

RAT GENETICS: ATTACHING PHYSIOLOGY AND PHARMACOLOGY TO THE GENOME

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During the past five years, the Rat Genome Project has been rapidly gaining momentum, especially since the announcement in August 2000 of plans to sequence the rat genome. Combined with the wealth of physiological and pharmacological data for the rat, the genome sequence should facilitate the discovery of mammalian genes that underlie the physiological pathways that are involved in disease. Most importantly, this combined physiological and genomic information should also lead to the development of better pre-clinical models of human disease, which will aid in the development of new therapeutics.

MULTIFACTORIAL GENETICS

For the past decade, the Rat Genome Project (RGP) has been organized by an international consortium of investigators and funding agencies, particularly the **US National Heart, Lung, and Blood Institute**. This project has benefited from strategies and technologies that have been developed and perfected in the human and mouse genome-sequencing projects, which have allowed the Rat Genome Project to move forward with lower costs. Before establishing the rat as a model organism in the Human Genome Project (HGP), there was much discussion of its use as a principal model organism, and about the benefits of having the rat genome sequenced, owing to the similarities between the rat and the mouse. However, because rat research has had a different focus to that of mouse research over the past two decades, being more physiological in nature (as discussed below), the rat provides unique opportunities for disease-based research that can be integrated with the important resources that have been generated by the HGP. Through international efforts, resources for the rat genome are largely now in place (BOX 1) and the sequencing of the rat genome is well under way, with a draft sequence now at more than twofold coverage and the completion of the draft scheduled for 2002.

In this review, we discuss ways to link the wealth of physiological and pharmacological data that are available for the rat to its genome and, more importantly, to those of the human and the mouse. We discuss several ways to map biological traits to the rat genome, such as by using characterized **INBRED STRAINS** in **QUANTITATIVE TRAIT LOCI (QTL)** -mapping studies, by developing **CONGENIC** and **CONSONIC** rat strains, and by using comparative mapping to build better rat models of human disease.

History of rat research

Rat genetics had a surprisingly early start. The laboratory rat *Rattus norvegicus* was the first mammalian species to be domesticated for scientific research; work on the rat dates back to before 1850 (REF. 1). The first genetic studies were carried out between 1877 and 1885 by Crampe and focused on the inheritance of coat colour¹. In 1903, William Bateson used the concepts of Mendel's laws to show that a variant of a rat coat-colour gene was inherited in a Mendelian fashion. The first inbred rat strain, PA, was established by King in 1909 — the same year that inbreeding began for the first inbred strain of mouse, DBA¹. The mouse soon became the model of choice for mammalian geneticists, whereas the rat became the model of choice for physiologists,

INBRED STRAIN

A strain that is generated through systematic inbreeding, which fixes certain alleles in a strain so that they replace all other alleles that were present in an outbred population.

QUANTITATIVE TRAIT LOCUS

(QTL). A genetic locus that is identified through the statistical analysis of complex traits (such as height or body weight). These traits are typically affected by more than one gene and also by the environment.

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Box 1 | **Rat genomic resources**

Rat genomic tools

Genetic markers

- Rat Genome Database, MCW, USA <http://rgd.mcw.edu>
- Rat Genetic Database, National Institutes of Arthritis and Musculoskeletal and Skin Diseases <http://www.niams.nih.gov/rtbc/ratgbase/index.htm>
- The Wellcome Trust Centre for Human Genetics, Oxford, UK http://www.well.ox.ac.uk/rat_mapping_resources

Clone libraries

- Children's Hospital Oakland Research Institute, California, USA (BAC and PAC clones) ... <http://www.chori.org/bacpac/>
- Whitehead/MIT (YAC clones) <http://www-genome.wi.mit.edu/rat/public/>
- Max Planck Institute, Rat Genome Project (YAC clones) <http://www.molgen.mpg.de/~ratgenome/>

Maps

- MCW (RH maps) <http://rgd.mcw.edu/maps/>
- University of Iowa (RH maps) <http://ratest.uiowa.edu/>
- The Wellcome Trust Centre for Human Genetics, Oxford, UK (RH maps) http://www.well.ox.ac.uk/rat_mapping_resources/rat_RH_comprehensive_maps.html
- RATMAP at the Rat Genome database (FISH maps) <http://ratmap.gen.gu.se/Physical/Chromapnyph.html>
- MCW (comparative maps) <http://rgd.mcw.edu/VCMAP/>
- Otsuka GEN Research Institute, Japan (comparative maps) http://ratmap.ims.u-tokyo.ac.jp/cgi-bin/comparative_home.pl
- Genome Sequence Center, Canada (physical maps) http://www.bcgsc.bc.ca/projects/rat_mapping/

Rat genome sequencing

- Baylor College of Medicine, USA <http://www.hgsc.bcm.tmc.edu/rat/>
- Celera Genomics, USA <http://www.celera.com/>
- Genome Therapeutics Corporation, USA <http://www.genomecorp.com/>
- The Institute for Genomic Research, USA <http://www.tigr.org/>
- MCW (EST sequencing) <http://rgd.mcw.edu>
- University of Iowa (EST sequencing) <http://ratest.uiowa.edu/>

Rat databases

In addition to sites already listed:

- Rat UniGene <http://www.ncbi.nlm.nih.gov/UniGene/Rn.Home.html>
- NIH Autoimmune Rat Model Repository and Development Center <http://www.ors.od.nih.gov/dirs/vrp/ratcenter/>
- PhysGen (MCW) <http://dolphin.ifrc.mcw.edu/PGA/>

BAC, bacterial artificial chromosome; EST, expressed sequence tag; FISH, fluorescence *in situ* hybridization; MCW, Medical College of Wisconsin; MIT, Massachusetts Institute of Technology; PAC, P1 artificial chromosome; RH, radiation hybrid; YAC, yeast artificial chromosome.

CONGENIC

A strain that is produced by a breeding strategy in which recombinants between two inbred strains are backcrossed to produce a strain that carries a single segment from one strain on the genetic background of the other. The selected segment can contain a quantitative trait locus.

CONSOMIC

A strain that is produced by a breeding strategy in which recombinants between two inbred strains are backcrossed to produce a strain that carries a single chromosome from one strain on the genetic background of the other.

nutritionists and other biomedical researchers. Geneticists preferred the mouse because of its smaller size, which simplified housing requirements, and because of the availability of many coat-colour, and other, mouse mutants with Mendelian patterns of inheritance that had been collected by mouse fanciers². In 1929, the vision of Clarence Cook Little, the founder of **The Jackson Laboratory**, created a pre-eminent resource that has served the mouse genetics community well, providing investigators with access to inbred strains and mutant mice.

By contrast, rat strains were developed primarily by physiologists and by other biomedical researchers in their own laboratories, and were selected and bred to have traits of biomedical interest. By the 1960s and 1970s, for example, the field of rat genetics was dominated by research groups that were studying immunology. Over the years, many investigators from various fields have created more than 234 inbred strains of rat by selective breeding for disorders that range from hypertension to urological defects (see REFS 3,4 and the **strain query form at the Rat Genome Database** for more information on inbred rat strains). By the early 1990s,

many genetic markers had been developed and mapped to the rat genome⁵, allowing the mapping of QTL that contribute to these physiological phenotypes⁵⁻⁸. In 2000, the National Institutes of Health funded a rat model repository to preserve valuable rat strains and to make them available to the research community independently of commercial breeders.

So far, most rat models have phenotypic characteristics that are relevant to a particular human condition. These phenotypes were initially induced surgically or pharmacologically, but eventually they were developed by: phenotypic selection for certain traits, such as hypertension⁹⁻¹¹, and generating inbred strains; isolating spontaneous mutants that model human disease, such as type I diabetes mellitus^{12,13}; and transgenesis¹⁴. Although these models have served the research community well, and have helped to advance biomedical research in general, they do not always recapitulate the clinical outcomes of human disease, owing to species-specific differences. Nevertheless, animal models are extremely important, especially when little is known about the basis of a human disease, as they can provide entry points

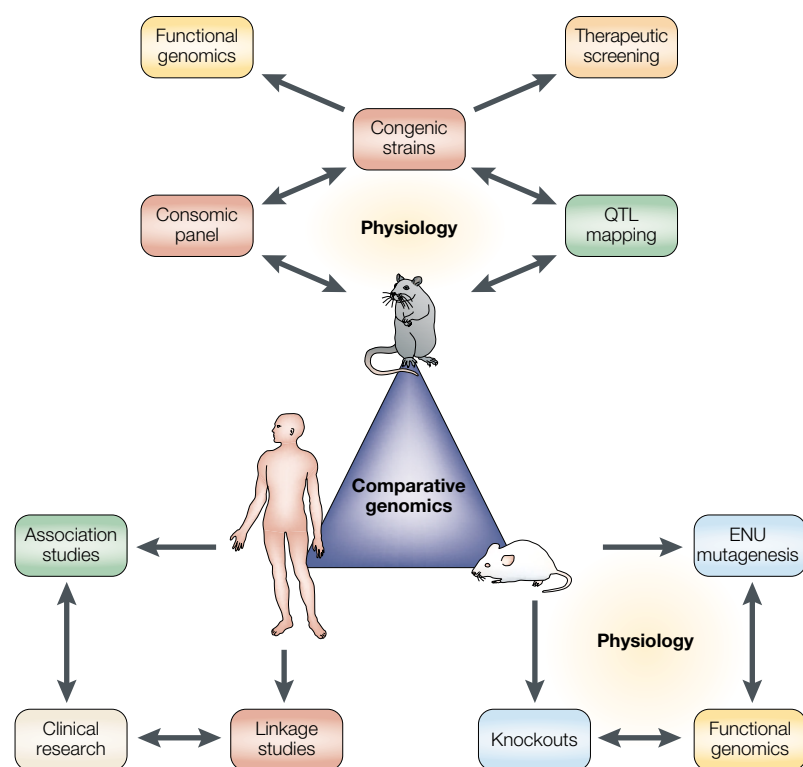


Figure 1 | Integrating data from the rat and mouse for studies of human disease. Through the study of comparative genomics, physiological, genetic and phenotypic data from the rat and mouse model organisms can be used to inform studies of human disease by providing candidate loci and gene-function data for gene-hunting studies. One of the primary advantages of the rat as a model organism is the amount of physiological and pharmacological information that is available for it. Inbred and transgenic strains of rat also provide models with clinically relevant phenotypes for the testing of new therapeutics. One of the key advantages of the mouse as a model organism is the availability of gene-knockout technology. ENU, ethylnitrosourea; QTL, quantitative trait loci.

into clinically relevant pathways, as exemplified by the identification of leptin in a mouse model of obesity¹⁵. So far, studies in humans have produced conflicting results as to the role of leptin in the genetic basis of human obesity, despite its involvement in obesity in both mice¹⁵ and rats¹⁶. Nonetheless, the elucidation of the leptin pathway has been very successful and has provided new insights into the pathways that mediate food intake and metabolism that, before these animal studies, were unknown in humans. So, animal models can provide important insights into human disease, besides being a means to investigate candidate disease genes. However, rat and mouse models often do not reflect nor develop all the clinical symptoms of human disease, and studies in a single inbred rodent strain often fail to sample a sufficient amount of genetic diversity to capture a complex phenotype. So, although an animal model can typify some aspects of a complex human disease, it often does not fully recapitulate it.

Attaching biological information to the genome

Gene identification and characterization are often the primary foci of genetic studies in humans and in the mouse, especially with the advent of mouse gene-knockout¹⁷ and

transgenic technologies. And, since the development of the first transgenic rat in 1990 (REF. 14), more than 2,000 publications that are indexed in PubMed report the use of transgenic rats in research. However, functional genomics ranges from gene transcription and regulation to overall SYSTEMS BIOLOGY at the whole-organism level. For investigators who are interested in, for example, the physiological or pharmacological aspects of systems biology, gene identification is not always an immediate concern because genomic tools can also be used to facilitate research into these areas of systems biology and to create better experimental systems. This is because the information that this research provides can be used to annotate the genome with physiological phenotypes. The mapping of many biological traits to the genome should help to accelerate our understanding of the biology of disease by guiding and facilitating the identification and functional understanding of disease genes.

We believe that any model organism has its strengths and limitations. However, comparative genomics allows us to integrate different types of information derived from various model organisms to generate a synergistic platform for understanding complex disease mechanisms. In this way, information gained from each organism — such as physiological data from the rat, and genetic and knockout data from the mouse — can guide the study of human disease phenotypes (FIG. 1). Obviously, this strategy need not be limited to three species. Indeed, it could, and should, be extended to other organisms, such as *Drosophila* and yeast, to facilitate the discovery process.

Quantitative trait locus mapping. The use of whole-genome scans to identify QTL is a powerful way to investigate the genetic basis of susceptibility to complex human diseases, such as autoimmune disorders^{18,19}, cardiovascular disease^{10,20}, cancer^{21,22}, infectious disease²³ and drug addiction^{24,25}. Although QTL mapping has become easier with the availability of many genetic markers, identifying the genes that underlie the QTL that are associated with common phenotypes has proved to be a challenge²⁶. Too many genetic variants in humans, too few alleles in inbred animal models and the potential involvement of many genes in a single QTL have hindered our ability to unequivocally prove the causal role of a gene in a QTL. Nevertheless, genes that contribute to quantitative traits and complex diseases have been found — the first in tomato²⁷ and, more recently, for **type II (non-insulin-dependent) diabetes** and **Crohn disease**^{28–30}.

However, gene identification is not the only goal of QTL mapping. Knowing where a QTL maps can allow researchers to transfer it to another genetic background to reduce the complexity of a polygenic trait. This can be done, for example, by using MARKER-ASSISTED SELECTION to develop congenic strains. Animal models that are created in this way can be useful for physiological and pharmacological studies, even before the gene(s) that underlies a QTL has been identified, providing improved animal models for research. From our perspective, QTL mapping, particularly in the rat, is a

SYSTEMS BIOLOGY

The investigation of all of the elements of a particular biological system, rather than of individual genes or proteins. Systems biology aims to investigate the behaviour and relationships of all of the elements in a particular biological system.

MARKER-ASSISTED SELECTION

The use of genetic markers to predict the inheritance of alleles at a closely linked trait locus.

powerful way in which to annotate genomic sequence with information that relates to complex biological processes. Why do we believe that this approach is useful? Because once biological traits have been mapped to the rat genome, this information can be ‘transferred’ to other organisms by comparative mapping (as discussed in more detail below). Furthermore, QTL mapping in inbred rat strains can provide — within a reasonably short time — biological information for annotation. Although transgenic and gene-knockout studies have been useful for studying the function of single genes, they can be slow at providing information for annotation, given the time that it takes to generate mutant animals and because only one or a few genes can be studied at a time. Finally, QTL mapping in inbred rat strains can identify regions of the genome that carry allelic variants that collectively contribute to a phenotypic end point, as do QTL studies of complex human diseases. By contrast, single-gene or compound knockout mutants do not necessarily provide good models of such diseases, despite producing phenotypic end points that seem to be similar to a clinical phenotype in humans. This is because the mechanism that causes the mutant phenotype in an animal model might be unrelated to that which causes a complex disease in humans. This does not necessarily mean that a particular inbred rat strain is likely to be any closer to a complex human disease than is a knockout mutant. However, it can provide a more analogous model of such a disease, as its phenotype is more likely to be caused by the action of many allelic variants than by a single null allele. So, despite the many challenges of QTL mapping for the purposes of gene discovery, we believe that this mapping information can still be used to build better animal models of disease.

Finding genes underlying complex phenotypes

Technical advances have allowed many investigators to map QTL, but surprisingly little progress has been made in finding the genetic variants that contribute to quantitative phenotypes. There are many potential reasons for this (see REFS 26,31 for a detailed discussion). Nevertheless, we believe that there is some cause for optimism despite increasing evidence that single QTL can be genetically complex. Many investigators, including us, once believed that a single gene was responsible for the phenotypic effect of a QTL. However, it seems that for many traits, several genes contribute to the phenotypic effect of a single QTL^{32–34}, perhaps owing to the effect of selective pressures during the development of inbred strains or, possibly, because a phenotype is caused by several closely linked, interacting genes²⁶. In the latter case, we need to learn how these gene clusters are regulated if we are to understand complex disease, as single-gene models might not be able to replicate the phenotypes of such diseases. Unfortunately, how to identify a causal role for each gene in a QTL that consists of a cluster of genes has yet to be resolved. One possible solution to this problem is to measure additional phenotypes to dissect a QTL into subregions that are associated with specific functions. Physiological data in the rat

offer several advantages in this respect. QTL can also be further investigated by generating congenic strains, but these studies can be limited by losses or changes in a phenotype when a QTL is transferred to a different genetic background or when a transferred interval is reduced in size. This presumably occurs owing to the loss of other loci in the original genetic background that contribute to or modify the phenotype²⁶, and is a problem that could be addressed by developing congenics that carry multiple QTL to preserve epistatic and additive effects. The use of additional, more discrete phenotypes, or pharmacological agents (or other stressors) that can accelerate or exacerbate a trait, can also be used to overcome the problem of lost or altered phenotypes. Congenics can also be generated in both directions, INTRODUCING the susceptible QTL onto the resistant genome background, and the resistant QTL onto the susceptible background.

Although congenic strains and detailed physiological information are available for the rat, knockout and knock-in technology is not, and this limits the ability of researchers to validate a single gene as being causal in a quantitative trait. Although this shortcoming has hampered enthusiasm for the rat as a model organism, we believe that there are several ways to overcome this limitation. First is the use of transgenic technology. This has been successfully used in the rat to validate *Cd36* (REF. 35) — a positional candidate in a QTL interval that is associated with insulin resistance in the inbred spontaneously hypertensive rat (SHR) strain³⁶. In microarray expression studies³⁷, this gene showed differential expression between the SHR parental control strain and congenic animals, which contained the QTL region from the control BN strain that was introgressed onto the SHR background. These findings led to the discovery that SHR animals carry a null allele of *Cd36* (REF. 37). In transgenic complementation studies, the insulin-resistance phenotype of SHR rats was eliminated when a wild-type *Cd36* transgene was introduced into them³⁵.

In this example of transgenic COMPLEMENTATION TESTING, rescuing the SHR insulin-resistance phenotype with wild-type *Cd36* was equivalent to rescuing a knockout because the SHR rats carry a null *Cd36* allele. A more challenging goal is to use transgenesis to study rat quantitative traits that are caused by natural allelic variation, a more likely occurrence than null alleles. Functional complementation is a potentially powerful approach to address this issue, as exemplified by Eddy Rubin and colleagues^{38,39}. These investigators created a panel of transgenic mice that carry human YAC (yeast artificial chromosome) clones, which encompass a QTL for asthma on chromosome 5. They then looked for quantitatively modified asthma-associated phenotypes, such as total plasma immunoglobulin E (IgE) levels and broncho-constrictor responses to a methylcholine challenge, in these transgenic mice compared with non-transgenic wild-type littermates to identify genes or regulatory elements in the human YACs that could contribute to an asthma-associated phenotype^{38,39}. This functional approach allowed these investigators to screen genomic regions for functional elements that

INTROGRESSION

Transfer of genetic material from one strain to another by repeated backcrosses.

COMPLEMENTATION TEST

A breeding strategy to test whether two mutations are non-allelic when combined in an organism. If the resulting phenotype is wild type, the mutations are non-allelic and said to complement each other.

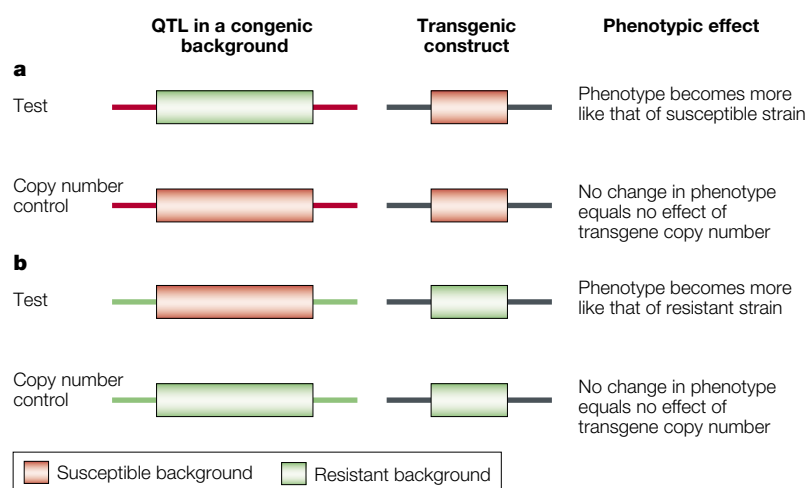


Figure 2 | Functional complementation by transgenesis. The role of a candidate gene in a quantitative trait locus (QTL) that is carried by congenic rat strains on a reciprocal genetic background can be tested by transgenesis, in a similar approach to that used by Symula *et al.*³⁹. **a** | A rat congenic strain with a resistant QTL (green) that has been introgressed onto a susceptible background (shown in red), and a transgenic construct, such as a YAC (yeast artificial chromosome) or a BAC (bacterial artificial chromosome), that carries rat genomic DNA from the susceptible background. If such a construct, when introduced into the above congenic strain, makes the phenotype of progeny more like that of the susceptible strain, then this construct might carry a gene that contributes to the QTL. The copy number control experiment can be done to rule out the likelihood that the phenotypic effects of the transgene have been brought about by introduction of an abnormal number of gene copies (that is, greater than the diploid state). **b** | In the reciprocal experiment, a resistant-strain-derived transgenic construct is introduced into a congenic strain that carries a susceptible QTL on the resistant background. The copy number control experiment is also shown.

might have a role in asthma, before the identification of a particular candidate gene or mutation. Interestingly, what they found was that their phenotype was modified by a mutation in a regulatory element that affected several closely linked genes. This approach could also be applied to the rat by introducing YAC or BAC (bacterial artificial chromosome) clones that carry a QTL into congenic animals. The same functional complementation approach could be used to show the causal role of a single candidate gene in a QTL, by using single-gene transgenes rather than large-insert YAC or BAC clones, as shown in FIG. 2.

However, these approaches have their limitations. For example, a phenotype can be altered by gene-dosage effects that are caused by additional copies of a gene rather than by the effects of a particular variant or allele used in the experiment. This problem could be addressed by using reciprocal congenic animals that contain an allele-specific transgene, as shown in FIG. 2. Because the generation of BAC libraries from each strain being studied is a labour-intensive process, the need for such strain-specific libraries can be avoided by using a BAC-engineering technique called ET cloning^{40,41}, in which PCR-generated constructs are introduced into BACs by homologous recombination in *Escherichia coli*. In this way, a transgenic construct that contains a candidate gene from either the susceptible or resistant rat strain could be made by PCR amplifying the gene from genomic DNA of either strain being studied. The gene

could then be introduced into BACs by homologous recombination using the ET cloning approach. This would remove the need to generate BAC libraries from the congenic and parental strains, and could allow the further study of specific point mutations. Although these techniques are still being refined, they have the potential to provide investigators with an effective way to study sequence variants in defined genetic backgrounds.

QUANTITATIVE COMPLEMENTATION has been proposed as a way to identify which genes in a QTL contribute to its phenotypic effects. This approach was originally developed in *Drosophila*⁴², and has also been proposed for use in the mouse³¹, but its use in the rat is prevented by the lack of knockout technology for this organism. Whether embryonic stem (ES) cells will ever be isolated for the rat is unknown; however, several groups are actively engaged in trying to clone rats^{43,44}. In the interim, investigators will probably need to limit their studies to QTL that have a recessive mode of inheritance and that, therefore, cause phenotypes that are amenable to transgenic rescue.

The rat genes that are thought to contribute to a QTL effect could also be tested in the mouse by knocking out the mouse homologue, and could be investigated in humans by conducting an association study to look for a relationship between the human homologue and an analogous human phenotype. However, both of these alternatives require that these species respond to a gene knockout or to a gene variant in a similar manner to that seen in a rat multifactorial model. One should also consider that although knocking out or altering a candidate gene in another species might not necessarily produce an identical phenotypic end point, it might still be useful. For example, a gene that is associated with hypertension in the rat might not produce a change in blood pressure when knocked out in mice, unless the knockout mice are placed on a high-salt diet. In this example, the phenotypic change is consistent, but not identical. Similar patterns should be taken into account when using data from animal models to inform studies of human disease.

Finally, the use of intermediate phenotypes has also been indicated as a solution to this problem⁴⁵. Intermediate phenotypes are those that are presumed to lie between the primary action of a gene and the end phenotype. Because they are believed to lie closer to the primary action of the gene than the end phenotype, they are deemed to be useful for gene-finding purposes. However, it is not necessarily known for many intermediate phenotypes if this is the case, and this term is frequently misused to describe phenotypes that might contribute to a QTL, in the absence of evidence that they do so. We propose that the definition of 'intermediate phenotype' be modified to include only those phenotypes for which there is genetic evidence that the phenotype maps to a QTL; when these data are not available, phenotypes that are believed to contribute to a QTL could be called likely determinant phenotypes. If a likely determinant phenotype maps to a QTL, it does not immediately qualify as an intermediate phenotype, as it might still be too distal from the primary deficit caused by a

QUANTITATIVE COMPLEMENTATION

A breeding strategy to identify genes that contribute to a quantitative trait locus (QTL). A QTL allele is crossed to animals that carry null mutations at each candidate gene in the QTL. If the phenotypic effect of the QTL allele on the progeny is not the same in mutant and wild-type backgrounds, allelism (quantitative non-complementation) between the mutant gene and the gene in the QTL is indicated.

functional genetic variant to be useful in positional cloning studies. In fact, it could be argued that it requires the cloning of a gene that contributes to a QTL to know whether a phenotype is a true intermediate phenotype. We recommend that such likely determinant phenotypes should be used more widely as some of them might lead to the identification of intermediate phenotypes that can then be used for studies in humans and other mammals.

Mutagenesis screens

Although QTL mapping is a powerful way to dissect the genetic components of complex disease, it is not the only way in which physiological data from the rat can be attached to the genome. Recently, with the advent of *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis in the mouse⁴⁶, there has been an increase in the screening of large numbers of phenotypes. Although this is a high-throughput approach to evaluate single-gene function in model organisms, the many animals that must be screened require that global assessments of their phenotypes are made, and such general phenotypic screens do not always reflect the direct function of a gene. For example, an investigator who is studying the genetic basis of a complex trait, such as blood glucose level or behaviour, is evaluating a phenotype that is typically a clinical end point. This phenotype could be quite distant from the effects of the gene that is responsible for, for example, sensing altered glucose levels or alterations in neuronal transmission.

The combination of QTL mapping and ENU mutagenesis offers considerable potential for accelerating the discovery of genes and gene function. So far, ENU has been predominantly used in the rat to cause neoplasia⁴⁷. However, it is likely that it will soon be used as a mutagen in phenotypic screens, as has been done in the mouse. A particularly useful approach could be to use congenic animals that carry a QTL as sensitized strains in ENU mutagenesis screens, to look for ENU-induced mutations that can modify the effect of the QTL. In this approach, ENU-induced modification of a quantitative trait might lead to the identification of a gene that contributes to a QTL effect or that acts in the pathway that regulates the trait. ENU-induced mouse mutants that have similar phenotypes to quantitative traits being investigated in the rat can also inform rat studies as investigators can use comparative mapping data to see if the ENU mutation maps to a region that is in conserved synteny with the rat QTL being studied (see links to the different mouse ENU mutagenesis programmes and to the [virtual comparative maps at the Rat Genome Database](#)). In cases in which such mouse mutants exist and the identity of the mutated gene is known, the ORTHOLOGOUS RAT GENE can be further studied to see if it contributes to the effect of a QTL. Conversely, for investigators using the mouse, determining if mouse mutants map to a region of conserved synteny in the rat genome that contains a QTL might provide additional phenotypes that could be used to facilitate gene identification. In this way, both species can be used as a source of additional alleles, which can be used in the validation of candidate genes.

Mechanistic mapping

There are two other approaches to generating physiological information that can be mapped onto the genome: breaking down a complex response with mathematical modelling, and the use of physiological challenges to elicit specific responses. Most molecular genetic studies of complex disease have focused on genes that are involved directly in generating the phenotype, rather than on physiological control or buffering systems, which can also affect a trait. Understanding the role of regulatory reflexes in systems biology often requires the use of mathematical modelling to capture and characterize these complex events, as has recently been done to characterize the BARORECEPTOR REFLEX in rats (C. M. Kendzierski *et al.*, unpublished observations). Evaluating this, and other similar reflexes, such as the chemoreceptor pathway for controlling respiration, requires the recording of several physiological responses at many time points. This is because these reflexes are not easily captured as a single end point or trait because they often involve a series of steps — such as detecting a change in homeostasis, neuronally transmitting the recorded change, processing the message, transmitting a counter-response to effectors and response of the effectors to restore homeostasis. The use of mathematical models allows such complex responses to be reduced to several parameters that correspond to quantitative measurements that can be mapped as discrete traits, thereby allowing the reflex to be attached to the genome. Although such mathematical parameters do not directly implicate the involvement of specific genes in a response, having such mapping data to hand will inform investigators that a gene(s) in the region might have a role in a particular reflex.

Another approach to producing physiological information for mapping purposes is the use of pharmacological agents that stimulate or inhibit various biological pathways to assess the genetic basis of the pathway that is affected by an agent, and the reflexes that compensate for its effects. This approach could be used in genetic crosses between disease and control strains, and in various inbred strains of rats, including congenics. Using this approach, Vincent *et al.*⁴⁸ have found an interaction between L-type calcium channel antagonists and a blood pressure locus on rat chromosome 2. Interestingly, this locus was not detected in a search for QTL that are linked to blood pressure. The drug created a stimulus that unmasked a genetic difference in a blood-pressure response to this agent in the two rat strains studied. This approach allowed these investigators to dissect overall changes in blood pressure into a more specific phenotype: a change in blood pressure in response to an L-type calcium channel antagonist. The combined use of pharmacological challenges with QTL mapping allows various components of a complex phenotype to be mapped to the genome and can facilitate the discovery of the pathways that are involved in disease pathogenesis.

Collectively, these strategies in the rat offer a means by which to start annotating genomic sequence with physiological functions. Some believe that attaching

ORTHOLOGOUS GENE

Homologous gene in different species, the lineage of which derives from a common ancestral gene without gene duplication or horizontal transmission.

BARORECEPTOR REFLEX

A reflex with a negative-feedback loop system; as blood pressure increases or decreases, the baroreceptor detects the event and initiates a cascade of physiological mechanisms that results in a return to baseline blood pressure.

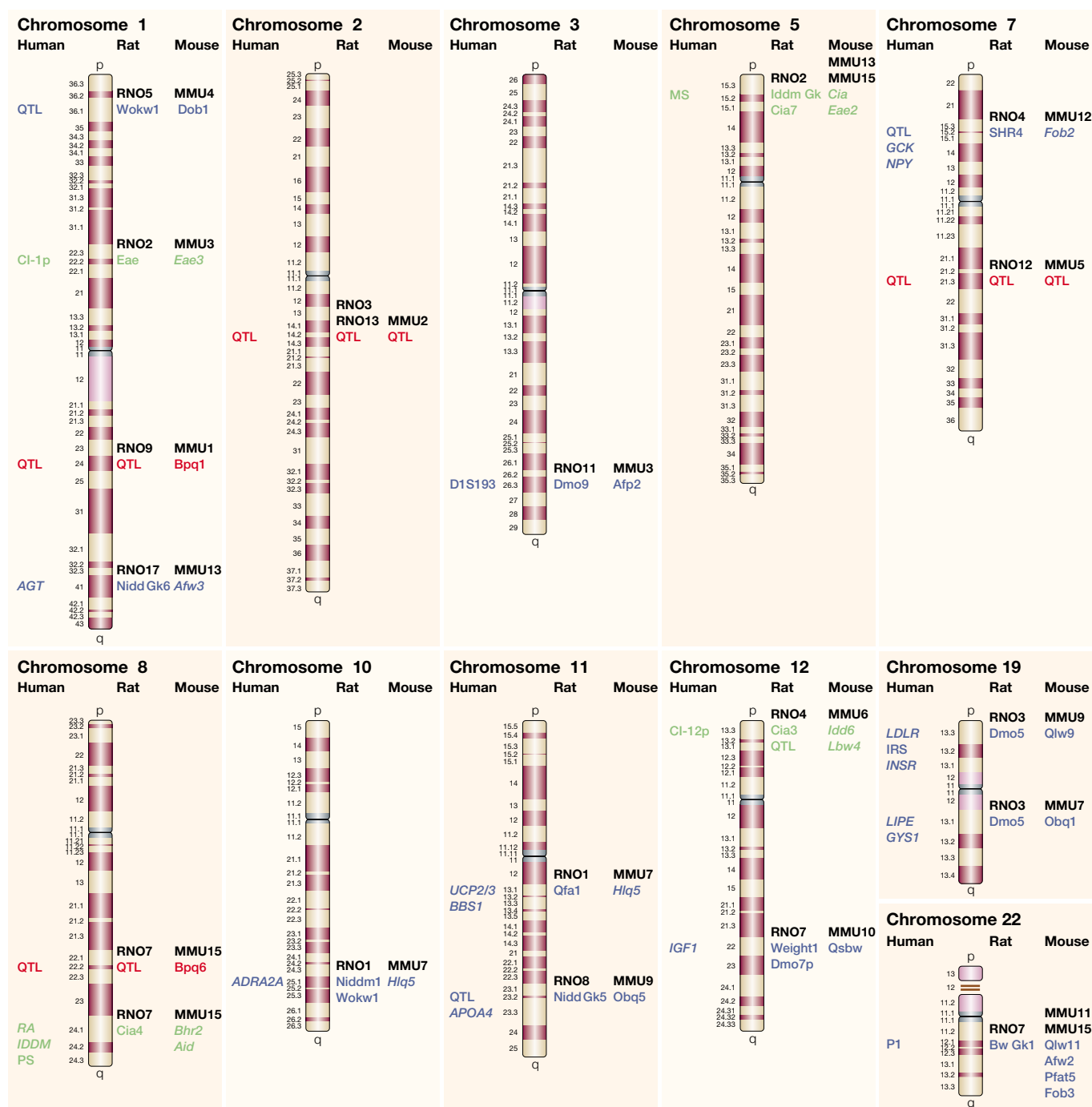


Figure 3 | QTL associated with complex disease phenotypes that map to regions of conserved synteny in the human, rat and mouse genomes. Human ideograms annotated with loci associated with obesity (shown in blue), hypertension (shown in red) and autoimmune/inflammatory phenotypes (shown in green) in humans, rats and mice. Quantitative trait loci (QTL) and comparative mapping information were obtained from the literature; map information relating to metabolic syndromes (blue) was obtained from *The Human Obesity Gene Map: The 2000 Update*¹⁷; that for hypertension phenotypes from REFS 51,53; and for autoimmune/inflammatory disorders from REFS 67,68. Each QTL shown has been mapped in one species and linked to a region of conserved synteny that contains a related locus in another species. Human loci (left of each ideogram) are shown by their locus name when available and by QTL when not. Homologous rat QTL are shown to the right with their rat chromosomal (RNO) location and by locus name or QTL. Mouse homologous QTL (far right) are shown with their likely chromosomal (MMU) location and locus name. Only QTL that have been mapped in all three species are shown. As genetic intervals associated with QTL are often not reported, chromosomal locations, rather than specific intervals, are shown. *ADRA2A*, adrenergic, α -2A-receptor; *Afw3*, abdominal fat weight QTL 3; *Afp2*, abdominal fat per cent 2; *AGT*, angiotensinogen; *Aid*, activation-induced cytidine deaminase; *APOA4*, apolipoprotein A4; *BBS1*, Bardet–Biedl syndrome 1; *Bhr2*, bronchial hyper-responsiveness 2; *Bpq*, blood pressure QTL; *Cia*, collagen-induced arthritis; *Dmo*, diabetes mellitus OLETF; *Dob1*, dietary obesity 1; *Eae3*, susceptibility to experimental allergic encephalomyelitis 3; *Fob2*, F-line obesity QTL 2; *GCK*, glucokinase; *GYS1*, glycogen synthase 1; *Hlq5*, heat loss QTL 5; *Idd6*, insulin-dependent diabetes susceptibility 6; *IDDM*, insulin-dependent diabetes mellitus; *IGF1*, insulin-like growth factor 1; *INSR*, insulin receptor; *IRS*, insulin receptor substrate; *Lbw4*, lupus NZB \times NZW 4; *LDLR*, low-density lipoprotein receptor; *LIPE*, lipase; *Nidd Gk*, non-insulin-dependent diabetes glycerol kinase; *Niddm1*, non-insulin-dependent diabetes mellitus 1; *NPY*, neuropeptide Y; *MS*, multiple sclerosis; *Obq5*, obesity QTL 5; *P1*, P blood group; *Pfat5*, adiposity QTL; *PS*, psoriasis; *Qfa1*, weight QTL; *Qlw*, QTL late weight gain; *Qsbw*, Quackenbush–Swiss body weight QTL; *RA*, rheumatoid arthritis; *SHR4*, SHR (spontaneously hypertensive rat) body weight QTL; *UCP2*, uncoupling protein; *Wokw*, body weight QTL.

Box 2 | **The PhysGen Project**

The Medical College of Wisconsin Programs for Genomic Applications (see link to [The PhysGen Project](#)) is generating publicly available consomic rat strains and is characterizing each strain for more than 200 heart, lung and blood phenotypes. Consonic strains offer increased mapping power because all the animals in a consomic panel are genetically identical, except for the substituted chromosome, in contrast to F_2 populations (in which each animal is unique) or recombinant inbred strains (in which the animals are identical, but the genetic background is varied). By comparing phenotypes between the consomics and the parental strains, the PhysGen project aims to map these traits to a particular chromosome. In addition, consomics can be rapidly converted to congenics and, collectively, can carry a tremendous degree of genetic variation; the two consomic panels being developed by PhysGen capture, on average, nearly 50% of the genetic variation in the rat.

systems-level biology to the genome might be premature, but we believe that such additional data can offer insight into the location of genes that underlie complex disease and can facilitate physiological studies. If such genes are to be found, then many more phenotypes, such as reflexes, will need to be mapped to the genome to dissect disease mechanisms further, especially if functional gene clusters in QTL are a hallmark of complex traits.

The power of comparative genomics

Despite the growing physiological data set, there is still a need to create better animal models for the pharmaceutical industry and for the annotators of the human genome alike. Nevertheless, we are entering an era in which comparative genomic data can be used to develop animal models that better recapitulate human disease at both the phenotypic and the genomic levels. For example, several years ago, a rat QTL that is linked to high blood pressure was mapped near to the rat angiotensin-converting enzyme (*Ace*) gene^{5,8}, and it was suggested then that another gene(s) near *Ace* might be the causative gene. After these publications, *ACE* was reported to be not linked to hypertension in humans⁴⁹; however, genes in the vicinity of *ACE* could not be ruled out. Several years later, two publications reported hypertension QTL in the region of *ACE* in humans^{50,51}, indicating that the QTL identified in the rat might be indicative of where QTL for human hypertension might be found. Wilson *et al.* have recently shown that the *PRKWINK4* (protein kinase, lysine deficient 4) gene, which is located in the QTL that contains *ACE*, is responsible for the human hypertension disorder **pseudohypoaldosteronism type II** (REF. 52). The rat orthologue of *PRKWINK4* should now be investigated to see if it has a role in hypertension in the rat.

To investigate QTL that are associated with hypertension in rats and humans, we have studied seven different rat crosses, which involve five hypertensive inbred rat strains, and have identified 57 QTL for 33 blood-pressure traits⁵³. Other investigators have reported the same loci in other rat crosses^{10,20}, which indicates that these loci might be important for hypertension in the rat. We then determined which of these rat hypertension loci are conserved between rats, humans and mice, and found that of the six QTL for hypertension that have been reported in

humans, five are in regions that are in conserved synteny with mapped hypertension QTL regions of the rat genome⁵³. Different QTL contribute to blood-pressure variation depending on how the phenotype is measured (by tail cuff or by catheter); age at which phenotyping occurs also influences the number of genetic loci that contribute to hypertension⁵⁴. Because the different hypertensive rat strains show different aetiologies of hypertension, we believe that by integrating the results from all the crosses, we can recapitulate the heterogeneous clinical picture of hypertension in humans and identify homologous human regions for further investigation. Sugiyama *et al.* have carried out a similar study in the mouse and have also found significant concordance between mice, humans and rats for blood-pressure QTL⁵⁵.

Examples of QTL that are conserved between species are not restricted to QTL for hypertension. In both animal models and humans, loci for obesity, autoimmune-related disorders and hypertension phenotypes have been identified and published, together with comparative map information^{53,54,56–58}. FIG. 3 shows QTL for specific disorders that map to equivalent regions in the rat, human and mouse genomes. It is striking that 11 obesity loci, 4 autoimmune loci and 4 hypertension loci lie in potentially evolutionarily conserved regions of the rat, human and mouse genomes (see REFS 53,54,56–58 for further details). Furthermore, the number of QTL that are in evolutionarily conserved segments, between at least two of the three species, is large: for 52 out of the 59 human chromosomal regions that are reported to contain genes that are involved in obesity-related phenotypes, a QTL for a similar phenotype has been mapped to a conserved region in another species⁵⁶. To facilitate the identification of QTL that might be conserved across evolution, we have generated comparative maps between rats, humans and mice using an automated tool⁵⁹. These maps are publicly available, are maintained at the Rat Genome Database (BOX 1) and have links to detailed comparative map information and to other public databases. This useful tool will help to identify regions in the human or mouse genome, for example, that might contain orthologous genes involved in similar disease pathways as those identified by QTL mapped in the rat.

Therefore, the genomic sequencing of the rat, in parallel to that of the human and mouse, offers new opportunities for comparative and functional genomics. At present, it is difficult to know which diseases should be studied in the rat or any other animal model because the cause of most diseases and the biological distinctions between each species are poorly defined. Nonetheless, it is more likely that an animal model will better reflect a human disorder if it recapitulates the disorder phenotypically and genetically, rather than simply phenotypically. There are, however, some criticisms of the comparative genomics approach, including: how likely is it that the same gene will cause the same disease after many years of divergent evolution? And what is the likelihood that an intermediate phenotype in one species will occur in the other species? We know that there are many physiological responses that are common to rats and

LINKAGE DISEQUILIBRIUM

The condition in which the frequency of a particular haplotype for two loci is significantly greater or less than that expected from the product of the observed allelic frequencies at each locus.

humans. Consequently, it is possible that a trait in one species will correspond to a disease, or an altered intermediate phenotype, in the other. What is needed is enough biological knowledge to determine when the rat, or any other animal model, is not sufficient.

Linkage disequilibrium mapping

LINKAGE DISEQUILIBRIUM (LD) mapping might offer another means by which to annotate genomic sequence with function and has been used by several investigators^{60–62}. A recent paper by Grupe *et al.* describes how genome-wide single nucleotide polymorphism (SNP) genotyping data from 15 inbred strains of mice were used to predict QTL locations⁶³ by comparing genotype and phenotype data *in silico*, in a manner that is comparable with LD mapping. This paper illustrates the potential power of conducting extensive phenotypic characterization of existing inbred mouse strains. However, there are many controversial issues related to this strategy that need to be addressed before it can be widely used, which include the SNP marker density and the number of disease alleles that are present in the various inbred strains. However, if this approach does work, it will be a tremendous tool that could also be applied to rats.

Several years ago, we characterized 48 commonly studied inbred rat strains by determining allele sizes for 4,338 of the 5,214 simple sequence length polymorphisms then available, and made these data publicly available⁶⁴. The 48 characterized rat strains are models for alcoholism, abnormal behavioural phenotypes, cancer, and cardiovascular and neurological disorders. The average polymorphism rate was found to be 46% (allele difference = 2 bp), and the average number of alleles was six (range 2–13 alleles)⁶⁴. This study revealed that many long (extending for more than ten consecutive markers), conserved haplotypes are shared between closely related strains. We also observed that the greater the rate of polymorphism between two strains, the fewer the common haplotypes⁶⁴. These data indicate that LD mapping could be used to study these different inbred rat strains, as has been proposed in the mouse. The advantage of having characterized so many rat strains is that each strain is expected to carry differently sized blocks of LD, which should help to narrow down QTL regions. The extent of LD will need to be determined in the rat genome, as has been done for the human genome⁶⁵.

An alternative to using LD to map QTL in inbred rat strains is to characterize recombinant inbred^{66,67}, recombinant congenic^{68,69} or consomic strains instead⁷⁰. The consomic strategy has proved to be powerful for mapping QTL in mice⁷⁰, and work so far indicates that this will also be the case in rats (BOX 2).

Future prospects

In recent years, the field of comparative mapping has made tremendous advances⁷¹. Once genomic sequence is available for various vertebrates, including humans, mice, rats and zebrafish, it will be possible to convert comparative genomics to sequence-based biology — the ability to assign variation in function to particular alleles. Such comparative genomics studies are likely to provide data for annotating the human genome sequence, for building better animal models, for assisting in the development of new therapeutic agents and for understanding gene regulation. As the genomic sequence is annotated with more and more function, by QTL mapping, ENU mutagenesis, transgenic and future approaches, it will become increasingly easy to formulate testable hypotheses.

Finally, given the many inbred rat strains that have been developed to model common human disease, and with the increasing power of sequence- and genome-based biology, the rat is well positioned to continue its tradition as an important animal model for studying human disease, particularly with respect to drug development. In short, the rat race is getting faster, but the tools for discovery are keeping pace, offering a means to improve our understanding of human disease and, hopefully, of developing improved therapies.

Update — added in proof

Stoll *et al.*⁷² have recently published an article in which 239 likely determinant cardiovascular and renal phenotypes were mapped in a genome-wide scan. The study found several regions of the rat genome to which more than five cardiovascular-associated QTL mapped, which indicates that several genes in the region contribute to cardiovascular function. A new analytical technique, called physiological profiling, allowed the investigators to assess changes in the system biology of the cardiovascular system in response to allelic substitutions. The complete QTL maps and physiological profiles are available online (see online links box).

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 Online links

DATABASES

The following terms in this article are linked online to:

LocusLink: <http://www.ncbi.nlm.nih.gov/LocusLink/>
Ace | *ACE* | *ADRA2A* | *Atw3* | *AGT* | *Aid* | *APOA4* | *BBS1* | *Bhr2* | *Cd36* | *Eae3* | *Fob2* | *GYS1* | *Hlg5* | *Idd6* | *IDDM2* | *IGF1* | *INSR* | *Lbw4* | *LDLR* | *LIPE* | *NPY* | *PRKWINK4* | *RA* | *UCP2*
OMIM: <http://www.ncbi.nlm.nih.gov/Omim/>
 Crohn disease | pseudohypoadosteronism type II | type II (non-insulin-dependent) diabetes

FURTHER INFORMATION

Baylor College of Medicine ENU mutagenesis screen:
<http://www.mouse-genome.bcm.tmc.edu/ENU/ENUhome.asp>

GSF ENU Mouse Mutagenesis Project:
<http://www.gsf.de/ieg/groups/enu-mouse.html>

Harwell ENU mutagenesis programme:
<http://www.mgu.har.mrc.ac.uk/mutabase/>

McLaughlin Research Institute ENU mutagenesis screen:
<http://www.montana.edu/wwwmi/enump.html>

Medical College of Wisconsin: <http://www.mcw.edu/>

PubMed: <http://www.ncbi.nlm.nih.gov/PubMed/>

QTL maps and physiological profiles: <http://bcr.mcw.edu/phyprf>

Rattus norvegicus:
[http://animaldiversity.ummz.umich.edu/accounts/rattus/r_norvegicus/\\$narrative.html](http://animaldiversity.ummz.umich.edu/accounts/rattus/r_norvegicus/$narrative.html)

Strain query form at Rat Genome Database:
<http://rgd.mcw.edu/strains/>

The Jackson Laboratory: <http://www.jax.org/>

The PhysGen Project: <http://pga.mcw.edu/>

US National Heart, Lung, and Blood Institute – Programs for Genome Applications:
<http://www.nhlbi.nih.gov/resources/pga/>

Virtual comparative maps at the Rat Genome Database:
<http://rgd.mcw.edu/VCMAP/>

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