Quest for arthritis-causative genetic factors in the rat

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Joe, Bina. Quest for arthritis-causative genetic factors in the rat. Physiol Genomics 27: 1–11, 2006. First published June 27, 2006; doi:10.1152/physiolgenomics.00034.2005.—Experimental rat models of arthritis are extensively studied with a view to understand the genetic underpinnings of rheumatoid arthritis (RA). Genome scans using these models have led to the detection of arthritis regulatory quantitative trait loci (QTLs) on all but three chromosomes of the rat. Whereas some of the QTLs are model specific, others overlap between models. Some arthritis susceptibility and/or severity QTLs identified by genetic linkage analyses are corroborated by substitution mapping using congenic strains, whereas others are not. In these cases, testing alternate arthritis models proved to be useful to identify QTL effects. Nevertheless, development and testing of congenic substrains containing progressively shorter introgressed regions have not only fine mapped the location of the arthritis QTLs but also resulted in the identification of multiple QTLs within several originally identified individual QTL. Most of these studies progressed rapidly since 2001, when the rat genome sequence was published. Proof of principle for substitution mapping as a successful method for QTL gene discovery is provided by the positional cloning of Ncf1 as one of the arthritis QTLs in rats. This finding is encouraging for similar sustained dissection of all the other arthritis QTLs mapped in the rat. Identification of rat arthritis QTLs is expected to pave the way for discovery of yet-unidentified arthritis-causative genetic elements and/or pathways for RA in humans and potential development of targeted therapeutics. This review catalogs some of the recent advances made in QTL discovery projects of experimentally induced rat models of arthritis.

IDENTIFICATION OF disease-causative loci for polygenic traits such as rheumatoid arthritis (RA) in humans is confounded by several factors including genetic heterogeneity, complex interactions of environmental and genetic factors, and low disease penetrance. The use of inbred strains of rats for genetic analyses minimizes several of these factors. Among rats, there are no known inbred strains that spontaneously develop arthritis. For this reason, researchers have used induced models of arthritis in experiments involving genetic analyses. Both rats and mice are actively investigated for arthritis quantitative trait loci (QTLs). The contents of this review are limited to rat models of experimental arthritis. Irrespective of the species used for identification, comparative mapping will allow for cross-species detection of homologous regions rendering arthritis susceptibility.

There are several rat models of experimentally induced arthritis (18, 20). Among these, collagen-induced arthritis (CIA), mycobacterial adjuvant-induced arthritis (Mbt-AIA), pristane-induced arthritis (PIA), and oil-induced arthritis (OIA) are the most prevalently used models for genetic analyses. Genetic studies have also been reported for squalene-induced arthritis (SIA) and streptococcal cell wall-induced arthritis (SCWIA) although not to the extent of studies conducted using the models mentioned earlier. The hallmark of each of these rat models is the simulation of the development of joint inflammation seen in human RA. The similarities and differences in incidence, progression, and severity of arthritis in each of these models is the simulation of the development of joint inflammation seen in human RA. The similarities and differences in incidence, progression, and severity of arthritis in each of these models are discussed elsewhere (18, 20). The expectation from genetic analyses using these models is to be able to gain insight into the genetic control of RA in humans.

GENETIC ANALYSIS USING INBRED RAT STRAINS

Conducting whole genome genetic linkage analysis in segregating populations derived from arthritis-susceptible and relatively arthritis-resistant inbred rat strains constitutes the primary step toward identification of arthritis QTLs. With the use of this approach, information on chromosomal location, the magnitude of the arthritis effect, and the mode of inheritance of each causative locus can be obtained. Inbred strains of rats vary widely in their susceptibility to and the severity of the development of experimental arthritis. Examples of the susceptible inbred strains include the DA and LEW rats. These two inbred strains are highly susceptible to many experimentally induced models of arthritis (39). Therefore, these rats were obvious choices as the arthritis-susceptible parental strains for conducting genetic analyses of arthritis.
Table 1. QTLs for experimental arthritis in rats identified by linkage studies

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RESULTS OF QTL ANALYSIS OF EXPERIMENTAL ARTHRITIS

Remmers et al. (34) reported the first complete genome linkage analysis for arthritis in rats using microsatellite markers on an F2 population derived from CIA-susceptible DA rats and CIA-resistant F344 rats (34). Subsequently, multiple genetic linkage analyses have been performed using various combinations of arthritis models and arthritis-susceptible and -resistant inbred strains. As a result of this, several QTLs controlling different aspects of arthritis incidence and progression were identified to be present on several rat chromosomes. Some of the QTLs identified by genetic linkage analysis are corroborated by substitution mapping studies using congenic rat strains. Table 1 lists the names and chromosomal locations of the experimental arthritis QTLs identified by genetic linkage analyses and/or corroborated by substitution mapping studies.

NOMENCLATURE OF EXPERIMENTAL ARTHRITIS QTLs

As with the genetic analysis of any complex polygenic trait, the issue of nomenclature of experimental arthritis QTLs is not consistent between publications from laboratories and databases. Most of the QTLs are identified by the name of the experimental model followed by a number, which denotes the chronological order of its identification/publication among the QTLs identified for the same experimental model. For example, “Cia1” through “Cia5” are CIA QTLs 1–5, which were reported in 1996, whereas “Cia25” and “Cia26” are CIA QTLs 25 and 26, which were reported in 2004. However, this nomenclature is not followed for curation in the Rat Genome Database (RGD; http://www.rgd.mcw.edu). For example, Cia20, Cia21, Cia22, Cia23, and Cia24 do not exist as published literature from research laboratories but are arbitrary names used by RGD. When QTLs identified by linkage analysis are tested by substitution mapping, the name of the QTL as mentioned in the linkage analysis is maintained, often within the parenthesis following the lettering describing the recipient and donor strains of the congenic strains constructed for substitution mapping. For example, the name DA.F344(Cia5) denotes the QTL Cia5 that is being tested in a congenic strain that contains alleles from the F344 rat at the Cia5 region introgressed into the DA rat genomic background. This follows the rules for nomenclature detailed by the International Committee on Standardized Genetic Nomenclature for Mice and Rats (http://www.informatics.jax.org/mgihome/nomen/strains.shtml#congenic). It is at the stage of further fine mapping that nomenclature is problematic. More often than not, the originally identified single QTL region comprises multiple QTLs, for which there are no strict guidelines for nomenclature, as this was somewhat unanticipated. Different laboratories have handled the nomenclature issue at the fine-mapping stages differently. Irrespective of the names of the congenic substrains used, most laboratories have retained the original name of the QTL and added alphabets in small case as suffixes to it. All the names of QTLs studied by substitution mapping are compiled in Table 2.

The objective of this article is to review the results of the genetic linkage analyses and substitution mapping studies that 1) provide evidence for the presence of arthritis QTLs in rats and 2) indicate the location of these arthritis QTLs on the rat genome. For purposes of clarity, the results are sorted by rat chromosome (RNO).

RNO1

Four different genetic linkage analyses have resulted in the identification of QTLs for CIA and PIA on RNO1 (Table 1). The earliest of these was the identification of Cia2 in a genetic linkage analysis of F2(DA × F344) rats (Fig. 1, RNO1) (34). (Note: as a matter of convention throughout this article, populations are represented as arthritis-susceptible strain × arthritis-resistant strain.) A study using an F2 population raised from BioBreeding diabetes-resistant rats [BB(DR)], which are CIA susceptible, and Brown Norway rats (BN), which are CIA resistant, resulted in the identification of a CIA QTL in the same approximate region as Cia2 (10). Therefore, this QTL was not assigned a new name but is depicted in Fig. 1 as Cia2*. Both of these CIA QTLs regulated the severity of CIA. DA alleles decreased arthritis severity. The mode of inheritance of Cia2* is reported as BN additive. The gender effects of Cia2 identified in the F2(DA × F344) are not reported, but the arthritis effect of Cia2* in the F2[BB(DR) × BN] population is observed in both sexes.

Significant linkage to anti-collagen (CII) antibodies was identified on RNO1 in (DA × E3) backcross to DA rats (30). This QTL is called Ciaa6 (Fig. 1, RNO1) (30).

Two PIA QTLs were also localized on RNO1. Pia8, a QTL that regulates arthritis severity, early joint erosion, and maximum clinical score, was located toward the proximal region on RNO1 (Fig. 1). This QTL was identified using an F2 population of DA and a recombinant inbred strain (DXEC) that is PIA resistant (26). The mode of inheritance for this QTL is dominant for E3 alleles. Pia8 does not significantly overlap with
Cia2 or Cia2* (Fig. 1, RNO1). Another PIA QTL, designated Pia9, was located closer to the region of Cia2 (Fig. 1, RNO1) (24). Pia9 was identified using an F2(LEW.1F × E3) population. The mode of inheritance of Pia9 is stated as LEW recessive. There are two distinct features associated with Pia9, i.e., it is more effective in females and interacts synergistically with another PIA QTL, Pia11, which is located on chromosome 16 (24). Pia9 was confirmed using (LEW.1F).E3 congenic strains (Table 2) (24).

**Table 2. Substitution mapping of QTL for experimental arthritis in rats**

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**RNO1**

RNO, rat chromosome no.; SNP, single nucleotide polymorphism. From Marker and To Marker bracket the introgressed QTL genomic sequence. These markers were extracted from the original QTL plots of each corresponding publication. Start and End Positions (in bp) were obtained by basic local alignment search tool (Blast), searching the markers/SNP on the Ensembl Rat database (http://www.ensembl.org/Rattus_norvegicus) in April 2006.

Cia2 or Cia2* (Fig. 1, RNO1). Another PIA QTL, designated Pia9, was located closer to the region of Cia2 (Fig. 1, RNO1) (24). Pia9 was identified using an F2(LEW.1F × E3) population. The mode of inheritance of Pia9 is stated as LEW recessive. There are two distinct features associated with Pia9, i.e., it is more effective in females and interacts synergistically with another PIA QTL, Pia11, which is located on chromosome 16 (24). Pia9 was confirmed using (LEW.1F).E3 congenic strains (Table 2) (24).

**RNO2**

Three CIA severity QTLs are reported on this chromosome (Table 1). Two of these, Cia7 and Cia10, were identified by genetic linkage analysis of the same F2(DA × ACI) population...
As can be seen in the physical map of RNO2 on Fig. 1, Cia10 lies distal to Cia7. By substitution mapping with DA.AC1 congenic strains, Cia10 is now localized to a 52.6-Mb region on RNO2 (Table 2) (4). Cia7 is not yet corroborated by substitution mapping. A region overlapping with Cia7 was also identified to regulate arthritis severity in females from an F2[BB(DR) × BN] population (10). This QTL was also designated as Cia7* and is represented in Fig. 1 as Cia7*. The modes of inheritance for Cia7, Cia7*, and Cia10 are BN dominant, DA dominant, and DA dominant/additive, respectively.

Griffiths et al. (12) performed a genome-wide linkage analysis in F2(DA × BN) rats and identified a CIA severity QTL on RNO3 that was called Cia11 (Table 1, Fig. 1, RNO3). DA recessive is the mode of inheritance of this QTL. The gender effects of Cia11 are not specified. Another arthritis-regulating locus overlapping with Cia11 was identified in a (DA × E3) backcross to DA population (30). This QTL is also referred to as Cia11 (Fig. 1, RNO3, Cia11*). There are no reports of substitution mapping of either of these QTLs.
As can be seen in Fig. 1, RNO4 has the highest number of experimental arthritis QTLs detected to date. QTLs representing five arthritis models, AIA, CIA, SCWIA, OIA, and PIA, are found on this chromosome (Table 1 and Fig. 1, RNO4). Because the locations of several of these QTLs differ, there is reason to believe that there are multiple experimental arthritis regulatory genes on RNO4. Complex interactions of QTLs on RNO4 with other chromosomes are also observed. For example, the phenotypic expression of Pia7 is dependent on the presence of DA alleles at Pia6 (RNO14) (26). Several of these QTLs identified by initial genetic linkage analysis are corroborated and/or fine mapped by substitution mapping studies (Table 2). CIA, and to a lesser extent PIA, is lowered in DA.E3 (Pia7/Cia13) congenic strains. This confirms Pia7 and Cia13 (30). Analysis of E3.DA reciprocal congenic strains in an intercross experiment between (E3.Pia4 × E3.Pia7) F1 congenic rats also corroborates the effect of the genomic region containing Pia7/Cia13 on CIA but not on PIA (30).

The Mbt-AIA QTLs Aia2 and Aia3 were studied independently by constructing DA.F344 congenic strains (17). The arthritis-lowering effects of Aia2 and Aia3 were sex influenced and observed only in males and females, respectively. However, when F344 alleles of these two non-major histocompatibility complex (non-MHC) QTLs were introgressed into DA rats containing additional F344 alleles at Aia1, the MHC-containing AIA QTL region on RNO20 demonstrated a dramatic lowering of arthritis severity in both males and females (17).

F344 alleles at the Cia3 on RNO4, when introgressed into DA rats, significantly reduced severity of CIA in females but not in males (Table 2) (33). However, these DA.F344(Cia3) congenic rats exhibited marked lowering of PIA and OIA severity in a sex-independent manner (Table 2) (33).

Pia2, a QTL influencing the onset of PIA, also resides on RNO4 (Fig. 1, RNO4) (37). This QTL was identified using an F2(DA × E3) population. There is no report of substitution mapping of Pia2.

Oia2 regulates arthritis induced both by the nonimmunogenic immunostimulant squalene and by collagen (2). A search for genetic loci linked to OIA in an F2(DA × LEW.1AV1) population led to the identification of Oia2 (Fig. 1, RNO4; Table 1) (23). The location of Oia2 was confirmed using another population consisting of (DA × PVG.1AV1) backcrossed to DA rats (16). Reciprocal transfer of Oia2 between DA and either LEW.1AV1 or PVG.1AV1 rats confirmed the identification of Oia2 by linkage analysis (Table 2) (35). Oia2 influences arthritis in both males and females, with a more pronounced effect observed in males. Fine substitution mapping eliminated the rat natural killer cell gene complex (NKC) as Oia2 and resulted in the localization of Oia2 to <1 cM on RNO4 between the markers D4Got126 and D4Got136 (Table 2) (35).

Aside from the above-mentioned arthritis QTLs, Ciaa4, the autoantibody QTL that regulates autoantibody titer to rat type II collagen more strongly in females than in males, was described by Furuya et al. (10) in an F2[BB(DR) × BN] population (Fig. 1, RNO4).

There are no experimental arthritis QTLs detected by linkage analysis on this chromosome in rats. However, in the F2[BB(DR) × BN] population of rats studied for CIA, a QTL for anti-collagen antibody titer designated as Ciaa5 was identified (10). The name Ciaa5 is also given to a QTL that controls production of rheumatoid factor (RF) in (DA × E3) backcross to DA population (represented as Ciaa5* in Fig. 1, RNO5) (30). Both of these QTLs are broad regions on the physical map of RNO5 (Fig. 1, RNO5). Further localization is required to determine whether these two QTLs overlap.

PIA is the only model wherein QTLs have been identified on RNO6. Three different linkage analyses have located PIA QTLs on RNO6. Genetic analysis of two F2 populations, F2(DA × E3) (37) and F2(DA × DXEC) (26), resulted in the identification of the same region on RNO6 as QTLs that control the day of onset of arthritis (note: DXEC is a recombinant inbred strain derived from DA and E3 rats). Because the QTL regions were overlapping, they were identified by the same name, Pia3. Pia3 identified by the studies in the above-mentioned F2 populations are individually represented as Pia3 and Pia3*, respectively, in Fig. 1. The common feature of Pia3 in these two populations is that E3 alleles are dominant and cause earlier onset of arthritis. Pia3 identified by the analysis of F2(DA × DXEC) was predominantly observed in male rats. Pia3 identified by the analysis of F2(DA × E3) rats interacts with Pia4 on RNO12. Yet another PIA QTL, Pia12, was identified by genetic linkage analysis of a (DA × E3) backcross to DA population (27). As shown in Fig. 1, on the physical map of RNO6, Pia12 maps to a large genomic segment. E3 alleles are dominant for the functionality of Pia12, which is distinct from Pia3 in that Pia12 predominantly controls ankle swelling.

Initial attempts to corroborate Pia3 by substitution mapping using DA.Pia3 congenic rats failed, as the congenic rats and the control DA rats developed similar patterns of disease onset and severity (32). On the basis of computational analysis, the genetic interaction between Pia3 and Pia4 was strongest when alleles at Pia3 were homozygous for E3 and alleles at Pia4 were heterozygous, DA/E3 (32). To test this, Olofsson et al. (32) introduced heterozygosity at Pia4 in the DA.Pia3 congenic rats. These double congenic rats had significantly higher arthritis severity compared with the parental rats. Thus it is clear that the background effect of DA homozygous Pia4, which is identified as Ncf1 (28), completely masks the effect of Pia3. This epistatic interaction can be exploited for positional cloning of the Pia3 gene.

There are three distinct QTLs reported on RNO7. Two of these are CIA QTLs, and the third is a PIA QTL (Table 1). Of these, the CIA QTL, Cia4, was first reported by linkage analysis of an F2(DA × F344) population (34). The Cia4 QTL peak was near D7Arb5 (Myc), which is located at ~98 Mb on RNO7. Since the submission of this report, additional polymorphic markers allowed for more precision in the mapping of Cia4 to a more proximal region on RNO7 (Fig. 1, RNO7) (8).
This extended analysis also identified a second CIA severity QTL on RNO7, Cia18, which is located toward the p-terminus of RNO7 (Fig. 1, RNO7) (8). The modes of inheritance of Cia4 and Cia8 were better fitting for DA recessive and additive rather than DA dominant.

Substitution mapping studies using DA.F344(Cia4) congenic strains did not corroborate the identification of Cia4 by linkage analysis, because this congenic strain developed as severe CIA as the arthritis-susceptible DA rat (Table 2) (33). However, DA.F344(Cia4) congenic strains had significant arthritis-modulating effects in males when tested for PIA or OIA (Table 2) (33). Therefore, PIA or OIA could be utilized as arthritis models to further dissect Cia4.

Linkage analysis for PIA QTLs in an F1(DA × E3) backcross to DA population detected Pia13, a PIA QTL that controls clinical score and paw swelling (Fig. 1, RNO7) (27). DA alleles at Pia13 are disease promoting. Pia13 partially overlaps with Cia4 but does not overlap with Cia8 (Fig. 1, RNO7).

RNO8

A suggestive CIA severity QTL on RNO8 was first identified in F2(DA × F344) rats (34). Because of the “suggestive” nature of this QTL, it was not assigned a name. The location of this QTL on the physical map of the rat genome is represented in Fig. 1, RNO8, “no name.” Subsequently, by linkage analysis with an F2[BB(DR) × BN] population, a CIA severity QTL was again detected in the region overlapping with this RNO8 CIA QTL and named Cia6 (Fig. 1, RNO8) (10). Sex-segregated analysis suggested that Cia6 regulated arthritis severity more strongly in males than in females (10).

Although DA.F344(Cia6) congenic strains did not corroborate the CIA-modulating effect of Cia6, results from studying OIA in these congenic strains confirm the sex-segregated analysis of Cia6, because only males were significantly protected from OIA (Table 2) (33).

In the study of another model of arthritis, PIA, using F1(DA × E3) × DA backcross population, arthritis phenotypes such as onset and clinical score were found to be significantly linked to a QTL on RNO8, Pia14 (Fig. 1, RNO8) (27). Pia14 is not yet reported as corroborated by substitution mapping. Nevertheless, RNO8 harbors QTLs for at least two experimental models of arthritis.

RNO9

There are very few reports for experimental arthritis-related QTLs on RNO9. Cia15, an arthritis severity QTL, was identified as a CIA severity QTL in F2[BB(DR) × BN] rats (10). Cia15 regulated arthritis severity more strongly in females than in males. Aside from this, in the same study, a suggestive QTL for autoantibody titer was identified on RNO9 (10). This suggestive QTL overlapped with Cia23 (shown as Ciaa3* in Fig. 1, RNO9), which is an autoantibody QTL identified in an F2(DA × BN) cross (12).

RNO10

A QTL controlling the severity of CIA in F2(DA × F344) rats was identified on RNO10 (34). This QTL was named as Cia5 (Fig. 1, RNO10, Cia5). In a study of an F2(DA × BN) population, a QTL for severity of CIA was detected in the same region as Cia5 (12). Therefore, this QTL was also referred to as Cia5 (represented as Cia5* in Fig. 1, RNO10). Data obtained by both of these genetic linkage analyses were reanalyzed after segregation of the population by gender. The results strongly indicated that Cia5 comprises two QTL peaks Cia5a and Cia5b (19). Cia5a was located distal to Cia5b. The logarithm of odds (LOD) scores for Cia5a were higher for females, whereas the LOD scores for Cia5b were higher for males (19).

Genetic linkage analysis of CIA in an F2 population derived from BB(DR) and BN rats also resulted in the identification of a CIA QTL on RNO10, Cia16 (Fig. 1, RNO10) (10). Cia16 was detected only in males. As seen in Fig. 1, RNO10, Cia16 covers a broad region of RNO10 but is located more proximal to Cia5. Linkage analyses using two other experimental models of arthritis, OIA and PIA, have reported QTLs on RNO10. Genome-wide scan of progeny from an F2 intercross between DA rats and MHC-identical but arthritis-resistant LEW.1AV1 rats identified Oia3, a QTL determining susceptibility to OIA (23). Recently, Oia3 was also linked to SIA in an F2(DA × LEW.1AV1) intercross (7). Oia3 overlaps with Cia5 (Fig. 1, RNO10). Genetic linkage analysis of F1(E3 × DA) rats backcrossed to DA rats detected Pia10, a PIA-controlling QTL (27). On the physical map of the rat genome, this PIA QTL does not appear to overlap with either Cia5 or Oia3 but has significant overlap with Cia16 (Fig. 1, RNO10). A QTL linked to collagen-induced autoantibody production, Cia2, also maps to a region overlapping with Cia16 and Pia10 (22). Cia2 was identified by linkage analysis of F2(DA × F344) rats (22).

Substitution mapping studies using DA.F344 congenic rats have not only confirmed Cia5 but also provided evidence for the existence of at least two sex-influenced CIA regulatory loci within the region denoted as Cia5* in Fig. 1. Overall, the results indicate that replacing DA alleles at the distal segment of Cia5 with F344 alleles protected CIA in females better than in males, whereas replacing DA alleles at the proximal segment of Cia5 with F344 alleles protected CIA in males (19). Similar to these results, DA.F344 congenic strains containing F344 alleles at Cia5 and Cia5a protected DA rats from severe CIA, but the female specificity of the Cia5a region was not found for either PIA or OIA (Table 2) (33). Recently, Brenner et al. (5) not only confirmed the arthritis-protective effects of Cia5 and Cia5a but identified that there are at least three QTLs embedded within the originally identified Cia5 QTL-containing region. These are Cia5a, which is fine mapped to 10.1 Mb; Cia5d, which spans 47.3 Mb; and a third QTL with no name assigned to it but spanning the region from the microsatellite marker D10Arb20 to the microsatellite marker D10Wox22 (Table 2) (5).

(LEW.1AV1).DA congenic rats were constructed to confirm Oia3 (15). The expectation was that the arthritis-susceptible DA alleles of Oia3 would render the (LEW.1AV1).DA rats susceptible to OIA. But only low incidence and severity were observed in these Oia3 congenic rats. By using squalene, a more potent inducer of arthritis, Holm et al. (15) were able to confirm that the Oia3 congenic rats indeed developed higher incidence and severe arthritis compared with the LEW.1AV1 arthritis-resistant parental rats. Thus SIA was used as the appropriate model to further dissect Oia3. Three different genomic segments (Oia3a, Oia3b, and Oia3c) together repre-
senting Oia3 were derived from the Oia3 congenic rat (15). Concomitant studies of SIA in these congenic substrains indicated that Oia3b contained genes that regulate SIA in both males and females, whereas Oia3c does so only in females (Table 2) (15). Therefore, it is clear that Oia3 is indeed comprised of multiple arthritis regulatory genes.

Genetic analysis of Mbt-AIA in segregating populations did not detect QTLs on RNO10. However, a DA.F344 congenic strain containing the Cia5 QTL was protected from Mbt-AIA compared with the parental DA rat (17). The reason for the discrepancy between linkage analysis and substitution mapping is not known. However, the Mbt-AIA QTL identified by use of congenic strains is designated Aia5 (Table 2). To determine the cumulative effect of the AIA QTLs, Mbt-AIA severity was studied in a DA.F344 polycongenic rat that contained F344 alleles at Aia1, Aia2, and Aia3 (Table 2) (17). Compared with the DA rat, the severity of arthritis was lowered but not abolished, implying that there are other yet-undetected Mbt-AIA QTLs on the rat genome. One of these is Aia5 on RNO10 (Table 2).

**RNO11**

There are no arthritis QTLs reported on this chromosome.

**RNO12**

The search for arthritis genes on rat chromosome 12 began with the genetic linkage analysis in an F2(DA × E3) population, which resulted in the identification of Pia4, a PIA QTL (37). This QTL affects severity of arthritis during the early stages of joint erosion. Pia4 was substitution mapped using several iterations of congenic strains and finally isolated in a congenic strain with minimal introgressed region (Table 2) and reported as Ncf1, a gene coding for a component of NADPH oxidase (28, 30). Pia4 is the first QTL for which the underlying genetic variation has been identified (28, 29). The ATG-to-ACG polymorphism resulting in M153T substitution is located in the Pia4 congenic rats (28). This is contrary to previous observations that elevated NADPH oxidase activity compared with the DA rat was associated with the serum levels of IL-6 (31). Pia4 was isolated in a DA.E3 congenic strain called DA.Pia4 (Table 2) (38). The introgressed fragment from the E3 rat was ~30 cM between D14Wox8 and D14Rat64. This congenic strain was specifically protected from the chronic phase of arthritis.

**RNO15**

A suggestive QTL for severity of Mbt-AIA was identified in F2(DA × F344) rats (Fig. 1, RNO15; Table 1) (21). Because of the suggestive nature of this QTL, there was no name assigned to this QTL.

**RNO16**

A novel PIA QTL, Pia11, with significant linkage to severity of PIA is reported (24) (Table 1 and Fig. 1, RNO16). Pia11 was detected in the genetic linkage analysis of F2(DA × LEW.1F). The severity-predisposing allele of Pia11 was derived from the arthritis-resistant E3 strain. During the acute phase of the disease, Pia11 and Pia9 (on RNO1) show a synergistic effect in both females and males. Rats homozygous for the LEW.1F allele at Pia9 and homozygous for the E3 allele at Pia11 show a more severe disease than rats that are homozygous at only one of these loci (24).
Both PIA and CIA QTLs are identified on RNO18 (Table 1 and Fig. 1, RNO18). Cia17, a CIA severity QTL in females, was detected in a linkage analysis of F2[BB(DR) × BN] rats (10) (Table 1 and Fig. 1, RNO18). The mode of inheritance of Cia17 is described as BB(DR) dominant. Recently, another novel QTL, Cia26, influencing CIA during the more chronic stages, was described in an F2 intercross between DA and ACI (25). An epistatic interaction between Cia26 and Cia7 on RNO2 has been observed (25).

The PIA QTL described on RNO18 was identified using a DA backcross to (DA × E3) population (27). This QTL, called Pia15, operates with a dominant effect on severity. E3 alleles at Pia15 promote paw swelling. Unlike the CIA QTLs reported on RNO18, gender specificity of Pia15 is not described.

A QTL on RNO18 around the marker D18Mgh1 controls the level of serum IL-6 on day 35 of PIA in F2(DA × E3) rats (31). This QTL overlaps with the arthritis QTLs described on RNO18.

There is no reported substitution mapping studies of experimental arthritis QTLs on RNO18.

RNO19

Genetic linkage analysis of CIA in F2(DA × BN) rats identified the only reported experimental arthritis QTL on RNO19 (Fig. 1, RNO19; Table 1) (12). This QTL is called CIA14 and controls arthritis severity with the mode of inheritance described as DA recessive. No congenic strains are described for the analysis of CIA14.

RNO20

Five different models of experimental arthritis identified major QTLs on RNO20 (Table 1 and Fig. 1, RNO20). This is not surprising, considering that the rat MHC, which is a major regulator of arthritis, is located on RNO20. Aia1, the Mbt-AIA QTL on RNO20, was reported by Kawahito et al. (21). On the physical map of the rat genome, Aia1 spans a large region toward the p-terminus of RNO20 (Fig. 1, RNO20). The CIA QTL on RNO20, CIA1, was first identified in an F2(DA × BN) linkage analysis (34) and also identified in two other linkage analyses of F2(DA × BN) (12) and F2[BB(DR) × BN] (10) populations (Fig. 1, RNO20). CIA1 identified in the F2(DA × BN) and F2(DA × BN) populations are linked at the Tnfa locus (Table 1). Therefore, Fig. 1 does not include these reports.

Vingsbo et al. (36) determined that RNO20 is also involved in the genetic control of PIA. Comparison of MHC congenic LEW strains showed that the severity and chronicity of arthritis varied among the different MHC haplotypes. Rats with RTI/I haplotype showed a significantly higher susceptibility to PIA (36). Evidence for a suggestive linkage to Pial, the PIA QTL on RNO20, is also provided using an F2 cross between DA and E3 recombinant inbred strain (Table 1, Fig. 1, RNO20, Pial) (26). Reanalysis of the data obtained from F2(DA × E3) rats for possible epistatic interactions revealed that the phenotypic expression of Pial is dependent on the presence of DA alleles at RNO18 (Fig. 1, RNO20, Pial*) (26). Pial was confirmed by subsequent genetic linkage analysis of F1(DA × E3) × DA rats (Fig. 1, RNO20, Pial**) (27). This dominant locus delays arthritis onset and affects early severity.

The name Oial was assigned by Lorentzen et al. (23) to a previously described arthritis susceptibility locus on RNO20 (6). The LOD plots of Oial are not published and therefore are not represented in Fig. 1.

Cia14 depicted in Fig. 1, RNO20, is a QTL that regulates autoantibody responses to type II collagen in CIA, which is localized on RNO20 using F2(DA × F344) (11, 22), F2(DA × BN) (12), and F2[BB(DR) × BN] (10). Additional corroboration for Cia14 is provided in the QTL analysis of DA backcrossed to (DA × E3) rats (Table 1) (30).

There are several reports of substitution mapping for experimental arthritis QTLs on RNO20. Prominent among these are congenic lines with either the F344 alleles at Cia1 on the DA background or the DA alleles at Cia1 on the F344 background, which were resistant to CIA, PIA, and OIA, confirming contributions from both MHC and non-MHC regions of the rat genome to the genetic control of multiple experimental arthritis models (Table 2) (33).

RNOX

In a genetic linkage analysis of F2[BB(DR) × BN] rats, two CIA QTLs were identified on the X chromosome (10). A suggestive severity QTL was identified near the centromere and called CIA18 (Fig. 1, RNOX). The more significant QTL was located toward the telomere and designated as CIA19 (Fig. 1, RNOX).

RNOY

Cia19 (Fig. 1, RNOY) is a CIA QTL reported on RNOY (11). This QTL was identified using an F2(DA × ACI) population, wherein relative resistance to CIA was observed by inheriting either of the parental Y chromosomes (13). This resistance to CIA was independent of the source of the X chromosome (13).

DISCUSSION

Numerous genetic linkage analyses have pointed at specific genomic regions of the rat as distinct experimental arthritis and/or related QTLs. Some of the QTLs identified so far by genetic linkage analyses are overlapping regions on the physical map of the rat genome. Clearly, further studies involving substitution mapping either are in progress or are needed to resolve each of these QTLs and identify the underlying genetic causes for susceptibility to experimental arthritis. Application of substitution mapping to localize experimental arthritis-causative genes can be viewed as belonging to three stages, based on the extent of localization achieved. These stages are as follows: 1) construction and characterization of primary congenic strains with large introgressed segments (>20–30 cM); such experiments are primarily focused on corroborating the initial arthritis QTL localization by genetic linkage analysis; 2) construction and characterization of congenic substrains to further localize an experimental arthritis QTL within a smaller genomic region (<5–20 cM); and 3) construction and characterization of congenic substrains to fine localize an arthritis QTL to a region <1 cM on the rat genome. Most of the studies conducted have advanced from stage 1 to stage 2. Very few have, however, progressed into stage 3. So far, there is only one report that provides a reasonable “proof of principle” for substitution mapping using congenic strains as a successful approach for advancing from QTL localization to arthritis-
causative gene identification (28). In consideration of the fact that evidence from genetic linkage analyses and substitution mapping indicate the presence of a large number of arthritis QTLs (Fig. 1 and http://rgd.mcw.edu/), coupled with the fact that the identity of only one of them is known, an obvious question faced by investigators is whether to proceed with substitution mapping as a valid approach for finding the remaining arthritis-causative genes. One of the major driving forces in favor of searching for arthritis-causative genes using substitution mapping is the availability of the rat and mouse genome sequence data. This is because, in the past, a major limitation for the construction of congenic substrains was that the development of microsatellite markers in a given target region was difficult. The availability of mouse and subsequently rat genome sequences has facilitated the identification of microsatellite markers in any targeted region of the rat genome, which can then be easily tested for polymorphisms and used to construct congenic strains. Additionally, now that the genome sequence is annotated for human, mouse, and rat genes, single nucleotide polymorphisms that are useful for prioritizing candidate genes in a QTL region can sometimes be found by searching a database. The real challenge is, therefore, not so much in finding the genomic sequence variants but in pinpointing a particular sequence variant unequivocally as being the QTL. Although there is no single definition for positively identifying a gene underlying a QTL, the Mouse Complex Trait Consortium suggested that a candidate gene should meet more than one of these eight criteria: 1) its polymorphisms in either coding or regulatory regions have been found, 2) its function has been linked to the quantitative trait being analyzed, 3) its function has been tested in vitro, 4) its function has been tested in transgenic animals, 5) its function has been tested in knock-in animals, 6) its function has been assessed in deficiency-complementation test, 7) its function has been tested by mutational analysis, and 8) a homologous QTL for the same phenotype in another species has been found. In addition to these, complementary techniques such as combining differential gene or protein expression patterns are often used to arrive at conclusions for the superior candidacy of any given QTL over the other positional candidate genes.

The recent reports discussed in this article indicate that considerable effort from different laboratories has resulted in the identification of several regions of the rat genome as potentially harboring causative genetic factors for experimental arthritis. The ability to develop induced models of arthritis is greater in rats than in mice, which has allowed for research in the genetic studies of rat models of arthritis to progress faster than in mice. However, the pace of progress was curtailed relative to that of the mouse until the genome sequence for the rat became available in 2001. Despite this setback, as exemplified by several studies since 2001, fine mapping and identification of arthritis loci in rats have progressed considerably and even more so than in mouse models. Interestingly, several of the arthritis regulatory loci in rats overlap RA susceptibility loci in humans (11). Improving the resolution of localization of each of these QTLs in rats is an important ongoing activity that is not only essential for advancing from arthritis QTL detection and confirmation to arthritis QTL identification but also helpful for comparative mapping of homologous RA regulatory loci in humans with potentially improved resolution. To conclude, “is it possible to identify arthritis-causative genes using the rat as an experimental model?” The answer is “yes,” as proved by Olofsson et al. (28). This proof of principle of applying mapping strategies to gene identification for experimental arthritis has set the timely precedence for the ongoing quest of identifying all the other arthritis QTLs in the rat.

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REFERENCES


