an important role in refining knowledge of pathophysiology: they have been used to study process of intervertebral disc degeneration, and the development of therapeutic options that can restore the structure and function of degenerative discs.

Thus, the knowledge obtained on the AM has led to significant progress in understanding the biological basis of disc degeneration and the therapeutic possibilities of cell transplantation, gene therapy, the use of supportive biomaterials and bioactive factors, or a combination of both, which is critical for the development of new therapeutic approaches for clinical application (Mern *et al.*, 2021).

Diabetes

The need for current research is to develop successful and robust diabetic animal models for understanding disease susceptibility and pathogenesis. The tremendous success of using animal models has already been recognized to identify key genetic and environmental factors such as IDD loci and microbial exposure, including the gut microbiota. In addition, animal models have also helped in identifying many therapeutic targets and strategies for immune intervention. It should be noted that despite some success, many researchers recognize that many of the discovered immunotherapeutic agents work in animals and have not had a significant effect on humans. In addition, due to poor initial screening and evaluation for non-equivalent AMs, the percentage of potential drugs that successfully passed clinical trials was very low. Therefore, it is important to bridge this gap between preclinical studies and clinical trials by testing existing animal models for consistency. Evaluation of the importance of animal models in diabetes research and clinical trials, according to the published literature over the past decade, has shown the need for some improvement in the diabetic animal model for a smooth transition from preclinical studies to clinical trials.

Recently, there has been a breakthrough in the field of preclinical research, transplantology and genetic engineering. Current technological capabilities suggest that the future of modeling various pathophysiological conditions in research may lie in the creation of a highly predictive animal model using patient specific organs, either on a chip or on a sensor device (Pandey & Dvorakova, 2020).

Cancer

The variety of malignant oncological neoplasms is influenced by complex genetic and molecular signaling pathways produced by tu-

mor cells in coordination with the tumor microenvironment (Onaciu *et al.*, 2020). Mouse models for preclinical testing of new therapeutic strategies for the ultimate goal of clinical implementation are of paramount importance in modern research practice. Mouse models are widely used in cancer research due to their low cost, availability, and variety of immunocompetent and immunodeficient strains. Over 95% of in vivo cancer research is done in mice. However, translational research is limited and hampered by many biological aspects, such as animal behavior and species differences, which can lead to misinterpretation of the results. Currently, in vivo studies are focused on induced and spontaneous disease models in small animals, and are more limited in large animals. Cancer incidence and development is the result of an interaction between extrinsic or exogenous factors such as lifestyle and environment and intrinsic factors such as genetics (Onaciu *et al.*, 2020; Rudolph *et al.*, 2016; Zimta *et al.*, 2019). The implementation of induced cancer models has attracted a lot of attention due to the ease of having different protocols and methods. These studies focus on physical/chemical factors. These physical (irradiation) and chemical factors (cancer cells, tumor tissue, various genetic constructs including viruses, homologous recombination, and gene editing) can cause the desired disease. The most effective strategy is the use of genetic engineering to develop genetically programmed models of cancer. Depending on the characteristics of the cancer, some protocols involve using a combination of physical and chemical factors to induce cancer in laboratory animals. Given the complexity of the existing foundations of oncology, the relevance of models in biomedical research is critical in light of the possibility of obtaining valuable data with their help. Cancer research is currently being carried out in a huge number of highly funded research projects focused on new early research, diagnostic methods and therapeutic drugs. The similarity of human characteristics in cancer models is directly proportional to the relevance and safety of clinical trials. These aspects have a direct ethical, social, and economic impact on the healthcare system, whereby a successful preclinical model can determine the rapid clinical application of results that affect the quality of life and survival of cancer patients. It should be noted that the topic of animal models for cancer research is an important area of study, with numerous studies still to be done in the future (Onaciu *et al.*, 2020).

Schizophrenia

Schizophrenia is a severe mental illness with a worldwide lifetime prevalence of 0.4% (Ang *et al.*, 2021, Saha *et al.*, 2005), affecting more than 21 million people worldwide. Schizophrenia is a complex neuropsychiatric disorder, the etiology and pathogenesis of which is associated with both genetics and the environment (Ang *et al.*, 2021). Symptoms usually begin between late adolescence and early thirties (Ang *et al.*, 2021; Saha *et al*., 2005). Symptoms of schizophrenia are usually divided into three groups: positive, negative and cognitive (Miyamoto & Nitta *et al.*, 2014; Winship *et al.*, 2018). Positive symptoms include hallucinations (false perceptions), delusions (abnormal beliefs), disorganized thinking, and experiences not characteristic of a normal mental state. Negative symptoms include social isolation, lack of motivation, impoverished speech, emotional dullness, and anomalies in social interaction, which are signs of a deficit in normal social functions (Ang *et al.*, 2021). Finally, cognitive symptoms include impairments in working memory, attention, and executive function (Ang *et al.*, 2021; Canetta & Kellendonk, 2018; Kellendonk *et al.*, 2009; Leung & Jia, 2016; Miyamoto & Nitta *et al.*, 2014; Nestler & Hyman, 2010; Ribeiro-Santos *et al.*, 2014; Simpson *et al.*, 2010).

At present, antipsychotics are mainly effective against positive symptoms, with little therapeutic success in alleviating negative and cognitive symptoms (Ang *et al.*, 2021; Nestler & Hyman, 2010; Simpson *et al.*, 2010). Even so, existing antipsychotics against positive symptoms have limited efficacy and have harmful side effects (Ang *et al.*, 2021).

It is known that both genetics and the environment play a role in its etiology and pathogenesis; to date, the etiology of schizophrenia

remains unclear. In addition, there are no biological markers for diagnosing schizophrenia, and a patient's diagnosis is based only on an established set of clinical symptoms (Ang *et al.*, 2021). In addition, the choice of drugs and the evaluation of treatment, prognosis and life functioning of patients with schizophrenia are primarily guided by clinical signs. The identification of these clinical symptoms in human patients is of paramount importance. Different animal models are needed to identify these diverse symptoms, which can be achieved by monitoring disease progression more rapidly than is possible in humans (Winship *et al.*, 2018). Nevertheless, it is difficult to fully reproduce the symptoms of schizophrenia in experimental animals (Ang *et al.*, 2021; Kellendonk *et al.*, 2009). One of the main approaches to identifying and understanding these diverse symptoms in humans has been to study behavioral phenotypes in a range of model animals. When creating animal models of schizophrenia, candidate models were assessed for schizophrenia-like behavior using several behavioral tasks for positive, negative, and cognitive symptoms designed to confirm symptoms of schizophrenia in humans. Such validated animal models have been provided as rapid preclinical opportunities for drug testing and mechanistic studies. Based on recent advances in this field, it is clear that multiple behavioral tests are needed to validate and assess the congruency of animal models with the multiple behaviors and clinical signs exhibited by patients with schizophrenia. Together, these tests reproduce the dysfunctions found in patients with schizophrenia and can reveal significant theoretical and neurobiological correlations between preclinical and clinical data. In fact, the formulation and application of these behavioral objectives allows for the advancement of research using model animals, which, in turn, contributes to the elucidation of the etiology of schizophrenia and the exact mechanisms underlying its development (Ang *et al.*, 2021; Leung & Jia, 2016; Simpson *et al.*, 2010).

Aging

Most studies of aging mechanisms are carried out on a very limited number of classical model species, i.e., on laboratory mice (Mus musculus), rats (Rattus norvegicus domestica), common fruit fly (Drosophila melanogaster) and roundworms (Caenorhabditis elegans). Obvious advantages of using these models are access to resources such as strains with known genetic properties, high-quality genomic and transcriptome sequencing data, versatile experimental manipulation capabilities, including well-established genome editing tools, and extensive animal husbandry experience. However, this approach can lead to interpretation biases due to the specific characteristics of the species being studied, which can lead to inappropriate or even false generalizations. For example, it is still unclear to what extent knowledge about the mechanisms of aging obtained from short-lived model organisms is applicable to long-lived species such as humans. In addition, other specific adaptations that contribute to a long and healthy life, from the huge set of evolutionary tools, can be completely missed. In this regard, studies are being conducted on new model animals, which have attracted the attention of gerontologists. Models shown include short-lived species such as killifish (Nothobranchius furzeri), long-lived species such as primates (Callithrix jacchus, Cebus imitator, Macaca mulatta), bathyergid diggers (Heterocephalus glaber, Fukomys spp.), bats (Myotis spp.), birds, olms (Proteus anguinus), turtles, Greenland sharks, bivalves (Arctica islandica) and potentially ageless species such as hydra and planaria. Any choice of non-canonical model organisms will necessarily be incomplete. Like African mole rats, mole rats (Spalax), for example, show a deviation from the correlation of lifespan with body weight (Holtze *et al.*, 2021; Tacutu *et al.*, 2018), they are also extremely resistant to cancer, possibly mediated by a concerted necrotic cell (Gorbunova *et al.*, 2012; Holtze *et al.*, 2021). In addition, elephants exhibit strong resistance to cancer, which has been associated with high copy numbers of the tumor suppressor TP53 and additional tumor suppressors (Holtze *et al.*, 2021; Sulak *et al.*, 2016; Vazquez & Lynch, 2021).

With regard to potential molecular targets for slowing the aging process, it is not surprising that the most promising targets from estab-

lished (short-lived) model organisms also appear to some extent in studies conducted on non-canonical species. However, it is interesting to note that, especially in long-lived alternative model organisms, other mechanisms seem to play an even more prominent role. This is especially true of enhanced DNA repair, for which there is ample evidence that various extremely long-lived mammals, turtles and planarians may be a critical factor in longevity. The same applies to evolutionary adaptations that are associated with the coordination of protein synthesis of the nuclear and mitochondrially encoded components of the respiratory chain, called mitonuclear balance. Such adaptations have so far been observed in diggers, bats, killifish and clownfish. Moreover, the results of studies of two long-lived genera of social mole rats (Heterocephalus and Fukomys) suggest that increased proteasome activity contributes to their longevity and health duration. There is also evidence that specific adaptations of the immune systems of mole rats, bats, and clownfish greatly contribute to their longevity. However, regarding the effect of oxidative stress and telomere maintenance on aging, the results of non-canonical model organisms are as mixed as those of canonical ones. It is no coincidence that canonical species have become a popular object of biological research; their rapid maturity and breeding make them economical to breed and raise in captivity. However, their rapid maturity and short life span are the result of millions of years of adaptation to a very different evolutionary landscape than long-lived species. Undoubtedly, non-canonical model organisms provide an opportunity to study mechanisms related to the increase in lifespan that canonical ones do not have. These include species-specific causes of resistance to cancer, such as those found in bathyergid mole rats, mole rats, and elephants, the ability of hydra and planarian stem cells not to deplete over time, the physiological and molecular mechanisms linking longevity and neoteny in amphibians, and probably many others that are yet to come. to be studied.

Thus, the presented species have an exceptional lifespan, a huge potential for regeneration, or a remarkable resistance to diseases associated with aging. Previous data on possible molecular causes support the mechanisms known in classical model organisms, but with a different weight to the various known signaling pathways associated with aging. In addition, there are some intriguing life extension mechanisms that have no equivalent in classical model organisms. Incorporating this multitude of evolutionary adaptations into future research is likely to expand our understanding of the aging process and may ultimately contribute to the development of methods and techniques aimed at increasing human life expectancy and improving their health (Holtze *et al.*, 2021).

Neurodegenerative diseases XI

Neurodegenerative diseases (ND) are a large group of neurological disorders including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Huntington's disease (HD). Neurodegenerative diseases are incurable and represent one of the most complex health problems, with increasing life expectancy worldwide, their incidence is rapidly increasing. Although this group of diseases exhibits heterogeneity with distinct clinical and pathological phenotypes, they share important pathological features characterized by age-dependent and progressive neuronal degeneration (progressive loss of specific populations of neurons in the aging human brain) caused by the accumulation of malformed proteins. The association of genetic mutations with neurodegenerative diseases has made it possible to establish various types of animal models that mimic genetic defects. However, most genetically engineered rodent models lack the overt and selective brain neurodegeneration seen in the patient, making it difficult to use small animal models to test the effectiveness of neurodegeneration treatments. Recent studies in pig and monkey models show that large animals can more accurately reproduce pathological conditions, features of neurodegenerative diseases (Butzlaff & Ponimaskin, 2016; Leung & Jia, 2016; Yang *et al.*, 2021).

AD, which is the most common form of neurodegenerative disease, affects about 7–8% of

people over 65 years of age. The main clinical manifestations of AD include progressive memory loss, cognitive dysfunction, behavioral disorders, and other comorbidities. Neuropathologically, AD is characterized by the presence of extracellular senile amyloid plaques and intracellular neurofibrillary tangles (NFT), along with other molecular changes such as neuroinflammation, brain atrophy, synaptic pathologies, and cerebral amyloid angiopathy (Dubois *et al.*, 2014; Serrano-Pozo *et al.*, 2011; Yang *et al.*, 2021). More than 90% of AD patients are sporadic and present with dementia at age 60 or later, and less than 10% of AD patients have an early form of the disease that can be caused by a single genetic mutation in the APP genes (presenilin 1, presenilin 2, and amyloid precursor protein – APP) (Lanoiselee *et al.*, 2017; Yang *et al.* 2021).

Parkinson's disease (PD) is the second most common neurodegenerative disease that affects more than 1% of people over 60 and is characterized by a progressive and selective loss of dopaminergic neurons. Its characteristic pathological features, movement disorders, are rigidity, resting tremor, bradykinesia, and postural instability. Current knowledge about the pathogenetic mechanisms of PD is mainly associated with various experimental models that can represent different aspects of the disease at different levels of cells and/or molecules, movement and immobility, electrical activity. Like AD, most cases of PD are sporadic, and mutations in the genes encoding alpha-synuclein, PINK1, parkin, LRRK2, etc., are found in 10–15% of cases of familial PD (Deng *et al.*, 2018; Yang *et al.*, 2021). Each currently existing experimental model has its own specific application, but none of them can fully enumerate the pathological and/or phenotypic features of PD. The choice of PD model largely depends on the aspect of the disease being studied and the type of therapy to be developed. Cellular models help to reproduce some of the main features of PD, in particular the overarching biochemical pathways such as oxidative stress, mitochondrial disorders, autophagy dysfunction, neuroinflammation, and apoptosis of dopamine neurons. Thus, cell models can provide various opportunities for identifying molecular pathogenesis and large-scale testing of potential compounds. Animal models provide valuable information about the pathogenetic mechanisms of PD development. Non-mammalian model animals contribute to the study of some of the common phenotypes involved in the development of PD. Given the complexity of ethical identification and high homology with human genes, rodents are often the subject of disease modeling. The ideal rodent model for PD depicts age-related and progressive loss of dopaminergic neurons, motor dysfunction, and abnormal α-synuclein (Deng *et al.*, 2018; Ke *et al.*, 2021; Leung & Jia, 2016; Yang *et al.* 2021).

Recent studies show that α-synuclein pathology originates outside the brain, occurs in the gastrointestinal tract, and then is transmitted to the brain via the vagus nerve in patients with PD. The work of the authors on rodent models demonstrated the role of the gut-brain axis in the initiation and spread of PD. Another study recently reported that enteric infection with Gram-negative bacteria in Pink1-/- mice act as a trigger event in PD (Ke *et al.*, 2021; Matheoud *et al.*, 2019). These new rodent models certainly provide valuable tools to further explore the role of the gut-brain axis in the occurrence and spread of PD.

ALS is also a progressive neurodegenerative disease that particularly affects motor neurons in the brain and spinal cord, resulting in loss of muscle mobility (Goldman, 2014; Ke *et al.*, 2021). Like AD and PD, most patients with ALS are sporadic, and about 5-10% of patients have a familial form of ALS. Familial ALS can be caused by various mutations at genetic loci, including DNA binding protein 43 TAR (TDP-43), superoxide dismutase 1 (SOD1), sarcoma fusion (FUS), and C9ORF72 (Braak *et al.*, 2003; Ke *et al.*, 2021; Lin & Farrer, 2014).

On the other hand, HD exhibits autosomal dominance with complete penetrance, which is caused by the expansion of CAG repeats (> 36 CAG) in exon 1 of the HD gene, which is translated into a polyglutamine (polyQ) repeat in the disease huntingtin protein (HTT) (Di Maio *et al.*, 2016; Ke *et al.*, 2021; Schiesling *et al.*, 2008). The expansion causes misfolding and

aggregation of HTT in the patient's brain, leading to preferential loss of medium spiny neurons in the striatum and extended neurodegeneration in various brain areas as the disease progresses (Di Maio *et al.*; 2016, Ke *et al.*, 2021). Currently, effective treatments for these neurodegenerative diseases are still lacking, and no proven treatment can stop or slow the progression of these diseases. Animal models that can replicate the key pathological changes that occur in the brains of patients will be important for developing effective therapeutic strategies. A newly developed genome editing tool has made significant progress in producing valuable large animal models for the study of neurodegenerative diseases. The study of large animal models made it possible to detect important pathological events that would not occur in the body of small animals. However, the creation and research of genetically modified models of large animals is still difficult, mainly due to the high cost of animals and the time spent on experiments. Further optimization of the existing genome editing system or the creation of new tools will increase the efficiency and accuracy of large animal genome modification. Moreover, based on large animal models, it is possible to create small animal models that can more accurately replicate important pathological features to study pathogenesis and develop effective therapy (Ke *et al.*, 2021; Leung & Jia, 2016; Yang *et al.*, 2021).

Ischemic stroke

Despite impressive efficacy demonstrated in preclinical studies, hundreds of potentially neuroprotective drugs have failed to provide effective neuroprotection in ischemic stroke in human clinical trials. The lack of a powerful model of human ischemic stroke may be a major reason for the failure to develop successful neuroprotective drugs for the treatment of ischemic stroke. Innovative animal models more similar to human strokes, improved methods for evaluating functional outcomes, and better experimental designs that provide clearer and more compelling evidence could help develop truly neuroprotective drugs that will benefit patients with ischemic stroke. This may include using newer molecules or revisiting older studies with new experimental designs. It will then be possible to test any resulting successes in human clinical trials with greater confidence and optimism. Although models other than rodents have been described that include larger and more advanced animal species that more closely resemble human strokes, experiments on these models are hampered by a number of practical considerations and ethical issues that make their use in animal studies much more difficult. Whether their use could lead to greater scientific progress and the development of drugs and devices useful in treating stroke in humans is a subject of intense debate. The author considers that better design of animal experiments and better publication of animal research guidelines are also absolutely necessary to minimize the publication of biased reports. Experimental studies and therapeutic interventions should be designed to resemble and be relevant to the human condition. With the help of optimized, ethically sound and evidence-based animal research models and research designs, it is possible to bridge the gap between laboratory and bedside stroke treatment (Narayan *et al.*, 2021).

Functional gastrointestinal disorders (FGID)

Functional gastrointestinal disorders (FGID), such as functional dyspepsia (FD) and irritable bowel syndrome (IBS), are characterized by chronic abdominal symptoms in the absence of an organic, metabolic, or systemic cause that easily explains these complaints. FGID are complex and multifactorial disorders involving a complex interplay between biological, psychological, and social variables, the pathophysiology of which is still not fully understood. Although none of the current animal models can accurately reproduce them, animal models are of great value in improving the understanding of complex biological mechanisms. Over the past decades, many animal models have been developed to further study the pathophysiology of FGID and test drug efficacy. The main limitation of these models remains the social component of FGID pathophysiol-

ogy, which is extremely difficult to reproduce in animals. However, animal models have provided key insights into its pathophysiology, including the complex interaction between the gut and the central nervous system, and represent essential tools for identifying new therapeutic targets and testing new generations of pharmaceutical and non-drug therapies. Over the years, a better understanding of the pathophysiology of functional gastrointestinal disorders has spurred the development of new animal models that are now more complex and include a combination of causes that produce signs of FGID that more closely resemble the human condition. An analysis of the literature suggests that stress, gastrointestinal mechanisms caused by either infection or another inflammatory trigger, food metabolic disorders or food hypersensitivity and allergies, a secondary effect of medical interventions, and spontaneous models that have general characteristics of the gastrointestinal tract and anxiety-related disorders. The latter are powerful models of brain-gut axis dysfunction and provide new insights into FGID and their comorbidities such as anxiety and depression (Accarie & Vanuytsel, 2021).

Wolfram syndrome

Wolfram syndrome (WS), also known as diabetes insipidus, early-onset diabetes mellitus, optic nerve atrophy, and deafness, is a rare autosomal disorder caused by mutations in the wolframin-1 (WFS1) gene. Previous studies have shown that the glucagon-like peptide-1 receptor agonist (GLP1 RA) is effective in delaying and restoring blood glucose control in animal models and in patients. Liraglutide GLP1 RA has also been shown to have neuroprotective properties in aged rats. WS is an early chronic disease. Therefore, its early diagnosis and lifelong pharmacological treatment is the best solution to control the progression of the disease. To assess the efficacy of long-term treatment with liraglutide on progression of WS, 2-month-old WS rats were treated with liraglutide until 18 months of age and changes in markers of diabetes, visual acuity, and hearing sensitivity were monitored over the treatment period. Treatment with liraglutide has been found to delay the onset of diabetes and protect against vision loss in a rat model. In this regard, the researchers concluded that early diagnosis and prophylactic treatment with liraglutide may also be a promising option for the treatment of patients with WS by improving the quality of life (Jagomäe *et al.*, 2021).

COVID

In addition to the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 has become the third deadly coronavirus to infect humans and cause the novel coronavirus disease (COVID-19). COVID-19 has already caused more than 6,5 million deaths worldwide and is probably the largest pandemic that humanity has faced this century. While many studies have been done on SARS-CoV-2, a detailed understanding of SARS-CoV-2 and COVID-19 is still lacking. Coronaviruses (CoV) are a group of enveloped, positive-sense, single-stranded RNA viruses in the family Coronaviridae, order Nidoviruses (Lin *et al.*, 2022; Weiss & Navas-Martin, 2005). CoVs are among the RNA viruses with the largest genome size. There are four genera of CoV: α-, β-, γ- and δ-CoV. While CoVs can infect a range of animals, including pigs, cattle, horses, dogs, cats, rodents, and birds, only α- and β-CoVs can infect humans (Lin *et al.*, 2022; Woo *et al.*, 2012). Novel SARS-CoV-2, first identified in Wuhan, China in December 2019 (Huang *et al.*, 2020), is causing an ongoing coronavirus disease (COVID-19) pandemic and the global death toll is now approximately 6.5 million people (https://covid19.who.int/), highlighting the urgent need to develop prevention and control strategies. It has been suggested that wild bats are potential vectors for three deadly coronaviruses, SARS-CoV, MERS-CoV and SARS CoV-2 (Cui *et al.*, 2019; Zhou *et al.*, 2020), and viruses can be transmitted between species through one or more intermediate hosts (Cui *et al.*, 2019; Lin *et al.*, 2022; Wu *et al.*, 2021). To date, primates (NHP) (Koo *et al.*, 2020; Lu *et al.*, 2020), hamsters (Chan *et al.*, 2020) and fer-

rets (Kim *et al.*, 2020) have been shown to be susceptible to SARS infection -CoV-2. Since the end of 2019, we have seen the enormous impact of the COVID-19 pandemic on global public health. Currently, new emerging variants of SARS-CoV-2 such as Delta and Omicron and others, combined with relatively slow vaccination, pose a constant threat worldwide. Therefore, it is extremely important to effectively speed up the vaccination process worldwide and evaluate existing vaccines urgently and carefully, as their safety and efficacy are still being debated. In addition, detailed understanding of SARS-CoV, MERS-CoV, and SARS-CoV-2 infections, host immune responses, and immune pathology is required to develop next-generation vaccines and new therapeutics. They can be greatly accelerated using preclinical animal models. Whether a model animal is suitable for the development of vaccines and antivirals depends on how well it mimics the corresponding human disease. This includes, first, the successful entry and replication of the virus in animals, and then the development of measurable clinical symptoms. Some patients with severe COVID-19 have chronic conditions such as diabetes, hypertension, and obesity. It would be ideal if these diseases were taken into account when developing and optimizing future MFs. Ideally, suitable animal models could also facilitate the evaluation of vaccines and antivirals. If vaccines and antivirals could be thoroughly tested in such animals to determine their efficacy and side effects, it would certainly minimize the risk that vaccines and antivirals pose to patients and volunteers participating in clinical trials. The authors expect that in the near future, thanks to model animals, progress will be made in establishing the cause and pathogenesis, in the development of vaccines and antiviral drugs for the prevention and treatment of diseases, which will ultimately help to control the current pandemic (Lin *et al.*, 2022; Wang *et al.*, 2021).

Atherosclerosis

Atherosclerosis remains a leading cause of global morbidity and mortality throughout the world, especially in industrialized countries. Despite ongoing efforts to study the pathogenesis of the disease and search for potential points of effective therapeutic intervention, understanding of the mechanisms of atherosclerosis remains limited. This is partly due to the multifactorial nature of the pathogenesis of the disease, when several such different factors as altered lipid metabolism, increased oxidative stress and chronic inflammation act together, leading to the formation and progression of atherosclerotic plaques. Model animals have proved indispensable for the study of human diseases, including atherosclerosis, and the search for new therapeutic approaches. Currently, several reliable models of atherosclerosis in rabbits and mice have been developed and tested. Most of them are based on genetic modifications of key genes involved in the development of atherosclerosis, such as the genes for apolipoprotein E or the LDL receptor. The models differ in blood lipid profile, the ability to develop atherosclerotic lesions spontaneously or as a result of a special diet, and the presence of complex and unstable plaques. While the induction of atherosclerotic lesions in animal models can be reliably achieved, modeling complex plaques with features such as calcification, neovascularization, intra-plaque hemorrhage, and thrombosis is more challenging. Future research should focus on creating models that would allow testing of new drugs aimed at stabilizing plaques.

Thus, adequate animal models are currently needed to study these processes and search for new treatments. Model animals (mice, rats, and rabbits) are important tools for studying the processes of atherosclerosis, opening up previously unknown possibilities for modeling the disease and selecting new methods of treatment (Poznyak *et al.*, 2020).

Migraine

Mouse models of rare monogenic forms of migraine represent a unique experimental system for studying the cellular circuits and mechanisms of primary brain dysfunctions that cause it. According to the literature data, studies of the migraine phenotype are being conducted, due to functional changes in the brain of five genetic

models of mice, four of which carry mutations obtained from patients with familial hemiplegic migraine (FHM), and the fifth carries a mutation from patients with both phenotypically normal MA and with family extended sleep phase syndrome (FAPS). Studies of mouse models with mutations affecting serine-threonine kinase, a voltage-gated calcium channel that controls neurotransmitter release at most brain synapses, and Na/K-ATPase, which is expressed primarily in astrocytes, have shown that increased susceptibility to experimentally induced pervasive cortical depression brain (CDB) is a key migraine-associated phenotype common to five models. In addition, the study of synaptic changes in the cerebral cortex of migraine genetic models will allow us to study the mechanisms underlying their increased susceptibility to CDB, and changes in the trigeminovascular pain pathway will allow us to study the mechanisms of pain in migraine and its pathophysiology (Pietrobon & Brennan, 2019).

Although the reviewed functional studies support the view that migraine is a brain disease characterized by dysregulation of the excitatory-inhibitory balance in specific neuronal circuits, much remains to be done in genetic models of mice, i.e. identify relevant impairments and establish whether and how changes in the function of specific circuits (in the cerebral cortex and/or other areas of the brain) are condition dependent and may, under certain conditions, contribute to the onset and a migraine attack. Migraine is much more than episodic headache and pain syndrome. This is a complex brain disease that primarily affects the sensory nervous system and is characterized by a general dysfunction in the processing and integration of multisensory information. Indeed, in most attacks, the typical throbbing unilateral headache is associated with increased perception from multiple senses, indicating increased sensory amplification. Hypersensitivity to sensory stimuli may persist in the interictal period during which some changes in sensory physiology are found in the migraine brain. Interestingly, the magnitude of some of these changes increases in the interictal period to the next attack and peaks the day before the attack, in temporal coincidence with prodromal symptoms (difficulty speaking, reading, concentrating, increased emotionality, irritability, sensory hypersensitivity), which many patients with migraine are significantly predictive of an attack (Burstein *et al.*, 2015; De Tommaso *et al.*, 2014; Pietrobon & Brennan, 2019).

The neurobiological mechanisms of causal brain dysfunction underlying the onset of a migraine attack and changes in multisensory processing remain largely unknown and are key unanswered questions in the neurobiology of migraine. It is believed that migraine is a complex polygenic genetic disease with an estimated heritability of up to 50% (Ferrari *et al.*, 2015; Pietrobon & Brennan, 2019; Sutherland & Griffiths, 2017). Although genome-wide association studies (GWAS) are providing more and more information about common genetic variants associated with migraine (Gormley *et al.*, 2016; Pietrobon & Brennan, 2019), the study of data obtained from GWAS is very difficult, if not impossible, given also the fact that they usually lie in throne or intergenic regions and therefore they are likely to affect gene regulation rather than directly protein function. Thus, "normal" migraine is not amenable to reproduction in a mouse model and is not amenable to attempts to determine the mechanism. In contrast, rare monogenic forms of migraine are caused by mutations that directly affect the function of proteins, and the functional consequences of disease-causing mutations can be studied in genetic models of the disease in mice. To date, five monogenic migraine mutations are known to be associated with murine knockout (KI) lines, allowing investigation of their underlying mechanisms. Four of these were from patients with familial hemiplegic migraine (FHM) and one from patients with migraine with aura (MA) and familial advanced sleep phase syndrome (FASPS), a rare sleep condition in which people go to bed unusually early in the evening and wake up early in the morning. Apart from motor weakness or hemiplegia during the aura and possible longer duration of the aura, typical attacks resemble ordinary MA attacks, and both types of attacks can alternate between patients and co-occur in families (Pietrobon & Brennan, 2019).

Thus, FHM and MA are considered part of the same spectrum and may share common pathogenic mechanisms. Mouse models of rare monogenic forms of migraine represent a unique experimental system for studying the cellular and contour mechanisms of primary brain dysfunctions that cause migraine disorders. Animal genetic models provide insight into how "spontaneous" may occur in the migraine brain and support the concept of migraine as a brain disorder characterized by dysfunction in the regulation of balance in specific neural circuits in the brain, cerebral cortex, and other structures. brain. Much work remains to be done to identify the relevant dysfunctional circuits and to establish whether and how changes in the function of specific circuits are state-dependent and whether, under certain conditions, may contribute to a migraine attack (Pietrobon & Brennan, 2019).

Autism Spectrum Disorders (ASD)

Autism Spectrum Disorders (ASD) present unique challenges in the field of genetics and neuroscience due to the clinical and molecular heterogeneity underlying these disorders. Genetic mutations found in ASD patients provide an opportunity to analyze the molecular mechanisms underlying autistic behavior using animal models. Studies of genetically modified models have provided critical insights into possible common mechanisms resulting from various mutations, but the relationship between molecular abnormalities and behavioral phenotypes remains elusive. The challenges posed in modeling autism in mice require a new analytical paradigm that combines behavioral analysis with schema-level analysis in genetically modified models with strong construct validity. Autism Spectrum Disorders (ASD) are a group of conditions primarily characterized by impaired social communication and limited repetitive behavior (Hulbert & Jiang, 2016; Leung & Jia, 2016). Common comorbidities include mental retardation, epilepsy, anxiety, sleep disturbances, sensory processing disorder, motor disturbances, and gastrointestinal complaints (Argyropoulos *et al.*, 2013; Hulbert & Jiang, 2016; Leung & Jia, 2016). ASD is heterogeneous in nature, as patients exhibit a wide range of symptom severity and prognosis (Howlin *et al.*, 2004; Lord *et al.*, 2000), as reflected by hundreds of identified causative or potentially causative genetic variants (Persico & Napolioni, 2013; Wilsey & State, 2015). Unfortunately, most genetic mutations are rare or frequent (i.e., only seen in one family). Both phenotypic and genetic heterogeneity present major barriers to understanding disorders, and attempts to link phenotypic severity to genetic differences have been mixed (Chang *et al.*, 2015; Chaste *et al.*, 2014). Although genetics undoubtedly play a significant role in the pathophysiology of ASD, the unexplained phenotypic heterogeneity and incomplete concordance between monozygotic twins (Hallmayer *et al.*, 2011) suggest that non-genetic factors may also contribute to the etiology (Hulbert & Jiang, 2016; Leung & Jia, 2016). Human population studies have been and remain critical to understanding the genetic and non-genetic contributions to ASD (Willsey & State, 2015). However, animal models are needed to determine the mechanisms leading to abnormal functioning. While human brain imaging techniques have identified regions and networks involved in disorders, animal models provide opportunities for direct manipulation of these brain regions and networks to test their precise functions. In modern clinical practice, ASD is defined by behavioral symptoms that are unique to humans, and so far not a single pathognomonic neuropathological feature has been identified, so it is difficult to determine the validity of an animal model of autism. However, recent advances in the identification of genes associated with ASD have paved the way for the study of the neurobiology underlying the disorders using animal models (Hulbert & Jiang, 2016; Karten & Hirsch, 2014; Leung & Jia, 2016).

Etiologically rare and frequent mutations clustered in selected molecular classes appear to be the major factor in the genetic basis in a subgroup of patients with ASD. At the molecular level, several pathways emerge from analysis of existing mouse models with ASD. These include disruption of overlapping signaling pathways mediated by mGluR5, BDNF, and

mTOR, although the extent of evidence varies across models. Moreover, the available data strongly support that dysfunctional synapses are a component of the pathophysiology of autism. However, despite the widespread impairment of synaptic function in mouse models of ASD, the direction of change and the magnitude of the effect are inconsistent between different models, as well as between different types of synapses in any given model. Although there are many obvious differences between the models, it is difficult to compare results obtained in different areas of the brain or at different times. For example, Fmr1 knockout mice have reduced spinal stability in the somatosensory cortex (Cruz-Martin *et al.*, 2010, Hulbert and Jiang, 2016), while Mecp2tm1.1Jae mice have increased spinal stability in the same area (Hulbert & Jiang, 2016; Landi *et al.*, 2011), but in the first the study used mice 10-12 days after birth, while the second study used mice 25-6 days after birth. Therefore, it is worth doing more side-by-side comparisons of different mouse models. One interesting finding in ASD genetic research is the frequent mutations in genes encoding epigenetic mechanisms. However, it is not immediately clear how a deficiency of these proteins contributes to the pathophysiology of autism. One of the widely tested hypotheses regarding the mechanism of ASD development is the change in the structural and functional connections of the brain (Belmonte *et al.*, 2004; Geschwind & Levitt, 2007; Kana *et al.*, 2014). Structural connections are physical connections between different areas of the brain, while functional connections refer to integrated relationships between spatially separated areas of the brain. Structural connections within the brain are thought to cause functional network activity as measured by coherence or information flow. Neuroimaging studies show that ASD is associated with impaired communication at both structural and functional levels (Minshew & Keller, 2010; Vissers *et al.*, 2012); however, the exact nature and nature of this aberrant neural connection remains uncertain due to conflicting results from neuroimaging studies in patients (Di Martino *et al.*, 2014; Kana *et al.*, 2014; Uddin *et al.*, 2013). While early studies reported reduced functional connectivity (Just *et al.*, 2004), recent studies point to hyperconnections in many areas of the brain and between neural networks (Keown *et al.*, 2013; Supekar *et al.*, 2013). In addition to methodological and conceptual inconsistencies, this uncertainty reflects the significant molecular heterogeneity of patients. Notably, these studies have been conducted primarily in patients with high-functioning ASD, whose etiology is largely unknown. For these reasons, animal models with autism offer a unique opportunity to test the hypothesis of functional association due to genetic defects. The combination of optogenetics and CRISPR/Cas9 genome editing tools in ASD models is expected to provide significant insight into whether there are common disorders in ASD models with different genetic defects. In addition, the spatial and temporal manipulation of ASD candidate genes will further delineate the patterns underlying ASD. In the future, manipulating the genomes of other species, such as rat or non-human primate models, will help overcome many of the limitations of mouse models. A more complete understanding of current genetic models of ASD will allow researchers to explore the role of non-genetic factors and their biological underpinnings. Ultimately, knowledge gained from animal models will lead to the development of effective clinical interventions that target specific molecular pathways and neural networks (Hulbert & Jiang, 2016; Leung & Jia, 2016).

Fragile X Syndrome (FLS) is a rare disease and a leading monogenic cause autism spectrum disorders (ASD). It is caused by the silencing of the fragile X mental retardation gene (FMR1) and the subsequent decrease or loss of mental retardation protein X (FMRP). The clinical effects seen in patients with FHL are few and far between, making them difficult to model. Fragile X Syndrome (FLS) is one of the most studied monogenic neurological syndromes over the past few decades, the leading monogenic cause of autism spectrum disorder (ASD). This is caused by the silencing of the Fragile X mental retardation gene (FMR1) and the subsequent decrease or loss of the FMR pro-

tein (FMRP). Expansion of CGG repeats in the 5'UTR of the FMR1 gene, followed by hypermethylation of the region, is the basis of the observed silencing in FXS1. This event occurs sequentially in subsequent generations, starting with a small re-expansion (55-200) causing a pre-mutation in one generation with toxic gain of function in mRNA, followed by further expansion (>200) to full mutation in subsequent generations with complete silencing. gene. FMRP is an RNA-binding protein, a well-known regulator of translation and is known to interact with more than 800 mRNAs in an adult neuron. Although some of the target mRNAs have not been fully characterized, it can be said that the loss of FMRP has a cascading effect across multiple pathways leading to the observed clinical features. Modeling a complex neurological syndrome such as FLS is an ongoing process and requires a set of organisms to model all clinical characteristics, study various pathobiological aspects, and screen potential drug development candidates. Modeling of most diseases and syndromes is needed in disciplines such as disease biology and drug discovery; however, given the monogenic etiology of FLS, these models could potentially be explored to understand several other aspects, including (but not limited to): a) developmental pathways for various cognitive abilities b) specific connective tissue organogenesis c) behavioral pathways such as anxiety, depression, irritability. Fragile X Syndrome is a classic example of a rare disease that, despite having multiple models and studies over decades, has not shown any therapeutic benefit for patients. In the case of drug development for rare monogenic diseases, in which disease manifestation is highly dependent on the underlying genetic background and the need to treat symptomatic syndromes in the first place, radically different approaches may be more fruitful (Kulkarni & Sevilimedu, 2020; Leung & Jia, 2016).

Monogenic immune disorders

Monogenic immune disorders may provide unprecedented insight into the molecular and phenotypic consequences of a disorder in individual genes in humans, thereby enriching our knowledge of immune function and disease. Genomics has accelerated the discovery of monogenic diseases, as well as the revelation of the complexity of human disease, in which multiple factors outside the genome can drive pathogenesis. Research will be of great value if the discovery of human disease genes is combined with mechanistic research using integrative omics and mouse modeling to exploit their unique strengths. With the introduction of next generation sequencing (NGS) technology, the number of detected innate immunity errors continues to increase, exceeding 400 different diseases worldwide, with at least 430 different gene defects (Barmada *et al.*, 2021; Tangye *et al.*, 2020), and not all of them are inherited according to Mendelian laws with complete penetrance. Although monogenic disease of the immune system is rare on its own, these sets of disorders are generally not uncommon, and include infection, allergy, autoinflammation, autoimmunity, and malignancy. In addition, the study of these disorders may reveal some other hidden mechanisms underlying the development of more common diseases with complex etiologies that are more difficult to decipher. The use of physiologically relevant findings in the field of monogenic human diseases, together with mouse modeling tools, offer an ideal approach to meaningful and interpretable findings. Over the past two decades, the development of «omics» technologies – transcriptomics, proteomics, metabolomics, epigenomics and metagenomics have enriched our knowledge in understanding health and disease (Karczewski & Snyder, 2018; Thaventhiran *et al.*, 2020). To obtain maximum information about monogenic immune disorders, it is necessary to ensure the comprehensive integration of genomic data with large-scale biochemical and epigenetic data, which will allow to accurately determine the underlying mechanisms of the disease and provide personalized therapy (Barmada *et al.*, 2021).

Obesity and metabolic syndrome

Obesity and metabolic syndrome are among the main causes of death worldwide, and their pathogenetic mechanisms are not fully under-

stood. Therefore, the priority task of scientists is the development of new research methods aimed at the prevention, control or treatment of these diseases. The use of experimental animals has been and is of great importance in medical research, including for the study of metabolism. However, the results obtained in preclinical studies are not necessarily the same as those obtained in humans. Transferring findings from animals to humans can be challenging, both because of the differences in physiology between species and the failure to adopt the research model itself. Therefore, the choice of a valid model for the study of any disease in order to achieve maximum similarity to what occurs in humans is of fundamental importance. An analysis of the literature data indicates that several animal species can be used to study metabolic disorders. However, the most commonly used rodent models are monogenic and as diet-induced obesity (DIO). Monogenic animals are the best choice when evaluating one aspect, and animals in which diet is induced obesity tend to show better interactions between disease, environment, and genetics. However, they are still not fully effective in understanding all the mechanisms of the disease, not yet fully effective in understanding these disorders. Thus, not always the results obtained with the help of models will lead to new effective methods of treating people. The authors assume that in the near future models will be developed that provide more effective methods of treatment (Fuchs *et al.*, 2018).

Alcohol addiction

Alcohol use and dependence disorder (AD) is a multifaceted neuropsychiatric illness that combines behavioral, psychosocial and neurobiological aspects. Modeling this disorder in animals has been challenging, but significant advances have been made in the past two decades—more complex behavioral models associated with modeling have been created: alcohol consumption, addiction, and seeking; compulsive aspects of alcohol addiction; individual differences, factors of vulnerability and resistance to alcohol dependence; relapse despite treatment; and prevention of relapse by manipulating alcohol-associated memory reconsolidations. The development of AD is characterized by a cycle of distress addiction leading from social/recreational drinking to compulsive drinking, alcohol seeking, consumption, and addiction through repetitive phases of preoccupation/anticipation (craving), drinking/drunkenness and withdrawal and negative affect (Koob & LeMoal, 2001). Although most people consume alcohol throughout their lives, only a fraction of them develop AD, suggesting that genetic and environmental factors, and their combination through the nervous system in particular, may predispose them to alcohol dependence. The combination of simple self-administration procedures with complementary procedures, and the development of new behavioral paradigms that will model the complex nature of AD, may further unravel the neurobiological mechanisms underlying AD-related behavior at the molecular and systemic levels (Abrahao *et al.*, 2017; Ron & Barak, 2016). The authors believe that the predisposition to AD is a critical point that should be further investigated in the AM. An analysis of the literature data indicates vulnerability and resistance factors in the formation of AD, and environmental/physiological variables have been identified as risk factors for AD of a similar phenotype in rodents. In this regard, additional studies on the search for genetic, epigenetic and factors, as well as factors of the development of the nervous system and their combination, which interact with environmental components, can provide a comprehensive study of the formation of AD. In addition, more research in animal models is needed to advance understanding of the mechanisms that drive relapse. Importantly, impaired drug memory reconsolidation as an approach to reduce relapse is still under development (Lee *et al.*, 2017) and only a few studies have demonstrated this strategy in alcohol dependence. Drug memory reconsolidation studies are usually lengthy and involve a combination of Pavlovian and operant learning components, which complicates both procedures and data interpretation. Indeed, even from the limited data available, it is clear that drug/alcohol memory impairment requires a well-controlled

set-up and fine-tuning of parameters that complicate the translation of this approach into humanitarian research and treatment development (Spanagel & Bohus, 2015). For example, effective impairment of reconsolidation has been shown to depend on the strength and age of memories, whereby older and/or stronger memories are less susceptible to reconsolidation manipulation. Moreover, memories associated with operant learning are more difficult to destroy (Zhang *et al.*, 2018). Indeed, alcohol/drug-related memories that trigger a relapse tend to be old, strong, and include operant components (Spanagel & Bohus, 2015). Thus, recent advances in the modeling of phenotypes with AD are associated with the use of behavioral models of rodents, modeling of the main psychological structures that characterize AD. These advances represent a general attempt to capture and understand the complexity and multidimensional nature of AD, as well as the study of behavioral aspects that better reflect this disorder (Goltseker *et al.*, 2019).

Degenerative-dystrophic diseases of the human retina

The strategy of experimental study of the possibilities and effectiveness of modern methods of treating retinal diseases is aimed at careful selection of an experimental model of the disease and analysis of its adequacy to the research objectives. This is the focus of the search programs for new technologies, drugs and methods for their preclinical study. Experimental models of degenerative-dystrophic diseases of the human retina in animals can be divided into genetic and induced. Genetic experimental animal models model hereditary and congenital dystrophies of the human retina, which are very diverse. These include hereditary retinal dystrophies (retinitis pigmentosa (RP)), Leber's congenital amaurosis, Stargardt's disease, Usher's syndrome, which are still incurable (Milyushina *et al.*, 2013).

Currently, age-related macular degeneration (AMD) is considered to be a disease whose etiopathogenesis involves genetic, epigenetic, and environmental factors. All of them interact with each other and contribute to the risk of developing the disease. To date, more than 100 genes have been sequenced, the mutations in which are responsible for the development of these diseases. Knowledge in each case of the genetic cause of the pathology makes it possible to select or create a genetically modified animal for detailed studies, and also suggests the possibility of gene therapy. Genetic animal models are a powerful tool for studying the development, functioning of the eye and various visual pathologies. It is known that more than 150 genes are responsible for the development of certain hereditary diseases of the retina. Changes affecting the work of these genes have in some cases been reproduced in animals. As a result, various lines of mice, rats were obtained, which can be divided into natural/spontaneous and transgenic/developed. Most null mutations in natural populations are manifested by decreased vision in homozygotes and usually resemble recessive forms of eye disease in humans. Animals with hereditary retinal pathology are used to study AMD and PR in humans (Milyushina *et al.*, 2013). Mice homozygous for the mutation, reflecting hereditary retinal degeneration, serve as a model for studying human PR: retinal rod degeneration begins to develop on the 8th day after birth and leads to the complete death of photoreceptors by the end of the 4th week after birth. Pigmentary retinal atrophy in Campbell rats with hereditary retinitis pigmentosa begins to develop 17–20 days after birth and leads to complete blindness by the end of the 2nd month after birth. Rats with hereditary retinal degeneration were bred from Campbell rats. As a result of the initial crossing of Campbell rats with albinos and subsequent crossings according to a certain scheme, lines of mutant and genotypically normal RCS rats of black hood, pink hood (color as in the original Campell rats) and albino were bred. RCS rats have an autosomal recessive degeneration that manifests itself at 2 weeks of age with irregular outer segments. Complete death of photoreceptors occurs by 3 months of age. A genetic defect in these rats was a mutation in the Mertk gene, which leads to impaired (reduced) phagocytosis by retinal pigment epithelium (RPE) cells of the outer segments of photoreceptors, which leads

to the accumulation of debris in the subretinal space (Milyushina *et al.*, 2013).

Models of autosomal dominant human eye diseases have been modeled on transgenic animals that cosynthesize altered and normal proteins. Thus, mutations in the rhodopsin gene are extremely common in PR, and they make up the majority of the known genetic forms of autosomal dominant PR, so much effort has been made to create models in which defective rhodopsin is expressed. Mutations can affect both the C- and N-terminus of rhodopsin, leading to various phenotypic manifestations observed in autosomal dominant forms of PR. Genetic models of degenerative-dystrophic diseases of the human retina make it possible to study the mechanisms of retinal degeneration. E. Pierce identifies four main ways of retinal degeneration (Inoue *et al.*, 2021; Milyushina *et al.*, 2013; Pierce *et al.*, 2001). First, these are mutations that disrupt the formation of outer segments of photoreceptors (rds mice expressing the human pro347Ser mutant rhodopsin and TULP1 knockout mice) and mutations that cause abnormal distribution of photoreceptor proteins (as occurs in rhodopsin mutants and in RPGR knockout mice), which also leads to improper disc formation. Violation of disc morphogenesis leads to the death of photoreceptors, the mechanism of which is completely unclear. The second mechanism of retinal degeneration is metabolic overload, as occurs in rd mice, rcd1 dogs, and γ-PDE deficient mice. In these animals and in patients with a mutation in PDE6B, the opening of cGMP-controlled channels in the plasma membranes of photoreceptor cells is suggested, leading to metabolic overload due to the constant damaging activity of Na+/K+-ATPase to maintain electrochemical gradients. It is hypothesized that elevated calcium levels may activate apoptosis. The third mechanism of retinal degeneration is RPE dysfunction due to internal defects or disruption of the visual cycle leading to RPE impairment (ABCR and RPE65). It is assumed that the accumulation of all-trans-retinal or its metabolites in the outer segments of photoreceptors and subsequent damage to RPE cells lead to the death of photoreceptors. The fourth mechanism of photoreceptor damage is associated with continuous activation of transduction, as occurs in Arrestin and RhoK knockout mice. Patients with Oguchi's disease with recessive PR due to the PDE α subunit and patients with Leber's congenital amaurosis due to mutations in reticular guanylate cyclase are thought to have chronic activation of the visual cascade. It is not yet clear how this leads to retinal degeneration. However, knowledge gained from preclinical trials in mice and dogs using a viral construct encoding the RPE65 gene has led to successful clinical trials in patients with Leber's congenital amaurosis, in which amazing innovative results in the preservation and restoration of vision have been obtained (Stein *et al.*, 2011). These results serve as a good starting point for current and future gene therapy approaches to other inherited human retinal diseases. Knowledge of the existing genetic experimental models of degenerative-dystrophic diseases of the human retina in animals allows the researcher to choose the most adequate one for solving his problem (Inoue *et al.*, 2021; Milyushina *et al.*, 2013).

Regenerative medicine and tissue engineering

The rapid development of regenerative medicine and tissue engineering indicates a growing desire for the clinical implementation of breakthrough technologies. However, advancing promising preclinical data for successful approval in the clinical market remains a bottleneck. One barrier to advancing promising preclinical data for successful clinical approval is the shift from small animal studies to extended large animal preclinical studies to test the safety and efficacy of products. Despite this, in order to obtain meaningful and reliable conclusions from animal experiments, it is essential that the type and chosen model of the disease are appropriate for both the research question and the clinical problem. Selecting the most appropriate animal model requires in-depth knowledge of specific species and breeds to ensure that the model is adequate and outcome measures accurately reflect the clinical situation. Traditional reductionist approaches in animal experiments, which often underrepresent the disease under

study, are still the norm and can lead to inconsistencies in the results observed between animal studies and clinical trials. Addressing these issues will require a revision of the approach. This should include a stepwise approach using in vitro and ex vivo experiments and in silico simulations to minimize the need for in vivo studies for screening and early development studies, followed by large animal models that more closely resemble disease person. Naturally occurring or spontaneous diseases in large animals remain a largely untapped resource, and given the similarities of pathophysiology with humans, they allow not only the study of new treatment strategies, but also the etiology and prevention of disease. Models of naturally occurring diseases, especially for long-lived large animal species, allow the study of disorders at the age when the disease is most common. Companion and large animal models offer realistic natural disease models that more accurately assess the safety and efficacy of new therapies as they share the heterogeneity of the human population, including genetic and physiological variation and their complex interactions with the environment. An increasing number of studies are being conducted on companion animals and large animal species, demonstrating that they have much to offer the human clinic in search of new drug or cell therapies and tissue engineering. The use of large animal models will allow more attention to be given to key issues. These include routes of administration, since it is not yet clear which routes provide optimal engraftment of injected cells in various diseases. It also needs to be determined whether multiple injections would be more beneficial, and if so, the question is whether there is an associated increase in the risk of an adverse immune response. Cell therapy likely operates through a paracrine mechanism, and alternative approaches such as cell-free fractions of extracellular vesicles or soluble factors, which may reduce some of the risks associated with administering cells, especially allogeneic cells, need to be explored. For tissue engineered implantation studies using animals of the same size and weight as humans, testing implants under appropriate biomechanical conditions is critical. To answer these questions, pre-clinical trials are needed in cohorts of sufficient size, which must be carefully designed to measure relevant safety and efficacy outcomes. Equivalent diseases in animals make them not only relevant models that offer a more accurate assessment of the safety and efficacy of new treatments, but at the same time are potential beneficiaries of new treatment approaches. Therefore, human and veterinary medicine can mutually benefit if their similarities are taken into account (Ribitsch *et al.*, 2020).

Conclusion

Animal models play an important role in understanding human disease and are critical to expanding our knowledge of the molecular pathways involved in human disease. By now, there is a wealth of both experimental and inferred good quality information for humans, including genome quality, orthologous relationships, biomedical literature, tissue expression data, gene annotations, and protein associations. Unfortunately, the same does not apply to mice, rats or pigs. While the mouse is very frequently mentioned in the literature and well covered by tissue expression data and GO annotations, very few experimentally determined protein associations are reported for it. Meanwhile, there is a shortage of most types of annotations and data for both rats and pigs. Thus, one of the biggest limitations of current analysis is the availability of publicly available data for model organisms. This could be improved in the future by encouraging researchers to publish more experimental, carefully selected, and high-quality results from non-human organisms, especially when these organisms are popular model animals such as mice and rats. Thus, the gradual accumulation of genome data of the most commonly used model animals, knowledge of orthological relationships, as well as expression in tissues, gene annotations and protein associations are the basis for a more detailed understanding of the complex biochemical, biophysical processes that underlie the normal functioning of complex organisms and systems, violation of the dynamic balance in which can lead to certain pathologies and diseases (Doncheva *et al.*, 2021).

The importance of animal models is undeniable in terms of in vivo studies for any biomedical research in humans. It serves not only humanity, but also the well-being of veterinary patients. Animal models play an important role not only in drug development, toxicity studies, pharmacokinetic studies of drugs, but also in preclinical studies of medical and tissue engineering devices intended for use in humans. Laboratory animal models are more economical and suitable for high-throughput testing compared to large animal models (Mukherjee *et al.*, 2022).

Whatever the ways in which animal models are used for biomedical research, they must follow the principles of the 3Rs, i.e. reduction, refinement and replacement of animals. The use of animals in research must respect ethical principles and the reduction of their numbers should occur whenever possible. Improvements to the specific and valid model will potentially reduce the number of animals as well as the amount of exercise with them and should therefore be encouraged (Hubrecht & Carter, 2019).

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