

Chapter 2

Genetic Mapping and Positional Cloning

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Abstract

Genetic mapping and positional cloning of genetically complex traits in the laboratory rat (*Rattus norvegicus*) has recently led to the identification of various susceptibility genes in different rat models. Rat genetics has benefited from revolutionary advances in molecular biology, genetics, genomics and informatics and provide an unparalleled resource for molecular genetic investigation of mammalian physiopathology and its underlying complex genetic architecture. In this review, we will consider different strategies that are being used in the successful positional cloning of rat complex trait genes in the context of recent progress in rodent and human genetics.

Key words: Rat, Genetics, Positional cloning, QTL

1. Why Map Genes in the Rat?

The genetic characterisation of model organisms has progressed from the study of genetic modes of inheritance to establish the causal relationship between genes and both physiological and pathophysiological traits. Among these, monogenic traits are inherited in a Mendelian fashion in which single genotypic changes result in robust and discrete phenotypic differences. However, the expression of many Mendelian traits is also influenced by the actions of modifier genes and the segregation of phenotypic traits in a Mendelian fashion is the exception, rather than the rule. Most phenotypic differences between individuals in morphology, physiology, growth, behaviour and disease susceptibility are quantitative in nature, exhibiting a continuous and nearly normal phenotypic distribution (1). These genetically complex traits arise from the interaction of multiple segregating genetic variants together with the environmental factors. In this chapter, we shall focus mainly on genetic mapping and positional

cloning of genetically complex traits in the laboratory rat (*Rattus norvegicus*).

Rat models have been used in the elucidation of human physiology, pharmacology, toxicology, nutrition, behaviour, immunology and neoplasia for over 150 years (2). Because of its size, ease of manipulation and breeding characteristics, physiological research on the rat has generated a wealth of experience and methodological sophistication for the accurate determination of quantitative phenotype measurements. Although mapping genes in the rat has also been successfully applied in the discovery of Mendelian traits, most of the positionally cloned genes in rats underlie genetically complex phenotypes.

Genetic mapping of complex traits offers a powerful and complete approach when compared to candidate gene-based cell biology in the elucidation of disease susceptibility mechanisms. Complex traits are controlled by loci that have quantitative effects on the phenotype and the observed ubiquitous quantitative variation in biology is often characterised by a strong genetic component. Lander and Botstein first described the use of interval mapping based on DNA markers in order to genetically localise quantitative trait loci (QTL) in natural or experimental populations (3). Guidelines were then established for interpreting the genome-wide linkage results that were reported in numerous studies, including those undertaken in segregating rodent populations (4). Since then, the road from QTL discovery to causative gene identification has been described as “long and bumpy” (5). The challenging attempts to characterise the molecular basis of QTLs in rodents led to the consideration of alternative approaches such as forward genetic approaches using mutagenesis in mice (6, 7). Today, it is generally accepted that the mutagenesis approach is complementary to the QTL approach and will significantly increase the identification of complex trait genes in rat models (8, 9). More generally, rat genetics has benefited from revolutionary advances in molecular biology, genetics and genomics; some of the most significant ones being the sequencing of the complete rat genome with >90% coverage (10), dense single nucleotide polymorphism (SNP) and haplotype mapping allowing surveys of genetic variation (11) and characterisation of distribution and functional impact of DNA copy number variation in rats (12). Since 2001, 16 additional genes have been positionally cloned in various rat models (8).

Human disease gene discovery has entered a new era where two new technological approaches are currently used in the genetic dissection of common diseases: genome-wide association studies (GWAS) and deep resequencing. Recent studies on common diseases using these state-of-the-art techniques confirmed the complexity of their underlying genetic architecture (13, 14). Indeed, low-effect genomic variants identified by GWAS studies

and designated as “common variants” constitute the genetic hallmark of many human common diseases. In September 2008, studies presented on “The Genomics of Common Diseases” meeting held at the Broad Institute of MIT and Harvard in Boston revealed that the rare variants with high penetrance identified using deep re-sequencing add to the complex architecture of human disease. Given the increased heritability, flexibility and statistical power of experimental rodent crosses over the corresponding studies in humans, rodent models provide an unparalleled resource for molecular genetic investigation of mammalian physiopathology and its underlying complex genetic architecture. In addition, the genome resources currently available for rat models form a solid basis for comparative genomics in order to study human physiology and disease (15).

In this review, we will consider different strategies used in the successful positional cloning of rat complex trait genes in the context of recent progress in genetics and genomics, and new statistical approaches complementing a range of high throughput techniques. We will also highlight the importance of rat positional cloning in terms of translational studies in humans.

2. Successful Positional Cloning in the Rat

A total of 22 positionally cloned genes over a 10 year period (Table 2.1) demonstrate the difficulty of positional cloning of genes underlying rat QTLs when one considers the nearly 1,000 QTLs identified in rat models. Table 2.1 shows that until 2004, positional cloning was mostly achieved by genetic mapping techniques (linkage and/or physical mapping) with functional *in vivo* complementation for some of these studies. After the publication of the genome sequence of the Brown Norway rat in 2004 (10), rat positional cloning was complemented with additional approaches such as computational analysis of the rat genome (16), association and functional studies in humans (17), expression QTL (eQTL), quantitative trait transcript (QTT) analyses (18–20) and RNA interference (RNAi) as an alternative approach for *in vivo* complementation (21). Recent studies using these relatively novel strategies have showed that the human orthologues of the rat positionally cloned genes are also susceptibility factors for human left ventricular mass (LVM) (19) and heart failure (18).

Standards of proof of gene discovery in complex traits have been previously described (22, 23) and all of the successfully positionally cloned genes in rat models (described in Table 2.1) fulfil the working criteria described in Glazier et al. (23). The burden of proof for complex trait mapping includes linkage, association

Table 2.1

List of positionally cloned rat genes between 1999 and 2008 and their positional cloning strategy. All underlined genes were studied in humans in parallel with rat models. Until 2002 positional cloning was achieved mainly by genetic mapping, and positional cloning of *Pkhd1* is the only example of translation of findings in rat models into human disease

Gene name	Positional cloning strategy	Year	Reference
<i>Cd36</i>	Linkage analysis, expression profiling, transgenic rescue	1999	(24, 74)
<i>Aspa</i>	Genome shift approach, biochemical analysis	2000	(75)
<i>Mertk</i>	Genetic mapping, adenovirus mediated gene transfer	2000, 2001	(76, 77)
<i>Atrn</i>	Genetic mapping, transgenic rescue	2001	(78)
<i>Cyp11b1</i>	High-resolution substitution mapping	2001, 2003	(25, 26)
<i>Cb1b</i>	Genetic and physical mapping, transgenic rescue	2002	(38)
<i>Gimap5</i>	High resolution physical mapping	2002	(79, 80)
<u><i>Pkhd1</i></u>	Linkage analysis, comparative genomics between rat and human	2002	(56)
<i>Ncf1</i>	Physical mapping, in vitro and in vivo functional complementation	2003, 2004	(28, 39)
<i>Rab38</i>	Linkage analysis, high resolution substitution mapping	2004, 2005	(30, 81)
<u><i>Ciita</i></u>	Linkage analysis, expression profiling, haplotype analysis, association and functional studies in humans	2005	(17)
<i>Gstm1</i>	Linkage analysis, expression profiling	2005	(27)
<i>Anks6</i>	Physical and genetic mapping, computational analyses of the rat genome	2005	(16)
<u><i>Fcgr3</i></u>	Linkage analysis, haplotype mapping, association studies in humans using genomic structural variation	2006	(33)
<u><i>Tmem67</i></u>	Linkage analysis in rats and humans, comparative genomics with humans	2006	(57)
<u><i>Fbx10</i></u> , <u><i>Frmpl1</i></u>	Linkage analysis, association studies in humans	2007	(31)
<u><i>Ephx2</i></u>	Linkage analysis, eQTL analysis, in vivo complementation using KO mice	2008	(18)
<u><i>Ogn</i></u>	Linkage and eQTL analysis, QTT analysis in rats and humans, in vivo complementation using KO mice	2008	(19)
<u><i>Jund</i></u>	Linkage analysis, expression profiling, in vitro RNA interference	2008	(21)
<i>Cd36^a</i>	Linkage analysis, eQTL, QTT analyses, transgenic rescue	2008	(20)
<i>Igl</i>	High resolution physical mapping	2008	(36)
<i>Srebfl</i>	Linkage analysis, transgenic rescue	2008	(29)

^a*Cd36* was initially positionally cloned as an insulin resistance gene in 1999 and identified as a blood pressure susceptibility gene using a different study design in 2008

and fine mapping followed by the functional tests of candidate genes in relevant animal models. We will first describe the gene identification procedures used in rodents that are applied after initial QTL mapping, emphasising the use of congenic, subcongenic, advanced intercross lines (AIL) and haplotype mapping in successful positional cloning. We will then focus on recent advances in fine mapping and functional tests of candidate genes underlying QTLs by highlighting the inflection points in rat genetics such as the sequencing of the rat genome, underlining the importance of recent approaches in rat positional cloning.

**2.1. Fine Mapping:
The Success
of Congenic,
Subcongenic,
Advanced Intercross
Lines and Haplotype
Mapping**

Once an initial linkage of a given trait to a discrete chromosomal location has been established, fine mapping is necessary to reduce the linkage interval and to test the plausible biological candidates. Congenic strains have been widely used in the fine mapping of rodent QTLs in various studies using rat models and they still constitute a powerful tool in QTL positional cloning (20, 21, 24–31). By repeatedly backcrossing one strain onto another, it is possible to produce rats that have a particular genomic region from one strain and the remainder of their genome from the other (*see* also chapter 17). The effect of the introgressed genetic region derived from one strain (generally corresponding to a QTL) can then be specifically tested on the genetic background of the other strain. Congenic strains are used primarily to:

- Confirm the established QTL.
- Fine map candidate gene(s) responsible for the phenotypic variance within the QTL.
- Characterise the biology of the QTL gene(s).

Human essential hypertension is one of the most extensively studied complex traits in rat models (32). According to the rat genome database, (<http://rgd.mcg.edu/>), 325 distinct blood pressure (BP) QTLs have been identified using various rat crosses. Congenic strains for blood pressure can be constructed in two ways with a given pair of parental strains. The low-BP strain can be the donor and the high-BP strain the recipient, or vice versa. The main difference between the two is the genetic background. In Dahl rats, for example, the genetic background of the Dahl salt-resistant rats is not very permissive for expressing BP differences and so most congenic strains using Dahl rats have been made on a salt-sensitive genetic background. On the other hand, successful congenic lines have been made in both directions with spontaneously hypertensive rat (SHR) and Wistar Kyoto (WKY) (32).

When complex traits map to a single locus, the use of congenic strains becomes crucial in the molecular identification of QTLs. The identification of *Cd36* as an insulin-resistance gene causing defective fatty acid and glucose metabolism is a striking

example showing the complete phenotypic effect of a QTL using congenic strains. For the defect in isoproterenol-mediated lipolysis in the SHR rat, Aitman and colleagues showed a complete rescue in the congenic strain carrying the region of the chromosome 4 QTL (linked to the defective catecholamine action) derived from the Brown Norway rat on an SHR genetic background (24). Similarly, Olofsson et al. showed a robust reduction in arthritis susceptibility using a congenic strain carrying the pristine-induced arthritis QTL 4 (*Pia4*) from the resistant E3 strain on a DA genetic background. The *Pia4* region has been found to be the only QTL associated with arthritis severity and joint erosions during the entire disease course (28). In addition, *Pia4* has been identified in models of multiple sclerosis and uveitis suggesting that it has a role in regulation of several inflammatory diseases. In contrast, when several loci are identified for a given phenotype, congenic strains do not show complete phenotypic correction. In a genome-wide linkage analysis for crescentic glomerulonephritis (*Crgn*), Aitman et al. identified seven QTLs (*Crgn1*–7) (33), and a follow-up study reported only modest phenotypic correction for *Crgn2* (Lod>8) derived from the *Crgn* resistant Lewis rat introgressed into the genetic background of the susceptible WKY rat (21). Altogether, these studies suggest that although phenotypic effects of QTLs vary according to rat disease model, congenic strains are powerful tools to confirm the QTL effects.

Congenic strains can also be used for the identification of interacting loci within a QTL and the elegant work by Samuelson et al. shows that *Mcs5a* locus is a compound QTL with at least two non-coding interacting elements (*Mcs5a1* and *Mcs5a2*) in a rat mammary carcinogenesis model (31).

Another application of congenic strains is the use of comparative microarray based expression analyses. Congenic strains differ from their parental progenitors by the introgressed genetic interval containing the QTL. Thus, comparative microarray analysis between congenic and parental strains may be informative on potentially and differentially expressed genes encoded by the introgressed interval. This approach was adopted by McBride and colleagues and led to the identification of *Gstm1*, which plays a pathophysiological role in hypertension and oxidative stress in the stroke-prone of spontaneously hypertensive rat (SHRSP), a well-characterized experimental model for essential hypertension and endothelial dysfunction (27, 34).

A subcongenic (or interval-specific congenic) strain is obtained by backcrossing the congenic strain to the parental strain until recombination events reduce the initial congenic interval. The limits of a QTL can be narrowed by constructing subcongenic lines retaining the QTL within progressively smaller amounts of donor chromosome. Subcongenic strains are generally used to fine map large

QTLs and have been successfully used to reduce a QTL to a single gene resolution in high-resolution mapping studies (25, 26).

AIL are obtained when two parental strains are crossed to produce an F1 which is then intercrossed to produce an F2. Subsequent generations are produced by intercrossing the F2 individuals according to a pseudo-random breeding protocol. This results in high recombination rates and genetic variation in the population (35). Undoubtedly, AIL offer high resolution mapping when compared to F2 crosses and Swanberg and colleagues demonstrated the advantageous use of AIL derived from DA and PVG rats in the positional cloning of *Mhc2ta*, in the genetic mapping of strain differences in expression of MHC class II molecules after nerve injury (*VRA* ventral root avulsion) (17). In this study, the high recombination frequency in a relatively small genetic interval in the AIL was used to narrow down the *Vra4* QTL by correlating the expression of *Cd74*, a marker of MHC class II molecules, and genotypes in the recombination interval. This approach reduced the number of candidate genes to 13 in *Vra4* (17). Similarly, the positional cloning of the *Igf* genes controlling rheumatoid factor production and allergic bronchitis in rats was achieved by highly recombinant (19–21 generations of intercrossing) AIL derived from F344 and GK rats (36).

Haplotype-based mapping (or haplotype mapping) uses sets of closely linked genetic variants (SNPs or microsatellite markers) between inbred rat strains to identify regions that co-segregate with a given phenotype. This approach requires having phenotypic and genotypic information for a panel of inbred rat strains and assumes that the genetic variants shared by a set of inbred rat strains are identical by descent. Haplotype mapping was used in the positional cloning of *Fcgr3* in an F2 population derived from glomerulonephritis-susceptible WKY and -resistant Lewis rats (33). This study showed an enhanced macrophage activation in the WKY rats when compared to Lewis rats. Macrophage activity was then delineated to a minimal genetic interval that contained only *Fcgr3* within the QTL where genetic variants at the *Fcgr3* locus co-segregated with macrophage activity in a panel of inbred rat strains showing either high or low macrophage activation (33). While *Fcgr3* was positionally cloned on the rat chromosome 13 QTL (*Crgn1*), a promoter polymorphism found in the AP-1 transcription factor *Jund* co-segregated with macrophage infiltration on the chromosome 16 QTL (*Crgn2*) in this rat model of human *Crgn* (21). The promoter polymorphism and other microsatellite markers were then used in a haplotype map and defined a region of 130 kb containing *Jund* where genetic variants co-segregated with macrophage infiltration.

The fine-mapping of QTLs can also be achieved by using an outbred population of rats. By generating a genetically

heterogeneous stock (HS) of rats, Johannesson and colleagues mapped a locus contributing to variation in a fear-related measure (two-way active avoidance in the shuttle box) to a region on chromosome 5 containing nine genes. By establishing a protocol measuring multiple phenotypes including immunology, neuroinflammation and haematology as well as cardiovascular, metabolic and behavioural traits, they established the rat HS as a new, powerful resource for the fine-mapping of QTLs contributing to variation in complex traits of biomedical relevance (37).

In summary, congenic, subcongenic, AIL and haplotype mapping have been successfully used in fine mapping of complex traits in various rat models of human disease. Given the importance of congenic strains in the verification of QTL-phenotype effects, we believe that in the future, positional cloning in the rat will still require a congenic strain strategy in combination with approaches allowing high recombination frequencies (subcongenic and AIL) and haplotype mapping in the genetic dissection of QTLs.

2.2. Recent Advances in Fine Mapping and Functional Tests of Candidate Genes

Generation of a high-quality draft of the sequence of the Brown Norway (BN) rat in 2004 was an important genome resource for rat geneticists (10). Rat positional cloning strategies have benefited from the generation of this comprehensive 7.5× sequence of the BN rat. Indeed, the positional cloning of *Pkdr1* in a rat model of polycystic kidney disease (PKD) combined traditional physical and genetic mapping methods with computational analyses of the emerging sequence of the BN genome (16). After fine-mapping and the determination of the critical interval responsible for spontaneous PKD in the *cy/+* rat, Brown and colleagues identified two genes by *in silico* mapping annotation. Following the identification of a C to T transition that replaces an arginine with a tryptophan at amino acid 823 in the protein sequence of SamCystin encoded by *Pkdr1*, the authors established the correct cDNA and protein sequences of the product. They then highlighted significant differences with all predicted *in silico* annotations of the *Pkdr1* gene and determined the expected sequences of the mouse and human orthologs (16). The publicly available rat sequence was also used in the fine mapping poorly annotated regions that are the subject to structural variations such as the *Fcgr3* locus on rat chromosome 13 (33). New informative microsatellite markers flanking the *Fcgr3* gene were identified from the rat genome sequence. Given the new sequencing technologies available, the sequence of other commonly used inbred rat strains will provide a basis for comparative genomics. The SHR rat is the most widely used strain for QTL mapping as a model of human metabolic syndrome, and a 10× sequencing of the SHR genome using massively parallel paired-end sequencing is currently being carried out as part of the EU-funded EURATools consortium. A complete sequence of the SHR genome will constitute an important

genomic resource for positional cloning studies in crosses where SHR rats have been bred with the reference BN strain in order to identify various QTLs related to metabolic syndrome.

As mentioned previously, genetic variation between inbred rat strains, usually assayed by using a limited set of microsatellite markers, can be used for testing co-segregation with a wide range of disease phenotypes in different rat strains. However, a dense set of polymorphic markers such as SNPs provide a more powerful tool for high-resolution mapping when compared to microsatellite mapping. The international STAR consortium reported a survey of genetic variation based on almost three million newly identified SNPs and obtained accurate and complete genotypes for a subset of 20,238 SNPs across 167 distinct inbred rat strains, two rat recombinant inbred panels and an F2 intercross (11). This detailed SNP map was used for fine mapping of QTLs in two recent studies: the positional cloning of *Ogn* as a regulator of LVM used SNP-based fine mapping to exclude *Hbld2* as a positional candidate in the refined LVM QTL on rat chromosome 17 (19). Another example was the identification of soluble epoxide hydrolase (*Ephx2*) that was achieved by SNP genotyping of F2 crosses between spontaneously hypertensive heart failure (SHHF) rats and reference strains (18).

2.3. Functional Tests of Candidate Genes

The availability of the BN genome sequence together with recent advances in SNP-genotyping in inbred rat strains and eQTL mapping in recombinant inbred (RI) strains has allowed the positional cloning of many genes in various disease models. Although these approaches enable the identification of sequence variants in candidate genes, the biological testing of causative variants remains as the biggest challenge in QTL mapping. Functional testing of candidate genes until 2002 was achieved by transgenic rescue in rats (24, 38). Nonsense mutations in *Cd36* and *Cblb* associated with insulin resistance and type 1 diabetes respectively resulted in strong reduction or complete loss of function of the gene products, and these were therefore functionally complemented with transgenesis of the wild type copy of the genes (24, 38). However, the biggest obstacle for functional testing of candidate genes is the lack of rat pluripotent embryonic stem cells, making the generation of stable knockouts by homologous recombination not possible. Although several positional cloning studies in rat models tested the candidacy of positionally cloned genes in mouse knockouts (18, 19, 39), recent advances in molecular biology offer alternative approaches for generating knockout rats and enable testing of the candidate genes in rat models. The use of somatic cell nuclear transfer to develop cloned rats as an alternative to using embryonic stem cells has been reported, but remains technically challenging (40). N-ethyl-N-nitrosourea (ENU) mutagenesis followed by a screening method to detect

single-nucleotide substitutions within the targeted gene was previously reported by several groups (41–43). However, the low induced mutation frequency, as well as the efficiency and throughput of the screening methods are the important drawbacks in generation of knockout rats by ENU mutagenesis. Indeed, most of the ENU-mutagenised rats are discarded within weeks after target genes have been screened, and the screening methods are based either on yeast (41, 43) or on high throughput resequencing that are currently expensive. A recent study by Mashimo and colleagues overcame these difficulties by combining a high throughput and low cost screening assay that uses phage Mu transposition reaction and intracytoplasmic sperm injection (ICSI) for the recovery of the rare heterozygous genotypes from a newly generated frozen sperm repository (44). This new approach is cost and resource effective and will allow functional testing of many candidate genes underlying QTLs in rat models.

RNAi is increasingly and widely used after the first description of this strategy in mammalian cells by introducing small double-stranded RNAs comprising 19–21 nucleotide complementary sequences (called small interfering RNAs, siRNAs) to silence gene expression with high specificity and without activating an interferon response (45). The positional cloning of vitamin K epoxide reductase (VKOR) is an outstanding example of the use of siRNA in gene identification (46). Although VKOR activity was first reported in 1974, the gene encoding VKOR was not identified until 2004. The previously mapped warfarin resistance (*R_w*) trait on rat chromosome 1 (47) and the mapping of a locus for combined deficiencies of vitamin-K-dependant (VKD) proteins to human chromosome 16, syntenic to the rat region (48), led the authors to focus on human chromosome 16p12-q21 for positional cloning. The 20.3 Mb region contained 190 predicted coding sequences and the authors prioritised 13 genes as they code for integral membrane proteins. An RNAi approach by using siRNA in vitro led to the identification of VKOR gene as only one gene among the 13 tested showed reduced VKOR activity following siRNA knockdown in A549 cells (46).

3. Translation to Humans

The ultimate goal of positional cloning using rat models is translation to related phenotypes in humans. The various existing examples in the literature show how findings deriving from rat models can be used to advance understanding of the genetic basis of complex human disease. Over the past 3 years, studies combining microarray and sequencing technologies have revealed that

the human genome is characterised by extensive and complex structural variation contributing to structural diversity among individuals (49–53). Naturally occurring variation in gene copy number is increasingly recognised as a heritable source of susceptibility to genetically complex diseases. The positional cloning of *Fcgr3* in a rat model of human *Crgn* gave new insights into the importance of structural variants in common human disease (33). In humans, low copy number of *FCGR3B*, an orthologue of rat *Fcgr3*, was associated with systemic, but not organ-specific, autoimmunity (54, 55).

Translational studies in humans can also be achieved by comparative genomics between rat and humans. Genetic analysis of a rat with recessive PKD revealed an orthologous relationship between the rat locus and the autosomal recessive polycystic kidney disease (ARPKD) region in humans. The identification of a mutation in the rat gene led to the screening of 66 coding exons of the human ortholog (PKHD1) in 14 probands with ARPKD and revealed 6 truncating and 12 missense mutations; 8 of the affected individuals were compound heterozygotes (56). A similar comparative genomic approach was undertaken in the gene identification for Meckel-Gruber syndrome (MKS), a severe autosomal recessive disorder characterized by the bilateral renal cystic dysplasia, central nervous system malformations, hepatic abnormalities and polydactyly (57). One of the MKS loci maps in humans to chromosome 8 (MKS3) and is syntenic to the *Wpk* locus in rat, which is a model with PKD, agenesis of the corpus callosum and hydrocephalus. Positional cloning of the *Wpk* gene suggested a MKS3 candidate gene, *TMEM67*, in which the authors identified pathogenic mutations in five MKS3-linked consanguineous families (57).

Genes underlying polygenic complex traits have also been successfully translated to humans and this includes the rat breast cancer susceptibility locus *Mcs5a*, as well as *Ogn* and *Ephx2* as risk factors for increased LVM and heart failure respectively (18, 19, 31). However, following the positional cloning in rat models, the translation studies in humans were achieved by association studies (*Mcsa5*) or by correlation with phenotype (*Ogn*, *Ephx2*) underlying the role of these genes as susceptibility factors rather than highly penetrant causative variants.

In high resolution genetic mapping studies carried out by John Rapp and colleagues, the *Cyp11b1* locus was identified as being responsible for the blood pressure difference between Dahl SS/Jr and SR/Jr rats in a minimal congenic strain. Although mutations in the *CYP11B1* gene in humans were initially well known to cause rare monogenic forms of inherited hypertension resulting from 11b-hydroxylase deficiency (58), common or rare variants within the *CYP11B1* locus contributing to essential hypertension have not been reported so far. None of the variants

previously associated with hypertension showed evidence for association in the recent Wellcome Trust Case Control Consortium (WTCCC) study (59) but deep resequencing have started to identify highly penetrant rare variants affecting blood pressure in humans (60).

4. New Approaches in Rat Positional Cloning

Segregating populations allow efficient identification of the genetic determinants of gene expression which may throw light on the molecular basis of complex traits. Quantitative variation in gene expression levels acts as an endophenotype (or intermediate phenotype) situated between the genomic DNA sequence variation and more complex physiological phenotypes. A number of studies indicate that the individual variation in gene expression (i.e. transcript abundance) is heritable in segregating populations (61–65) and gene expression levels can therefore be mapped to the genome using linkage methods, allowing identification of eQTLs. This approach has been termed genetical genomics (66, 67) as eQTLs represent genomic regions for the genetic control of gene expression. Although gene expression profiling is not a novel methodology for identifying causative genes in QTLs by itself, when combined with genetic linkage mapping, it can lead to new insights into the genes and regulatory pathways underlying a widespread range of complex whole-body phenotypes by elucidating metabolic, regulatory, and developmental pathways (67). eQTL analysis is particularly advantageous as it can discriminate between *cis*- and *trans*-acting influences on gene expression, which can help in identifying candidate genes whose expression is under local regulatory control (*cis*-eQTLs) and in dissecting complex regulatory networks comprised of multiple *trans*-eQTLs. A *cis*-acting eQTL maps to the physical location of the gene itself, whereas a *trans*-acting eQTL maps to a genomic region that is distant from the physical location of the gene being transcribed. The eQTL approach can be used as a complementary approach to the traditional physiological QTL (pQTL) mapping. Recent studies showed that the eQTL approach was indeed successful for the positional cloning of different genes in rat models. Thus, genome-wide expression profiling can be used as a trait in a segregating population allowing identification of eQTLs; and if the same study includes pQTL data, the combined approach significantly facilitates the molecular dissection of pQTLs. The key work by Hubner and colleagues integrating genome-wide expression profiling with linkage analysis in RI rat strains (68) led 3 years later to the positional cloning of *Ogn*, *Ephx2* and *Cd36* as

susceptibility factors for increased LVM, heart failure and blood pressure, respectively (18–20).

RI strains are derived by crossing two inbred rat strains in order to obtain an F2 population and following 20 or more brother–sister matings between F2 individuals, a panel of inbred animals each with a different combination of progenitor genomes is produced. In the early 1980s, the SHR strain was crossed with the normotensive Brown Norway (BN) strain to generate the BXH/HXB panel of RI strains (69–71). Although rat RI panels are powerful and renewable resources for genetic mapping that offer the opportunity to accumulate genetic and physiological data over time, to date, there is no reported study on the positional cloning of pQTL genes using exclusively linkage analysis for different metabolic phenotypes in RI strains. However, the combined eQTL–pQTL approach has been successfully used in positional cloning using RI strains as eQTLs identified in the heart highlighted *Ogn* as a candidate as it was the only eQTL within the chromosome 17 pQTL for LVM (19).

On the horizon, however, is an important change of perspective: from single gene(s) to gene networks and pathways. The relevance of a “systems biology” approach to identify pathways operational in common disorders has been suggested by Petretto et al. (19) where they inferred that *Ogn* may influence LVM through modulation of the TGF- β pathway. The role for the TGF- β signalling pathway in the regulation of LVM was supported by human data that show an association of LVM in humans with several genes that are important in this pathway. To date, integrated gene network approaches to positionally clone disease genes have been successfully carried out mainly in the mouse (72, 73) where perturbation signatures in gene expression data and networks were used to map genes for complex traits. These recent examples in the mouse raise the importance of gene networks in the pathogenesis of disease, suggesting that similar approaches can be employed to dissect the networks of *cis* and *trans* interactions involved in quantitative traits. The large QTL, eQTL and QTT studies published in the rat reveal that the datasets and study approaches are now also at hand in the rat model to accelerate identification of QTL genes through the integration of gene network and pathway analyses.

5. The Future of Genetic Mapping and Positional Cloning

Figure 2.1 summarises the present and future strategies in rat positional cloning of complex trait genes. It is noteworthy that a combination of different approaches such as eQTL–pQTL–QTT has been successfully applied in the identification of risk variants

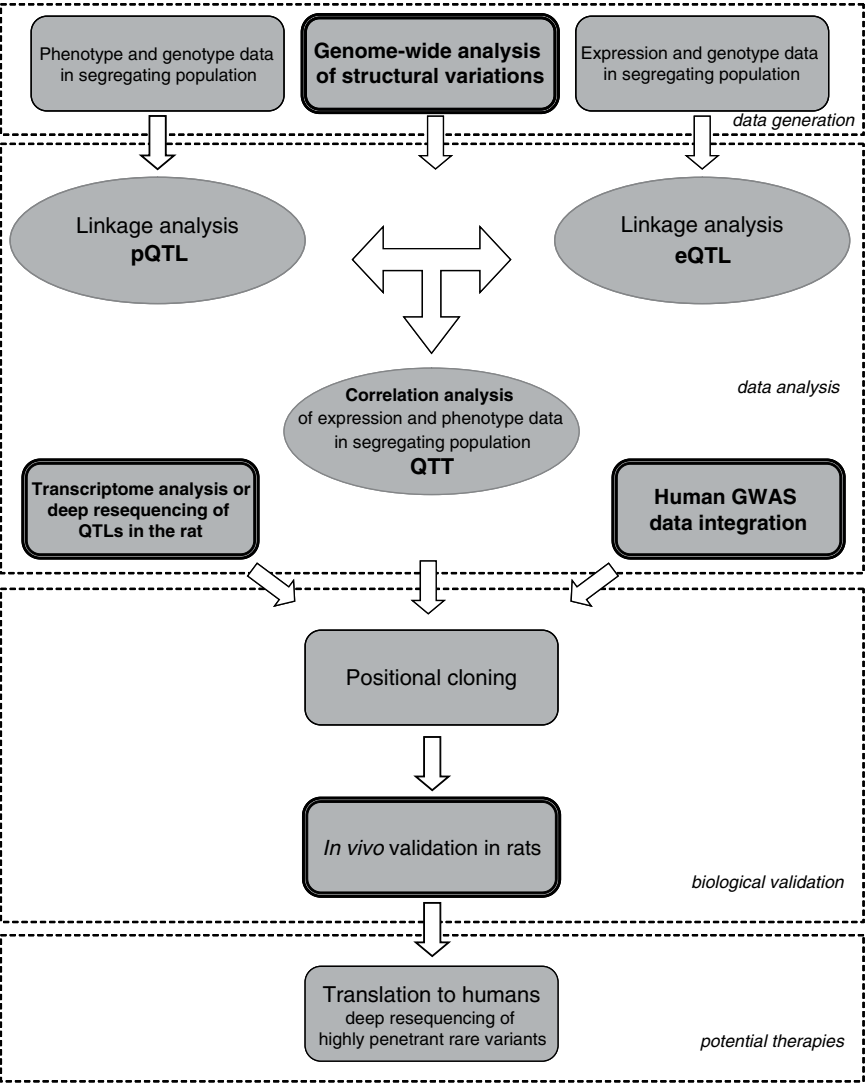


Fig. 2.1. The future of positional cloning in the rat. The combined pQTL–eQTL–QTT approaches were recently and successfully used in positional cloning of several complex trait genes (*see text*). The emerging deep resequencing, transcriptome analysis, genome-wide association (GWAS) and structural variation studies will allow integration of additional tools for positional cloning of complex traits in rat models. Given the recent advances in generating knock out rats, the *in vivo* validation is expected to be mostly carried out in the rat. The rat will constitute a valuable “reagent” for *in vivo* validation of genetic variants identified by human GWAS. Furthermore, rat positional cloning could provide rare genetic variants to be tested in human populations by deep resequencing (*pQTL*, physiological QTL; *eQTL*, expression QTL; *QTT*, quantitative trait transcript).

for different disease models. Figure 2.1 does not show the fine mapping strategies that could also lead to positional cloning (described in detail in “Successful positional cloning in the rat”). To date, translation to humans have been achieved by association or correlation studies; however we believe that in the near future, rat models will be a valuable “reagent” for the biological testing

of genetic variants identified in GWAS in human populations (Fig. 2.1). In addition to the common variants identified by these high throughput techniques, the emerging “rare variant hypothesis” of complex human diseases highlights the importance of rat genetics to facilitate elucidation of the underlying molecular basis of common human disease. Data from genetic studies in rodent models will suggest that genes whose orthologs should be prioritised for deep re-sequencing in order to identify rare variants that can be tested for disease association in human populations.

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