

Progress and prospects in rat genetics: a community view

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The rat is an important system for modeling human disease. Four years ago, the rich 150-year history of rat research was transformed by the sequencing of the rat genome, ushering in an era of exceptional opportunity for identifying genes and pathways underlying disease phenotypes. Genome-wide association studies in human populations have recently provided a direct approach for finding robust genetic associations in common diseases, but identifying the precise genes and their mechanisms of action remains problematic. In the context of significant progress in rat genomic resources

over the past decade, we outline achievements in rat gene discovery to date, show how these findings have been translated to human disease, and document an increasing pace of discovery of new disease genes, pathways and mechanisms. Finally, we present a set of principles that justify continuing and strengthening genetic studies in the rat model, and further development of genomic infrastructure for rat research.

The laboratory rat (*Rattus norvegicus*) has been used as an animal model for physiology, pharmacology, toxicology, nutrition, behavior, immunology and neoplasia for over 150 years (reviewed in ref. 1). Because of its size, ease of manipulation and breeding characteristics, it remained the preferred choice for most of these fields throughout the twentieth century, while the mouse became the leading mammal for experimental genetics. However, since the development of the first inbred rat strain by King in 1909 (ref. 2), over 500 inbred rat strains have been developed for a wide range of biochemical and physiological phenotypes and different disease models.

The US National Institutes of Health (NIH), realizing the potential of rat models in understanding basic biology and human health and disease, launched the Rat Genome Project in 1995, followed by the Rat Expressed Sequence Tag (EST) Project in 1997. These two programs, funded by 13 institutes and centers at NIH, produced a variety of basic genomic resources and provided powerful tools to link to and capitalize upon the data and resources in other model systems and humans. In 1999, the US National Heart, Lung and Blood Institute convened a meeting to discuss the opportunities needed to take full advantage of rat models and to define priorities. The meeting recognized the need to build the rat research community and create a rat genome database, and also made four major recommendations, listed in order of priority as shown in **Box 1**.

Since 1999, the community of investigators using rats as genetic models has grown and matured through its coordinated activities and the collective investments leveraged from funding agencies in the European Union, Germany, Great Britain, Japan and the United States. All of the recommendations of the 1999 meeting have been exceeded, with the exception of site-specific gene targeting, which is the ongoing focus of intensive investigation. The resources and infra-

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structure generated have been instrumental in accelerating the pace of biological research in the rat, in driving disease gene discovery in the rat model and in translating these discoveries to related diseases in humans.

In this article, we highlight the successes since the 1999 meeting and note where more infrastructure is needed. We then discuss the impact of advances in human genetics for continuing genetic studies in the rat and conclude with a vision for the future of rat research in the immediate post-genome era.

New genome resources

In the last decade, there has been an extraordinary increase in rat genomic resources, including the generation of a comprehensive 7.5× sequence of the Brown Norway (BN) rat genome; an increase from 5,000 genetic markers to millions of SNPs between different rat strains and from 93,000 ESTs to more than 800,000 ESTs and 5,000 annotated rat gene sequences; development of new methodologies for high-throughput genotyping and expression profiling; and construction of extensive database services in the Rat Genome Database (RGD).

Rat genome sequence. The goal of the genomic sequencing project was to develop a high-quality draft of a single strain. The Brown Norway strain (BN/NHsdMcwi) was selected as the reference strain because it is a well characterized inbred strain and is the founder strain for several important genetic panels, including the BXH/HXB recombinant inbred panel^{3,4} and the SS.BN and FHH.BN consomic panels⁵. The Brown Norway genome was sequenced with what was, at the time, a new strategy that combined a variety of sequencing and mapping resources (bacterial artificial chromosome (BAC) end sequences, whole genome shotgun (WGS) sequence and BAC fingerprint map) along with a new genome assembly program, Atlas. These combined approaches resulted in a draft sequence of the rat genome in which individual contigs are mostly of finished quality⁶. The draft sequence enabled the first detailed three-way sequence comparison between mouse, rat and human, providing new information regarding gene and genome evolution⁷.

Since the initial publication, the genome sequence has been upgraded once by replacing the draft with the available finished sequence from targeted BACs (~55 Mb). By using newer versions of the Atlas assembly software developed since the original assembly⁸ and combining unique sequence data from a WGS-only assembly with the data from the WGS plus BAC assembly, including additional finished BACs, a new assembly of the rat genome will be completed in 2008.

Given the new and increasingly inexpensive sequencing technologies available and the need in the community to sequence other commonly used inbred strains, further shotgun sequencing of more strains at the 2× to 4× level would provide a substantial increase in the number of known SNPs and copy number variants (CNVs). A pilot 2× sequencing of the spontaneously hypertensive rat (SHR) genome, using massively parallel paired-end sequencing, is at present being carried out by European investigators in the European Union-funded EURATools consortium. When combined with other techniques such as optical

mapping⁹, and applied to other commonly used strains, these resources will significantly enhance and further accelerate progress of genetics research in the rat.

Genetic markers, linkage maps and microarray resources. Since the publication of the first microsatellite maps of the rat^{10,11}, the collection of genetic markers has progressively increased^{12–15}. In this issue, the STAR consortium reports an additional survey of genetic variation, based on three million SNPs identified from a variety of sources and strains¹⁶. This consortium also selected a subset of 20,000 SNPs that were used to genotype a panel of over 300 inbred strains and hybrid animals. This has led to the creation of a large dataset of well characterized SNPs for quantitative trait locus (QTL) and disease gene mapping, and has allowed the construction of new high density genetic maps. As discussed in the accompanying manuscript¹⁶, the data further characterize the population structure, illustrate the degree of linkage disequilibrium and present an initial haplotype structure of the rat genome. These community resources are openly accessible and significantly augment the tools available for genetic studies of physiological and disease phenotypes in the rat. While the SNP discovery program is continuing within the United States and within the European Union EURATools consortium, the studies also demonstrate the clear need to extend further the collection of SNPs and markers for saturation genotyping and generation of a detailed haplotype map of the rat genome.

In a similar vein to advances with genetic maps, a recently updated cytogenetic map was constructed from G-banded prometaphase chromosomes. This map contains 535 individual bands, considerably improving the previous ideogram and enabling its better use for interpretation of biological data¹⁷.

Commercial rat microarray platforms have been built by Affymetrix, Agilent and Illumina that query expression of up to 31,000 genes at the whole transcript level, or 850,000 exon clusters on the Affymetrix exon arrays. Agilent and NimbleGen have built tiling microarrays for comparative genome hybridization or expression analysis, with up to 350,000 sequences represented, and commercial platforms for rat microRNA analysis have been developed by Ambion, Solexa and Exiqon. Upgrades to these technology platforms will be needed if the benefits of improvements in the genome assembly and annotation, and sequences from additional strains, are to be realized fully.

Database development. RGD (<http://rgd.mcw.edu>) was established in September 1999 to focus on the rat genome and its resources in conjunction with the principal genome databases: the US National Center for Biotechnology Information (NCBI); University of California, Santa Cruz (UCSC); Ensembl; and Mouse Genome Informatics (MGI). RGD supports the rat research community through comprehensive curation and nomenclature assignment for the complete rat gene catalog (position, protein identifiers, ontologies for function and pathways, links to expression and phenotypes), microsatellite markers, QTLs and rat strains. RGD also provides tools for data mining, analysis and presentation. As described in the accompanying paper by Twigger *et al.*¹⁸, all rat QTLs and associated QTLs from mouse and human—including

BOX 1 Recommendations of the 1999 Rat Genome Priorities meeting

- 1. Germ-line modifications in the rat:** to develop new technologies for site-specific gene targeting and nuclear transfer, cryopreservation of zygotes and sperm, and generation of *in vitro* fertilization techniques, including intracytoplasmic sperm injection.
- 2. Additional genomic resources:** to include expanding the Rat EST Project, development of a SNP map, increasing BAC clone resources, pilot genome sequencing, and development of a Rat Genome Database.
- 3. National Rat Genetic Resource Center:** to maintain and distribute current and future rat models as recommended in the NIH Rat Model Repository Workshop report.
- 4. Interaction with the NIH Mouse Mutagenesis and Phenotyping Program:** to yield significant interaction and collaboration on developing new rodent models of human disease.

Table 1 Genes for rat disease phenotypes identified by positional cloning since 1999

Gene	Phenotype	Susceptible strain	Date	Complementation ^b	Human trait	Supplementary references ^c
<i>Cd36</i>	Insulin resistance, hyperlipidemia	SHR/NCrI	1999, 2001	<i>In vivo</i>	Dyslipidemia, insulin resistance, hypertension	S1,S2
<i>Aspa</i>	Spongy leukodystrophy	TRM/Kyo	2000	ND	Canavan disease	S3
<i>Mertk</i>	Retinal dystrophy	RCS/Kyo	2000, 2001	<i>In vivo</i>		S4,S5
<i>Atrn</i>	Hypomyelination, mahogany coat color	Zi/Kyo	2001	<i>In vivo</i>		S6
<i>Cyp11b1</i>	Blood pressure	SS/Jr	2001	ND	Hypertension	S7,S8
<i>Ncf1</i>	Arthritis	DA/Rhd	2002	<i>In vivo</i>	Rheumatoid arthritis	S9,S10
<i>Cblb</i>	Type 1 diabetes	KDP/Tky	2002	<i>In vivo</i>		S11
<i>Gimap5</i>	Type 1 diabetes	BB/OK	2002	ND		S12,S13
<i>Pkhd1</i>	Polycystic kidney disease	PKD	2002	ND	Polycystic kidney disease	S14
<i>Rab38</i>	Platelet storage pool disease, renal failure	FH, LE/BluGill, FHH/EurMcwi	2004, 2005	ND		S15,S16
<i>Ciita</i>	MHC expression	DA/Slc	2005	ND	Rheumatoid arthritis, multiple sclerosis, myocardial infarction	S17
<i>Gstm1</i>	Hypertension, oxidant stress	SHRSP/Gla	2005	ND		S18
<i>Anks6</i>	Polycystic kidney disease	PKD/Mhn(cy/+)	2005	ND		S19
<i>Fcgr3</i>	Crescentic glomerulonephritis	WKY/NCrI	2006	ND	Systemic lupus erythematosus, systemic autoimmunity	S20,S21
<i>Tmem67</i>	Polycystic kidneys, brain malformations	WPK	2006	ND	Meckel-Gruber syndrome	S22
<i>Fbxo10, Frmpd1</i>	Mammary cancer	WF/NHsd	2007 ^a	ND	Breast cancer	S23
<i>Ephx2</i>	Heart failure	SHHF/Bbb	2007 ^a	<i>In vivo</i>	Heart failure	S24
<i>Ogn</i>	Left ventricular mass	SHR/NCrI	2007 ^a	<i>In vivo</i>	Left ventricular mass	S25
<i>Jund</i>	Crescentic glomerulonephritis, macrophage activation	WKY/NCrI	2007 ^a	ND	Macrophage activation	S26
<i>Vav1</i>	Neuroinflammation	DA/BIl	2007 ^a	ND	Multiple sclerosis	S27
<i>Srebf1</i>	Hepatic steatosis	SHR/Olalpcv	2008	<i>In vivo</i>		S28

^aPresented at the Cold Spring Harbor Rat Genomics and Models meeting, December 2007. ^bWhere indicated, complementation was by *in vivo* complementation in either rat or mouse. ND, Not done. ^cSupplementary references S1 to S27 are given in the **Supplementary References** online.

nomenclature assignment, map locations, strains or populations used in each study and phenotypes measured—are curated. The mapped traits are visualized in the genome browsers at RGD, Ensembl, UCSC and also in NCBI's map viewer. The comprehensive rat strain catalog maintained at RGD has become the foundation for all knowledge relating to over 1,000 strains, substrains and genetically modified rats. Reflecting the disease-focused nature of much of rat research, RGD has also created disease portals¹⁹ to provide easy access to genes, QTLs, pathways, phenotypes and strains associated with major disease areas such as obesity, cardiovascular, neurological and other diseases.

The community has been working to integrate data from scattered resources using Distributed Annotation Systems (DAS) and BioMart, both of which have proven highly valuable in other model organism and genome databases. Some significant developments centered on data integration are still needed, however. A common reference gene set is needed to ensure consistency across the genome databases. The main gene prediction and gene annotation groups (RGD, NCBI, Ensembl and UCSC) have been working together since mid-2007 to develop a process to tackle this issue. Phenotype data must be captured and described in a consistent manner both within a species and between species. Finally, there is a critical need to expand links between physiological data and genotype data across species, particularly as genome-wide association studies (GWAS) yield huge datasets that provide a rich resource for comparative data mining.

National Rat Genetic Resource Centers. The development of hundreds of inbred and mutant strains and the characterization of a wide range of disease models have necessitated and driven the creation of two major rat resource centers, in the USA and Japan. As a result of

recommendations of an international NIH workshop in 1998, the NIH Rat Resource Research Center (RRRC; <http://www.nrrrc.missouri.edu/>) was established in 2001. A similar approach on a larger scale was undertaken in Japan in 2002 with the National Bio Resource Project for the Rat, (NBRP-Rat; <http://www.anim.med.kyoto-u.ac.jp/NBR/>). Smaller repositories have been established in Europe at the Hannover Medical School (<http://www.mh-hannover.de/2652.html>) and by EURATools at the Czech Academy of Sciences (<http://www.euratools.eu>). The resource centers collect, preserve and supply unique and important rat strains for biomedical research to the local and international research community. In addition, all resource centers carry out research directed at improving repository functions, including better cryopreservation methods and assisted reproductive technologies, and they also develop new diagnostic screening methods for rat pathogens. Researchers at the NBRP-Rat have recently created a tutorial on DVD, in English and Japanese, on cryopreservation of embryos and spermatozoa and their rederivation, including vitrification of rat two-cell embryos and morulas, intracytoplasmic sperm injection (ICSI), cryopreservation of epididymal sperm and assisted reproduction techniques²⁰.

At present the RRRC contains over 300 transgenic, spontaneous mutant, complex trait models and inbred strains. The NBRP contains over 562 rat strains, and the ERRC and Prague repositories over 140 rat strains. These strain collections include a wide range of healthy inbred strains, as well as mutant, congenic, consomic, recombinant inbred and *N*-ethyl-*N*-nitrosourea (ENU)-mutagenized strains. Because of advances in cryopreservation and the recent development of robust protocols for ICSI and sperm freezing, it has also been possible to preserve ENU mutant lines as sperm and DNA collections. Sperm

from 5,000 ENU mutagenized F344 G1 animals are now integrated into the NBRP repository²¹. These strain repositories are important for the current and future research programs based around rat genetics.

Specialized mapping panels. Recombinant inbred strains have many advantages for QTL mapping, including the ability to increase trait heritability by phenotyping multiple genetically identical rats from each rat strain²² and the ability to accumulate data from genetically stable strains over years, obviating the need for *de novo* genotyping. Currently, two large recombinant inbred panels are available in the rat: the HXB/BXH set ($n = 31$) derived by reciprocal crosses of SHR/Ola and BN-Lx (refs. 3,4) and the LEXF/FXLE set ($n = 34$) by reciprocal crosses of F344/Stm and LE/Stm²³. Two smaller panels, PXO and LXB, have also been reported^{24,25}. The larger LEXF/FXLE and HXB/BXH panels have been proven to produce successful results in genetic mapping, and expression QTL (eQTL) and gene identification studies^{4,16,23,26–28}.

Rat heterogeneous stock panels allow fine-mapping of QTLs to sub-centimorgan intervals, significantly reducing the number of candidate genes found within each QTL. The best characterized rat heterogeneous stock panel was established at the NIH in 1984 and derives from eight genetically distinct and phenotypically diverse inbred strains (ACI/N, BN/SsN, BUF/N, F344/N, M520/N, MR/N, WKY/N and WN/N)²⁹. The heterogeneous stock colonies are maintained in a breeding scheme designed to minimize inbreeding and to reduce the size of haplotypes from each strain throughout the genome by recombination. After 50 generations of breeding, the estimated average distance between recombination events approaches a single centimorgan³⁰, enabling efficient and very high resolution QTL mapping. Heterogeneous stock colonies from this panel are now maintained at the Medical College of Wisconsin (Wisconsin, USA), the Autonomous University of Barcelona (Spain) and the Indiana University School of Medicine (Indiana, USA). Following the landmark study of Flint and colleagues³¹, who demonstrated successful fine mapping of 843 QTLs for over 100 phenotypes using heterogeneous stock mice, heterogeneous stock rat studies are now underway within the EURATools consortium and at the Medical College of Wisconsin and a wide variety of behavioral, cardiovascular, metabolic, inflammatory and other traits are being mapped. Many of these traits are preferentially studied in the rat because of accuracy and ease of high-throughput measurements in the rat model and similarity of the traits to human phenotypes.

Consonic rat strains are generated by introgressing an entire chromosome from one inbred strain into the isogenic background of another inbred strain using marker-assisted selection. The PhysGen (<http://pga.mcw.edu>) Program for Genomic Application at the Medical College of Wisconsin developed two panels of consonic rats using the SS/JrHsdMcwi, the FHH/EurMcwi and the BN/NHsdMcwi strains⁵. Comprehensive characterization (434,845 physiological data points) of these consonic strains, each carrying a chromosome from the sequenced Brown Norway strain, allows for immediate mapping of traits to a particular chromosome without the need for genetic crosses. From the consonic strains, congenic strains can be rapidly bred within 6 months to narrow the region on a specific chromosome to a region that can be targeted for gene identification. Several studies of this nature are now nearing completion. All strains are available from PhysGen.

From rat model to human disease

The infrastructure investment described above and the resulting genome resources have led to many disease gene discoveries in rats, frequently translated to humans, to an extent that could not have been anticipated

nine years ago (**Table 1**). In the early part of the past decade, most of the rat traits that were positionally cloned were monogenic, with only a few exceptions of more complex traits, including insulin resistance (*Cd36*)³², autoimmune diabetes (*Cblb*, *Gimap5*)^{33–35} and arthritis (*Ncf1*)³⁶. Identification of *Cd36* as an insulin resistance gene was the first example of cloning a complex-trait gene using a combined expression-microarray and linkage approach³². In the last 2 years, however, the integrated use of genetic mapping, gene expression and computational analysis has permitted identification of a steady stream of genes underlying rat polygenic traits, including, but not limited to, neuroinflammation³⁷, glomerulonephritis^{38,39}, mammary cancer⁴⁰, heart failure⁴¹ and left ventricular mass²⁶. In all of these studies, associations were also demonstrated to related traits or diseases in humans. The investments in animal models and genomic infrastructure, together with these recent reports, indicate that the discovery rate is accelerating. We predict that continued development of the genome infrastructure will further increase this rate of discovery.

New concepts in genomics are being rapidly applied to rat research, one example being gene CNVs. The observation that the molecular lesions of two of the earliest QTL genes identified in the rat (*Cd36* and *Fcgr3*) were gene CNVs^{32,38} has suggested that this type of structural genome variation may be an important evolutionary mechanism mediating development of genetically complex phenotypes in rats and other mammals. A recent genome-wide survey has revealed that at least 1.5% of the rat genome resides in regions that are variable in copy number⁴². Notably, these variable regions share many characteristics with regions that are variable in the human genome. In addition, 80 genes that are implicated in human disease were found to reside in variable copy number regions in both human and rat. Finally, the availability of data from recombinant inbred strains, including genome-wide gene expression data and high-resolution haplotype information, provides a platform for robustly linking copy number status to gene expression changes and phenotypic variability⁴². Construction of a more complete, higher resolution CNV map will provide further insights into the contribution of CNVs to the evolution of, and susceptibility to, complex phenotypes.

Genetic analysis of some rat traits, exemplified by polycystic kidney disease (PKD), has contributed to gene identification in humans and, in the case of PKD, has increased understanding of the pathophysiology to the extent of identifying treatment modalities for clinical trials. In the common infantile form of PKD, autosomal recessive PKD, the gene in the PCK rat model was found by mapping and positional cloning to be orthologous to that responsible for human autosomal recessive PKD, and analysis of the rat model enabled the human disease gene to be identified⁴³. Subsequently, the PCK model was shown to have ciliary abnormalities⁴⁴, reinforcing the link between primary cilia and PKD. Possible defects in planar cell polarity (mitotic spindle orientation) were also first associated with PKD in this model⁴⁵. The PCK model has also proven effective for screening potential therapeutic agents that have been targeted at the higher cellular amounts of cyclic AMP in PKD by way of the vasopressin V2 receptor (*Avpr2*) in the collecting duct^{46–49} or by use of somatostatin analogs⁵⁰. Clinical trials of a vasopressin receptor antagonist and the somatostatin analog octreotide are in progress.

Transgenesis and gene targeting

Although gene targeting technology remains under development, other technologies for disrupting genes have been adapted from the mouse to generate knockout rat models for functional annotation. Many of these strategies are derived from the mouse mutagenesis programs as recommended (see **Box 1**) and include traditional transgenesis (reviewed in ref. 51), viral vector-mediated DNA transfer^{52,53}, sperm-mediated DNA transfer^{54–57} and RNA interference^{58,59}. To date, it has not proved possible

to culture germline-competent rat embryonic stem (ES) cells, although ES-like cells have been isolated and reported to contribute to extraembryonic tissues *in vivo*^{60–62}. Nuclear transfer in the rat has also been problematic because cultured oocytes are prone to spontaneous activation, and so far there are few reports of live births after nuclear transfer^{63–65}. Meanwhile, chemical mutagenesis using ENU and gene-trap transposon insertional mutagenesis using the Sleeping Beauty transposon system have now generated dozens of mutant rat strains and potential disease models.

Traditional transgenesis by microinjection continues to be used for genetic manipulation of the rat to generate new models of interest. For example, one of the best models for Alzheimer's disease is a triple transgenic rat model that brought together two mutant amyloid precursor protein alleles with human presenilin-1 (ref. 66). Other examples include transgenic models for amyotrophic lateral sclerosis and human tauopathies^{67,68}.

Chemical mutagenesis. Small- and large-scale random chemical mutagenesis using ENU combined with a gene screening strategy has been used to generate gene knockouts in the rat. For example, two important cancer genes, *Brca1* and *Brca2*, were screened using a simple yeast-based screening assay that identifies premature stop codons introduced by ENU mutagenesis⁶⁹, and an *Apc* null line with a phenotype showing close similarity to human colon cancer has been reported⁷⁰. The Cuppen laboratory and the PhysGen program, using an alternative strategy⁷¹ involving high-throughput resequencing of PCR-amplified genomic targets to detect mutations⁷², have identified more than 200 ENU-induced mutant strains, including 18 putative knockout mutations. Finally, as reported in this issue and described above, the combination of ENU mutagenesis with a high-throughput screening assay using the Mu-transposition reaction (MuT-POWER) and ICSI for the recovery of the rare heterozygous genotypes from a frozen sperm repository²¹ has yielded large numbers of mutant strains and mutations.

Transposon insertional mutagenesis. Transgenic rats carrying components of the Sleeping Beauty transposon system have been bred to generate transposon-knockout mutations^{73,74}. Although the low mutation rate of Sleeping Beauty mutagenesis is less amenable than ENU to forward-genetic screening applications and is generally inapplicable to the site-directed approach, the efficiency of the Sleeping Beauty system is adequate for creating a resource of defined knockout rat strains by random mutagenesis. Combining this technology with visible markers such as fluorescent proteins or β -galactosidase has revealed expression patterns of trapped genes⁷³ and creates tools for cell lineage tracing and organ systems biology. From all transposon-induced mutations generated so far by PhysGen, more than 30 are being sent to RRRC for storage and distribution.

Although germline-competent ES cells have not been established in the rat system, spermatogonial stem cell lines^{75,76} offer an alternative for insertional mutagenesis *in vitro*. By combining transposition-mediated gene insertion, gene-trapping technology, high-throughput culturing and archiving, a library of gene knockouts in rat spermatogonial stem cells could be generated. The use of diverse transposon systems with distinct target site preferences makes full genome coverage with transposon-based insertional mutagenesis a realistic goal.

Phenotyping challenges

Arguably the greatest asset in rat biology is the detailed knowledge generated over many decades by phenotypic analyses of physiological, pathological and pharmacological traits. Most of these studies have been driven by specific hypotheses, and, as a result, rat strains until now have mostly been studied for very specific phenotypes, providing only a narrow glimpse of the (patho)physiology of each strain.

The mouse community has invested in creating standard operating protocols and establishing mouse phenotyping clinics (<http://www.eumorphia.org>; <http://empress.har.mrc.ac.uk>; <http://www.knockoutmouse.org>; <http://www.jax.org/phenome>), with the aim of permitting comparison of phenotypes across strains and laboratories. The growing number of mutant rat lines generated by ENU mutagenesis, and those anticipated from transposon-mediated mutagenesis, increases the importance of standardized phenotyping in the rat model. So far, the rat community has embarked on a limited number of discrete phenotyping programs, with similar aims to those of the mouse community in optimizing utility and comparability.

The Rat Phenome Project in Japan has recently recharacterized 163 rat strains as models of human diseases and collected strain data for 109 parameters across neurobehavioral, cardiovascular, biochemical, hematological and anatomical phenotypic categories⁷⁷. The PhysGen program has characterized a panel of 44 consomic rat strains for 213 mainly cardiovascular phenotypes measured across the entire consomic panel. Data from both programs are publicly available at <http://www.anim.med.kyoto-u.ac.jp/nbr/phenome> and at PhysGen. The EURATools consortium has developed a protocol to fine-map over 50 behavioral, metabolic, hemodynamic, hematologic, immunologic, morphometric and expression phenotypes in individual heterogeneous stock rats, and the generation of these phenotypes is now underway.

Development of a more comprehensive international rat phenome project would advance the use of the rat by new investigators and might improve design of existing studies by investigators already using the rat. In developing a phenome project, it will be important to consider the different requirements for screening mutant rat lines compared to phenotyping in mapping studies. The emphasis in screening mutant lines is to identify a single, often extreme abnormality, whereas in mapping panels and inbred strains, extreme values may represent poor-quality data. Another consideration is the challenge attached to obtaining several phenotypes from single animals, as recognized for example in the SHIRPA protocol⁷⁸ and in mouse heterogeneous stock mapping studies³¹.

The mouse phenome projects offer an example of a coordinated effort in phenotyping. A similar initiative in the rat should benefit from the experiences of the mouse community in refining strategies to expand the efficient characterization of large numbers of inbred and mutant strains and to enhance the use of specialized mapping panels. Furthermore, the use of standardized ontologies (for example, ref. 79) to describe rat phenotypes will facilitate the comparison of disease phenotypes in different model species and humans.

Building the community

The past 10 years have seen the growth of a committed and close-knit rat genetics and genomics community that spans many disease areas and several disciplines. The most important community activities are the establishment of annual international scientific meetings; the development of the genome database, RGD, as described above; and the establishment of a European rat research community around several European Union-funded projects, including the recently funded EURATools consortium.

Following 20 years of international meetings on rat alloantigens and other topics of interest to rat geneticists, the first Cold Spring Harbor Laboratory meeting on Rat Genomics and Models was held in December 1999. Subsequent biennial meetings have been held at Cold Spring Harbor Laboratory since then, with alternating meetings in the intervening years being held in Europe and Asia. Future meetings will be held at Cold Spring Harbor in 2009 and 2011, in Hinxton, UK in 2008 and in Japan in 2010.

In Europe, annual meetings have also been held centered around several consortia funded under the European Union's Sixth Framework Program (<http://cordis.europa.eu/era/>). Principal investigators from the targeted research projects, STAR (a SNP and haplotype map for the rat; <http://www.snp-star.eu>) and Med-Rat (New Tools to Generate Transgenic and Knockout Mouse and Rat Models; <http://medrat.abc.hu/>), and from the larger scale Integrated Project EURATools (European Rat Tools for Functional Genomics; <http://www.euratools.eu>), represent a critical mass of the European rat research community who have contributed widely to the progress described in this Perspective^{16,26,39,41}.

Vision for the future

A principal purpose for studying the biology and pathobiology of the rat is to translate this information to humans, particularly in the context of complex traits. Many inbred models have been developed by selectively breeding rats carrying a phenotype of interest, resulting in genetic models that capture natural variation leading to common disease phenotypes including cancer, hypertension, diabetes, arthritis, autoimmunity and alcohol preference.

Because of notable recent advances in human genetics, including identification of scores of susceptibility genes for common human diseases by GWAS, the need for a multispecies platform to integrate and investigate human disease at the level of both genotype and phenotype has become increasingly apparent. For although GWAS have identified many previously unsuspected human disease genes, most of the genes individually have small effects on disease susceptibility. Furthermore, many of the associations are in gene deserts or, alternatively, span several genes. Thus, identifying the exact genes and mechanisms that underlie the GWAS associations often remains a significant barrier to progress.

For genetic studies using animal models, the arrival of human GWAS is a major paradigm shift. For many years, the role of animal systems in genetic studies has been predicated on the increased heritability, flexibility and statistical power of experimental crosses over corresponding studies in humans. Animal studies could therefore map disease genes with greater ease and higher statistical confidence and could then inform the search for disease genes in the complex backdrop of the human genome. The new human GWAS and resequencing programs are now able to sift the genome of the human without the need for animal model data, therefore raising questions about the continuing value of animal genetics studies.

In our view, the case for continuing, and even strengthening, efforts in animal genetics is clear and is based on several fundamental principles:

1. The wealth of literature accumulated over 50–100 years in models such as the mouse and rat offer an unparalleled resource for molecular genetic investigation of mammalian physiology and pathobiology.

2. The genome resources currently and potentially available for animal models have increased the pace of gene discovery such that scores or even hundreds of traits now mapped as QTLs or defined in consomic or minimal congenic regions will be positionally cloned in the next 5 to 10 years.

3. Positionally cloned animal QTL genes offer a unique route to defining underlying mechanisms of gene action in a way that cannot be achieved directly for human disease genes. Animals also offer better systems for in-depth phenotyping and for studying mechanisms of action of human disease genes.

4. Identification of quantitative trait nucleotides that underlie animal QTLs provides insights not only into gene function in health and disease but also into the evolutionary history of animals and animal models and their genomes.

5. Although all genes identified as underlying animal disease phenotypes may not themselves underlie the related human diseases, the rat

QTL genes identified so far have shown remarkable relevance to related human disease phenotypes, and either the genes themselves or the pathways in which these genes reside are likely to continue to provide insights into human disease and new approaches to their prevention and treatment.

6. Rat models can and will be built on the basis of clinical disorders and genetic and environmental stimuli, increasing the reliability of these systems for predicting drug responses in humans.

This Perspective has summarized the development of rat genomic resources over the past 10 years and has described how these resources have led to considerable research successes and the establishment of a strong, dynamic and committed research community focused on use of genome tools to advance understanding of rat and human physiology and pathobiology. We believe, however, that the return on the investment of the past 10 years is at an early stage. The acceleration of research progress described in this paper has reached fruition for a handful of QTLs and disease genes, but scores of other rat QTL projects are on the brink of similar discoveries. With continuously advancing genome resources and new mapping strategies, the rate of discovery stands to accelerate further.

This predicted pace of discovery, however, will depend not only on support of the present community resources, but also on use of new technologies to generate more sequence from more strains, use of the emerging strategies for rat mutagenesis and gene targeting, improved use of comparative genomics for data mining, and further development of technologies such as tools for analysis of microRNAs. These approaches will broaden the resources that rat researchers can draw upon to complete QTL gene identification, pursue discovery of gene function, and investigate the evolutionary pressures and pathobiology that make the rat an invaluable animal model for understanding the mechanisms and pathways underlying mammalian health and disease.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to the production of this manuscript.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/naturegenetics/.

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