

VIEWPOINT

# Controlling complexity: the clinical relevance of mouse complex genetics

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**Experimental animal models are essential to obtain basic knowledge of the underlying biological mechanisms in human diseases. Here, we review major contributions to biomedical research and discoveries that were obtained in the mouse model by using forward genetics approaches and that provided key insights into the biology of human diseases and paved the way for the development of novel therapeutic approaches.**

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## INTRODUCTION

Developing treatments aimed at the causes of diseases such as cancer, infections and metabolic, autoimmune and psychiatric disorders requires knowledge of the underlying biological mechanisms. Such knowledge can only be obtained by functional studies in which the disease-relevant tissue and the course of disease are manipulated systematically, for example, pharmacologically, environmentally or genetically. Experimental models are fundamental for meeting these challenges, and biomedical scientists often select mice as models. That 'mice are not humans' is obvious and some studies claim that the mouse is not a good model for human diseases.<sup>1</sup> The use of experimental models in biomedical research serves two important functions: discovery of basic biological mechanisms and development of new drugs and treatments, both of which have been successfully employed for the development of novel clinical therapeutic concepts and treatments. In this article, the members of

the SYSGENET network recount some seminal studies on mouse models that provided key insights into the biology of human diseases and paved the way for the development of novel therapeutic approaches. SYSGENET represents a network of European scientists who use mouse genetic reference populations (GRPs) to understand complex genetic factors influencing disease phenotypes.<sup>2</sup>

## MOUSE STUDIES HAVE CONTRIBUTED TO MAJOR ADVANCEMENTS IN CLINICAL MEDICINE

One of the most important advances in immunology was the discovery of the major histocompatibility antigens, which turned out to be the key molecules in antigen recognition and tissue rejection. This landmark discovery came from studying tissue transplantations in different mouse strains and performing appropriate genetic studies, for which George Snell<sup>3</sup> was awarded the Nobel Prize in Physiology or Medicine in 1980. Four years later, research on mice was

awarded another Nobel Prize in Physiology or Medicine: Köhler and Milstein<sup>4,5</sup> were given the prize for developing one of the most innovative therapeutic approaches, the generation of monoclonal antibodies.

## THE VALUE OF FORWARD GENETICS USING MOUSE MODELS

Forward genetic studies rely on natural variations of the genomes to analyze complex genetic traits. They are generally performed on N2 backcross, F2 intercross mice or in mouse GRPs. These approaches resemble genome-wide association studies (GWAS) in humans. They allow detection of genomic regions (referred to as quantitative trait loci) with multiple genes that exert quantitative influences on the phenotype, an approach referred to as complex genetic trait analysis. The simplest GRP is a collection of inbred mouse strains, each of which generated by repeated brother–sister mating for at least 20 generations, rendering all members of a strain genetically identical and homozygous at all loci. In humans, such identity exists only between monozygotic twins.

The most advanced GRPs are the BXD recombinant inbred strains<sup>6,7</sup> and the newly generated Collaborative Cross population,<sup>8</sup> which covers a genetic diversity twice as large as that of the human population and enables high resolution mapping.<sup>8–13</sup> Furthermore, the frequency of functional mutations can be increased by treating inbred strains with mutagens such as *N*-ethyl-*N*-nitrosourea.<sup>14–22</sup> In this approach, a single gene determines the phenotypic alterations, which are generally referred to as Mendelian traits.

A typical forward genetics approach starts with the phenotyping of a GRP or a collection of mutagenized mice. Once a phenotypic difference is found, breeding and genotyping studies are performed to identify the genomic region, and eventually the gene locus, responsible for the phenotype. Below, we describe several examples in which forward genetics approaches led to important discoveries that are highly relevant for understanding human diseases.

## Infection and immunity

The story started from a simple observation in mice and ended with the award of the Nobel Prize for Physiology or Medicine to Bruce Beutler in 2011. Injection of bacterial lipopolysaccharide (LPS) causes a lethal toxic shock in most mouse strains. However, C3H/HeJ mice are resistant to LPS.<sup>23</sup> By performing breeding experiments and genetic mapping, they discovered that a

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defect in the *Tlr4* gene causes the resistance of C3H/HeJ mice to LPS.<sup>24</sup> *Tlr4* belongs to a family of genes that are essential for the detection of microbial pathogens and initiation of immune responses. TLR4 also has an essential role in sepsis, which causes >200 000 deaths annually in the USA.<sup>25</sup> Furthermore, *Tlr4* knockout mice are resistant to the development of neuropathic pain.<sup>26</sup> TLR genes also have roles in autoimmune disorders, and they are now used as therapeutic targets for the development of drugs.<sup>27</sup> The initial discovery of TLRs as pathogen-sensing molecules opened a new research avenue that led to the discovery of pathways in immune and non-immune cells that are crucial for the host defense.<sup>28</sup>

Wild-derived mouse strains are highly resistant to infection with West Nile virus, but many laboratory inbred strains are susceptible. Genetic analysis has revealed that the gene encoding the 2'-5'-oligoadenylate synthetase 1b isoform (*Oas1b*) contributes to this susceptibility.<sup>29,30</sup> The role of *Oas1b* was confirmed in laboratory mice by introducing the wild-derived allele into inbred mouse strains by generating knock-in and transgenic mice.<sup>31,32</sup> These studies paved the way to the discovery of genetic variants of the *OAS1* gene in humans as a risk factor for primary infections with West Nile virus.<sup>33,34</sup>

### Metabolism

To identify non-invasive markers of non-alcoholic fatty liver disease (NAFLD), Barr *et al*<sup>35</sup> performed a metabolomics study on serum samples from a mouse model of this complex disease. Their work identified several disease markers, which were later also found in a cohort of patients with NAFLD. These markers have been developed as a standardized clinical assay for diagnosis and classification of NAFLD in humans.

The obese mutant mouse *ob/ob* arose spontaneously in The Jackson Laboratories, and genetic studies identified a mutation in the leptin (*Lep*) gene as the cause. Leptin is a satiety factor, and its absence in *ob/ob* mice results in uncontrolled eating.<sup>36</sup> Subsequent studies on humans also found an association between mutations in *LEP* in a subset of morbidly obese people, confirming leptin's function in the regulation of appetite in humans.<sup>37,38</sup> Since then, leptin has been demonstrated to have other functions in humans, including regulation of hematopoiesis, angiogenesis, wound healing, and immune and inflammatory responses.<sup>39</sup> Leptin level or leptin responsiveness is altered in humans affected by diabetes, renal failure, hypothyroidism and

AIDS. Thus, the identification of leptin and its receptor (*Lepr*), which is mutated in the db/db mouse, has opened a whole new research field to understand the biology of obesity and its risk for several severe disorders in humans, including diabetes, cardiovascular diseases and cancer.

### Cardiovascular system

Koutnikova *et al*<sup>40</sup> described the mapping of quantitative trait loci (QTLs) for blood pressure in the BXD recombinant inbred mouse population and found a QTL on chromosome 9. By using a combined genetic analysis of candidate genes from this QTL interval in mice and syntenic regions on chromosome 3 in humans, they discovered that a polymorphism in the *UBP1* gene is associated with hypertension in humans. The gene product of *UBP1* and the pathways in which it is active could serve as targets for treatment of hypertension in humans.

Atherosclerosis is a complex human disease involving both genetic and environmental risk factors. Polymorphisms in genes directing lipid metabolism, inflammation and thrombogenesis are thought to be responsible for the wide range of susceptibilities in the general population to myocardial infarction, a fatal consequence of atherosclerosis. Genetic linkage studies have been carried out on humans and mouse models to identify genetic polymorphisms controlling related phenotypic traits. Until now, ~40 quantitative trait loci for atherosclerotic disease have been found in humans, and ~30 in mice.<sup>41</sup> Follow-up studies identified genes that are causally involved in the development of atherosclerosis. For example, the *Tnfsf4* gene, encoding the OX40 ligand, has been identified by positional cloning as a gene that influences atherosclerosis formation in mice. Subsequent analysis in a human cohort identified a polymorphism in the *TNFSF4* gene associated with increased risk of myocardial infarction.<sup>42</sup>

### Circadian rhythms

The first known mammalian gene regulating circadian behavior is *Clock*. It was discovered in mice in an ENU mutation screen.<sup>43–45</sup> These findings led to the discovery of a set of core circadian clock genes (*Bmal1*, *Npas2*, *Per1,2*, and *Cry1,2*), which function together with *Clock* in a negative feedback loop. They also paved the way to the concept of central and peripheral clocks regulating circadian biology. The discovery of *Clock* was the basis for genetic association studies in humans, which demonstrated an association of *CLOCK* and other circadian genes with

sleep/wake cycles and disrupted circadian rhythms in familial cases of advanced sleep phase syndrome.<sup>46,47</sup> More importantly, the discovery of *Clock* and other circadian genes provided the basis for the investigation of circadian clock-controlled mechanisms in diverse physiological and pathological conditions in humans, such as the regulation of blood pressure, cardiovascular functions, ischemia, diabetes, metabolism syndrome and regulation of endocrine and immune functions.<sup>48,49</sup>

### Central nervous system

Poot *et al*<sup>50</sup> studied the natural variation in the volume of the corpus callosum in mice from the BXD population. They found a QTL on chromosome 7 that influences this trait. Subsequently, they related the mouse genes located in this QTL interval to genetic data from patients with abnormal corpus callosum (ACC) development. Their analysis revealed that the *HNRPU* gene (*Hnrpul1* in mice) is strongly associated with corpus callosum abnormalities in humans. These results pinpointed a common genetic basis for corpus callosum development in the brain of mouse and man, and provided a foundation for using mouse models to elucidate the neurobiological mechanisms underlying behavioral disorders associated with abnormal development of the corpus callosum.

Narcolepsy is a rare human sleep disorder with a strong genetic component. However, genetic studies in humans only found a strong association with the human leukocyte antigen (HLA) system. Forward genetic studies in the dog and reverse genetics in the mouse found that the orexin/hypocretin system underlies this disease.<sup>51,52</sup> Subsequent studies on humans found that orexin-expressing neurons are lost in narcolepsy and are not detectable in cerebrospinal fluid. Orexin level in cerebrospinal fluid is now used to diagnose narcolepsy and to study the etiology of the loss of specific orexin neurons.<sup>53,54</sup>

### Psychiatric abnormalities

Psychiatric disorders are very difficult to study in humans as well as in experimental animals due to the difficulty of defining and assessing disease phenotype. However, comparative genetics has contributed to the discovery of translational genotype-phenotype relationships in humans and mice that are relevant for starting to understand these complex brain disorders.<sup>55</sup>

De Mooij-van Malsen *et al*<sup>56</sup> studied chromosome substitution strains of mice

(CSS)<sup>57</sup> to dissect complex behaviors into separate components. Subsequent genetic mapping of these traits revealed a genetic locus for avoidance behavior on mouse chromosome 15 that is homologous to a human linkage region for bipolar disorder. By integrating the mouse QTL data with genotypes from a large genome-wide association data set for bipolar disorders, they identified novel genes, such as *Adcy8*, that provide new insights towards understanding the neurobiological mechanisms underlying this complex mood disorder.<sup>56</sup>

Hovatta *et al*<sup>58</sup> performed behavioral analysis in different inbred mouse strains and related them to gene expression profiles in various brain regions. They identified 17 genes with expression patterns correlating with anxiety-like phenotypes. Subsequently, they tested 13 known human homologs as candidate genes for human anxiety disorders and showed that several of them are associated with human anxiety disorders as well.<sup>59</sup>

Malki *et al*<sup>60</sup> treated mice from different inbred strains with antidepressant drugs and measured gene expression levels in the hippocampus. Gene expression analysis of strain-by-drug interactions revealed 17 differentially expressed genes. Subsequently, they searched for SNPs in the corresponding genes in a human cohort in which the response to antidepressant drugs was investigated. This comparison identified in the human *PPM1A* gene polymorphisms that are associated with differential responses to nortriptyline, a norepinephrine reuptake inhibitor.

## REVERSE GENETICS AND THE MOUSE MODEL

In addition to forward genetic approaches, the mouse has been extensively used as a model for reverse genetics, that is, manipulating a gene and then determining the biological consequences. For the latter, the establishment of targeted mutagenesis in ES cells has made it possible to generate knockout (KO) mice in which a specific gene is deleted. The main advantage of KO models is studying the function of a single gene by comparing KO mice to wild-type mice, on a defined genetic background. KO models have contributed enormously to our understanding of gene functions.<sup>61,62</sup> Generation of a mouse KO for every mouse gene in combination with systematic phenotyping of these mutant lines by the International Mouse Phenotyping Consortium (IMPC)

will be extremely important for future forward genetic approaches.<sup>63</sup>

Complex genetics studies in mice and genome-wide association studies in humans identify QTL regions in which the causative gene(s) can be identified. Subsequently, the combination of extensive knowledge of many genes and the availability of the KO models is extremely important to find the best candidates and to demonstrate causality. One example is the confirmation of the consequences of the *Tlr4* mutation for LPS resistance.<sup>26</sup>

KO mice are generated on a single genetic background. However, the phenotypic manifestation of a gene KO might differ between genetic backgrounds due to differences in modifier genes. Such variations have been observed in diabetic mice with mutations in the *db* or *ob* gene.<sup>64</sup> Similarly, the severity of the diabetes caused by disruption of the *Wfs1* gene depends on the mouse genetic background.<sup>65</sup> Further, the *clock* mutation was found in a ENU screen in a B6 background, but was lost when transferred onto a C3H background. A subsequent QTL study identified a modifier gene that is part of the melatonin synthesis pathway.<sup>66</sup> Thus, forward and reverse genetic approaches are not mutually exclusive but highly complementary.

## 'THE HUMAN IS THE BEST MODEL FOR HUMAN DISEASES'

One criticism leveled against the use of mouse models for human diseases is that they do not reflect the full complexity observed in humans.<sup>67</sup> We very much disagree with this view, because it does not appreciate the reasoning behind using models, which is to reduce the complexity of a system and not to reproduce it. Only then is it possible to study the effect of individual causal components. In humans, diseases are in most cases multifactorial. Both genetic and environmental factors influence the outcome, so it is often very difficult to identify the genetic influences because they are masked by too many confounding factors. Experimental models are extremely useful because they 'simplify' the study by keeping most of the environmental factors constant (no differences in medication, diets, socioeconomic factors, stress at work, pollution, etc).

It is also important to reflect on the term 'model' in biomedical research. In basic research, the main purpose is to discover and understand biological mechanisms. In this case, it is not necessary that a model

reflects perfectly a human disease state. One should not always expect that a mutation in an orthologous gene in the mouse results in the same phenotype observed in humans. But even in these cases, one will obtain important insights into its biological functions. On the other hand, the expectations are different in drug research and development. In this case, a model should approximate as much as possible the human phenotype in order to reliably predict the outcome of clinical treatment. Here, many sophisticated disease models based on advances in mouse genetics have been developed. They include mouse lines with multiple gene knockouts and transgenic lines carrying human disease-associated alleles. An analysis of the 100 best-selling drugs showed that phenotypes of knockout mice correlate well with drug efficacy, and thus were crucial for target discovery and validation.<sup>68,69</sup> In addition, ~40% of animal studies could be reproduced in human trials. For those that failed, one of the reasons may have been inappropriate clinical trial design.<sup>70,71</sup> It should also be noted that using a single mouse strain and a single tissue for analysis may not be appropriate to draw general conclusions about the usefulness of the mouse model.<sup>1</sup> Thus, 'we should foster the possibilities of each model, not malign them.'<sup>72</sup>

Another argument against the validity of the mouse as a model system is that GWAS studies in humans make mouse genetics obsolete.<sup>73</sup> This may be true in some cases, but not in general. For obvious ethical reasons, experiments on humans are not possible for phenotypes such as susceptibility to infections, toxic substances, allergens or stress. Further, the regions identified in GWAS studies contain many genes and without prior knowledge of gene functions in the mammalian organism (described in experimental models) it will be impossible to find the causal gene variants. Once an association between genetic variations and disease states has been identified in human studies, it will be necessary to prove causal relationship. This is only possible in an animal model. Furthermore, environmental and other confounding factors are numerous and difficult to control in human studies. To address them, cohorts will have to be stratified into increasingly smaller groups, reducing the groups to sizes that do not allow any more significant associations. Last but not the least, studies in mouse models cost only a fraction of the cost of large-scale genetic studies in humans.

## CONCLUSIONS

Granting agencies and policy makers as well as clinical researchers and practitioners should all realize that mouse models are essential to advance our understanding of human biology and, in this way, obtain a better knowledge of human disease pathomechanisms and how to best treat them. Yet, we do recognize the limitations of mouse models and that one-to-one translation to humans in clinical trials is not the norm. Thus, joint translational research involving clinicians and basic researchers using mouse models will always be vital for the success of clinical research.

Furthermore, one should not be shortsighted and dictate research approaches solely by current clinical needs. Even experts in their fields were way off the mark when predicting the future potential of their developments. Gottlieb Daimler presumed that 'There will be a maximum of 5000 automobiles being built, because there are not enough chauffeurs to drive them.' IBM chief Thomas Watson estimated in 1943 'a world-wide need of about five computers', and in 1981, Bill Gates thought that '640 K are enough for everybody'.<sup>74</sup> Thus, looking back, it is very clear that without broad basic research approaches, and the use of mouse model systems, in particular, many modern clinical treatments and therapies would simply not exist. Therefore, only continuous efforts to understand mammalian biology in experimental animal models will allow biomedical research to make major discoveries that will considerably advance the development of novel strategies to diagnose and treat human diseases.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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