Brief Reviews

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Genetic and Molecular Basis of Quantitative Trait Loci of Arthritis in Rat: Genes and Polymorphisms[1]

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Rheumatoid arthritis (RA) is an autoimmune disease, the pathogenesis of which is affected by multiple genetic and environmental factors. To understand the genetic and molecular basis of RA, a large number of quantitative trait loci (QTL) that regulate experimental autoimmune arthritis have been identified using various rat models for RA. However, identifying the particular responsible genes within these QTL remains a major challenge. Using currently available genome data and gene annotation information, we systematically examined RA-associated genes and polymorphisms within and outside QTL over the whole rat genome. By the whole genome analysis of genes and polymorphisms, we found that there are significantly more RA-associated genes in QTL regions as contrasted with non-QTL regions. Further experimental studies are necessary to determine whether these known RA-associated genes or polymorphisms are genetic components causing the QTL effect. ***The Journal of Immunology,*** **2008, 181: 859–864.**

Rheumatoid arthritis (RA),[3] one of the most common autoimmune diseases, is characterized by chronic joint inflammation and variable degrees of bone and cartilage erosion. Like other autoimmune diseases, RA has a strong genetic component with a heritability of ~60% based on twin data (1–3). To elucidate the genetic and molecular basis of RA, tremendous efforts have been made in the mapping of quantitative trait loci (QTL) associated with the disease. Linkage analysis has identified 52 or more QTL that regulate experimental autoimmune arthritis in rat models for RA, including collagen-induced arthritis, pristane-induced arthritis, oil-induced arthritis, and adjuvant-induced arthritis (4). However, identifying the particular responsible genes within these QTL remains a major challenge. To our knowledge, only one gene, *Ncf1*, has been confirmed as a causative gene underlying the *Pia4* QTL (5). The availability of whole genome sequence and rapid advances in functional annotation of genes offer an opportunity to pinpoint the genetic factors within QTL that are essential for the development of autoimmune arthritis in rats.

Identification of QTL for experimental arthritis in various rat models

We found 52 experimental arthritis QTL identified by linkage studies in rat models by searching the PubMed database for every publication up to January 2008 using the key words "arthritis" and "QTL". Most data have been summarized by Joe (4). In accordance with accepted linkage criteria, a logarithm of the odds (LOD) score of >4.3 was considered significant, and a LOD score between 2.8 and 4.3 was considered suggestive for linkage (6). Therefore, in this review we only chose those QTL with a peak LOD score of 2.8 or above. For well-defined QTL, we defined the size of the QTL by the QTL region given by authors. For other QTL, the 2-LOD support interval, the chromosomal region in which the QTL is located with a confidence of ~95%, was used to establish the QTL region (7). The information of QTL is listed in Table I. For the QTL identified by multiple studies, we listed all of them in Table I as they usually have different flanking markers. We are aware that it is possible that we may not collect every relevant publication or QTL and apologize to authors whose work is not included.

Characteristics of genomic regions containing QTL as contrasted with non-QTL regions

The 52 arthritis QTL cover 1,486,846,486 bp of genomic sequence, which is roughly 54% of the total rat genome. Every autosome and the X chromosome (Chr), except Chr11, Chr13, and Chr17, contain at least one arthritis QTL. Within the 1,486,846,486-bp genomic sequences, a total of 16,222 genes have been located. The average gene density throughout the whole rat genome except ChrY is about one gene per 99,918 bp. Within the total of 1,486,846,486-bp genomic sequences representing arthritis QTL there is about one gene per 91,656 bp,

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[3] Abbreviations used in this paper: RA, rheumatoid arthritis; Chr, chromosome; LOD, logarithm of the odds; QTL, quantitative trait locus.

Table I. *QTL information for experimental arthritis in rats*[a]

QTL	LOD	Markers	Search Region (bp)	RAA Genes within QTL	RAAP Genes within QTL
Cia2 (8)	5	D1Arb15-D1Arb31	64,146,083–146,714,212	*Chrna7*, *Fcgrt*, *Ax1*, *Tgfb1*, *Gpi*, *Bax*, *Il4i1*, *Zfp36*, *Xrcc1*, *Gp49b*, *Il11*, *Il16*	*Zfp36*, *Xrcc1*
Cia2 (9)	4	D1Rat7-D1Rat35	15,999,443–124,749,065	*Chrna7*, *Fcgrt*, *Ax1*, *Tgfb1*, *Vip*, *Gpi*, *Bax*, *Ccr6*, *Ctgf*, *Il4i1*, *Pdcd5*, *Zfp36*, *Xrcc1*, *Gp49b*, *Il11*	*Zfp36*, *Xrcc1*
Ciaa6 (10)	3.3	D1Rat202-D1Mgh12	181,173,661–247,322,280	*Dkk1*, *Syvn1* (*Hrd1*), *Cd19*, *Fadd*, *Pten*, *Mb12*, *Htra1*, *Lip1*, *Il4ra*, *Il21r*	*Mb12*, *Il4ra*
Pia8 (11)	4.7	D1Mit15-D1Rat10	1,595,128–25,271,177	*Ctgf*	
Pia9 (12)	5.3	ΔD1Rat185 (D1Rat320)-ΔD1Mit13 (D1Rat193)	119,536,685–183,163,338	*Serpinh1* (*Ra-a47*), *Nox4*, *Il16*	
Cia7 (13)	4.6	Prlr-D2Mit23	59,681,382–146,659,672	*Tnfsf10* (*Trail*), *Crh*, *Il2*, *Ank*, *Il7*, *Il21*, *Tenr*, *Prlr*	*Crh*, *Il2*, *Il21*, *Tenr*
Cia7 (9)	3.2	ΔD2Mit24 (D2Mgh14)-D2Mit23	42866227–146659672	*Tnfsf10* (*Trail*), *Il6st* (*Gp130*), *Crh*, *C6*, *C7*, *Il2*, *Ank*, *Il7*, *Il21*, *Tenr*, *Prlr*	*Crh*, *Il2*, *Il21*, *Tenr*
*Cia10** (13)	3.4	D2Mit23-D2Mgh29	146659371–227310008	*Il12a* (*p35*), *Ptpn22*, *Tlr2*, *S100a9*, *S100a8*, *Gstm1*, *S100a4*, *Adam15*, *Gstm4*, *Sh2d2a*	*Ptpn22*, *Tlr2*, *Gstm1*, *Sh2d2a*
Cia11 (14)	5.6	D3Mit9-D3Mgh5	26674019–97524000	*Nr4a2* (*Nurr1*), *Cd44*, *Traf6*, *Itgav*, *Hoxd9*, *Lrp2*, *Mdk*	*Itgav*
Cia11 (10)	3.5	D3Mit12-D3Mgh6	48033364–50522717		
Aia2 (15)	5.8	D4Arb26-D4Arb30	36592629–73972591	*Irf5*, *Ptn*	*Irf5*
Aia3 (15)	3.9	D4Arb30-ΔD4Arb16 (D4Mit16)	73972454–130228614	*Aqp1*	
Cia13 (9)	10	D4Mit17-D4Mgh11	134858562–171204531	*Cd69*, *Cxcl12*, *Cls*, *Clr*, *Il17ra*, *Il17re*, *Il17rc*	
Cia13 (10)	10.8	D4Mit16-D4Rat112	130228505–182386736	*Cd69*, *Cxcl12*, *Cls*, *Clr*, *Il17ra*, *Il17re*, *Il17rc*	
Cia13 (14)	4.5	D4Mit12-D4Wox12	104,415,993–179,854,843	*Cd69*, *Cxcl12*, *Cls*, *Clr*, *Il17ra*, *Il17re*, *Il17rc*	
Cia3 (8)	4.8	D4Arb30-D4Arb4	73,972,454–154,937,227	*Cxcl12*, *Aqp1*, *Il17re*, *Il17rc*	
Cia3 (10)	5.2	D4Wox22-D4Mit16	78,042,682–130,228,614	*Aqp1*	
*Ciaa4** (9)	3	D4Mgh18-D4Mgh11	128,179,958–171,204,531	*Cd69*, *Cxcl12*, *Cls*, *Clr*, *Il17ra*, *Il17re*, *Il17rc*	
Oia2 (16, 17)		D4Mit24-ΔD4Mgh21 (D4Rat84)	78,039,508–185,398,530	*Cd69*, *Cxcl12*, *Cls*, *Clr*, *Aqp1*, *Il17ra*, *Il17re*, *Il17rc*	
Pia2 (18)	3.9	D4Mgh1-D4Rat22	17,617,957–60,974,511	*Irf5*, *Pon1*	*Irf5*
Pia5 (18)	4.5	D4Rat22-D4Mit16	60,974,347–130,228,614	*Aqp1*, *Ptn*	
Pia7 (11)	4.9	ΔD4Arb16 (D4Mit16)-D4Wox16	130,228,505–158,101,973	*Cxcl12*, *Il17ra*, *Il17re*, *Il17rc*	
Pia7 (19)	5.3	ΔD4Arb16 (D4Mit16)-D4Mgh11	130,228,505–171,204,531	*Cd69*, *Cxcl12*, *Cls*, *Clr*, *Il17ra*, *Il17re*, *Il17rc*	
*Ciaa5** (9)	3.5	D5Wox3-D5Mgh8	99,080,787–149,757,440		
Ciaa5 (10)	3	D5Mit14-ΔD5Wox17 (D5Wox10)	138,304,249–165,073,513	*Padi4*	*Padi4*
Apr2 (20)	3.7	ΔD5Wox17 (D5Wox15)-D5Mgh9	148,718,587–171,801,867	*Padi4*, *Tnfrsf14*	*Padi4*
Pia3 (18)	4.5	D6Mit1-D6Rat4	98,840,517–144,200,901	*Serpina1* (*Pi*), *Fos*	
Pia3 (11, 19)	3.8	D6Mgh10-D6Rat4	108,621,345–144,200,901	*Serpina1* (*Pi*), *Fos*	
Pia12 (19)	3.9	D6Mgh7-D6Wox5	47,039,213–117,362,003	*Fos*, *Prkch*, *Pik3cg*	*Prkch*
Cia20 (10)	2.9	ΔD6Rat16 (D6Rat19)-D6Wox5	81,682,368–117,362,003	*Fos*, *Prkch*	*Prkch*
Cia4 (8, 21)	5.3	D7Mgh22-D7Arb13	56,589,443–103,063,768	*Ifng*, *Myc*	*Ifng*
Cia8 (21)	5.1	D7Rat33-D7Mgh22	20,547,072–56,589,443		
Pia13 (19)	4.7	D7Mit9-D7Rat74	78,497,123–137,838,801	*Myc*, *Vdr*	*Vdr*
No name* (8)	3.2	D8Arb22-ΔD8Arb17 (D8Rat126)	103,815,825–124,310,006	*Myd88*	
Cia6 (9)	3.7	D8Mgh4-D8Rat71	86,627,870–127,907,265	*Cx3cr1*, *Myd88*	
Pia14 (19)	4	D8Rat21-D8Rat26	79,347,688–87,122,815		
Cia15 (9)	4.5	D9Rat44-D9Arb7	2,948,883–39,821,284	*Zap70*, *Vegfa* (*Vegf*), *Col9a1*	
Ciaa3 (9)	7.5	D9Rat44-D9Wox19	2,948,883–18,347,743	*Vegfa* (*Vegf*)	
Ciaa3 (14)	6.5	ΔD9Wox23 (0 bp)-D9Wox19	0–18,347,743	*Vegfa* (*Vegf*)	
Cia5 (8, 14)	4.9	D10Arb21-ΔD10Arb22 (distal end)	84,263,650–110,718,848	*Map3k14* (*Nik*), *Ace*, *Stat3*, *Socs3*, *Tbx21*	*Ace*
*Cia16** (9)	3.5	D10Rat95-D10Rat11	6,380,796–100,633,848	*Map3k14* (*Nik*), *Tnfsf13* (*April*), *Ace*, *Mefv*, *Ccl2*, *Stat3*, *Il4*, *Ccl3*, *Ccl5*, *Cxcl16*, *Il13*, *Tp53*, *I13*, *Tbs21*	*Ace*, *I14*, *I13*
Oia3 (16, 22)		ΔD10Mit13 (D10Rat92)-ΔD10Mgh1 (D10Rat135)	78,170,545–108,776,963	*Map3k14* (*Nik*), *Tnfsf13* (*April*), *Ace*, *Stat3*, *Socs3*, *Tbx21*	*Ace*
Pia10 (19)	3.1	ΔD10Rat26 (D10Rat27)-D10Rat44	24,731,831–77,091,892	*Tnfsf13* (*April*); *Ccl2*, *Il4*, *Ccl3*, *Ccl5*, *Cxcl16*, *Il13*, *Tp53*, *Il3*	*Il4*, *Il3*

(*Table continues*)

Table I. (*Continued*)

QTL	LOD	Markers	Search Region (bp)	RAA Genes within QTL	RAAP Genes within QTL
Cia12 (14)	4.6	ΔD12Wox5 (0bp)-D12Arb6	0–36,819,134	*Flt1* (*Vegfr1*), *Epo*, *Ncf1* (*P47-phox*)	*Ncf1* (*P47–phox*)
Cia12 (10)	8.3	D12Rat28-ΔD12Mgh6 (D12Rat19)	16,312,646–37,906,549	*Epo*, *Ncf1* (*P47-phox*)	*Ncf1* (*P47-phox*)
Cia25 (23)	4.7	D12Wox12-Nos1	20,932,556–39,869,383	*Ncf1* (*P47-phox*)	*Ncf1* (*P47-phox*)
Pia4 (18)	8.4	ΔD12Mit1 (D12Wox12)-D12Mgh5	20,932,556–29,130,304	*Ncf1* (*P47-phox*)	*Ncf1* (*P47-phox*)
Pia4 (12)	8.9	D12Rat72-D12Rat9	23,030,712–24,470,522	*Ncf1* (*P47-phox*)	*Ncf1* (*P47-phox*)
Pia4 (19)	53.1	D12Wox12-D12Mgh3	20,932,556–25,271,941	*Ncf1* (*P47-phox*)	*Ncf1* (*P47-phox*)
Apr1 (20)	6.1	ΔD12Rat17 (D12Mgh7)-D12Rat22	36,834,562–46,043,557	*Hspb8*	
*Piax** (12)	3.3	D14Rat32-D14Rat25	43,399,833–105,009,477	*Lif*, *Osm*, *Fgfr3*	
Pia6 (18)	4.9	D14Mgh3-D14Wox5	20,165,394–42,600,130	*Gc*	*Gc*
Ciaa7 (10)	3.2	D4Rat8-D14Wox12	12,400,909–20,178,280	*Gc*	*Gc*
No name* (15)	3	D15Mit6-D15Rat22	47,302,628–80,370,658	*Tnfsf11* (*Opg1*), *Htr2a*	*Htr2a*
Pia11 (12)	4.4	D16Rat64-D16Wox8	59,398,217–88,891,349	*Gas6*	
Cia17 (9)	4.3	D18Mit5-D18Rat82	32,458,819–73,666,556	*Dcc*, *Adrb2*, *Slc26a2*	*Adrb2*, *Slc26a2*
Cia26 (23)	3.6	ΔD18Rat60 (D18Rat13)-ΔD18Mit9 (D18Rat119)	66,720,050–80,564,142	*Dcc*	
Pia15 (19)	3.4	D18Mgh3-D18Mit6	69,013,304–82,920,522	*Dcc*	
*Cia14** (14)	3	D19Rat13-D19Mit8	24,725,553–45,692,656	*Il15*, *Nqo1*	
Aia1 (15)	17.9	D20Arb2-ΔD20Arb8 (D20Rat33)	2,811,409–14,052,767	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*, *Nfkbil1*	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*, *Nfkbil1*
Cia1 (8, 14, 15)	78.5	D20Arb2-ΔD20Arb8 (D20Rat33)	2,811,409–14,052,767	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*, *Nfkbil1*	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*, *Nfkbil1*
Cia1 (9)	9.4	D20Wox3-D20Rat60	3,660,646–14,605,852	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*
Pia1 (11)	3	D20Rat41-D20Rat33	4,740,610–14,052,767	*Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*	*Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*
Pia1 (19)	4.8	D20Wox3-D20Rat33	3,660,646–14,052,767	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*
Ciaa1 (9)	39.9	D20Wox3-D20Rat4	3,660,646–12,141,740	*Tnf*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*	*Tnf*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*
Ciaa1 (10)	5.8	ΔD20Mgh4 (0bp)-D20Rat15	0–20,264,413	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*, *Nfkbil1*	*Tnf*, *Mif*, *Tap2*, *Tap1*, *Hla-dma*, *Hla-dmb*, *Nfkbil1*
*Cia18** (9)	3.2	DXRat4-ΔDXWox3 (DXRat66)	23,494,725–31,245,802		
Cia19 (9)	4.5	DXMgh9-ΔDXWox3 (DXRat66)	31,245,802–88,514,169	*Ar*	*Ar*

[a] An asterisk (*) denotes a QTL with suggestive but not significant, linkage. Delta (Δ) denotes that the marker is not mapped to the assembly in the current Ensembl database; alternatively, we choose a marker near the target marker for Ensembl searching. The information on the distance between those two markers is from Rat Genome Database (rgd.mcw.edu/maps/); RAA gene: RA-associated gene; RAAP gene, gene containing RA-associated polymorphisms either in coding region or in regulatory region.

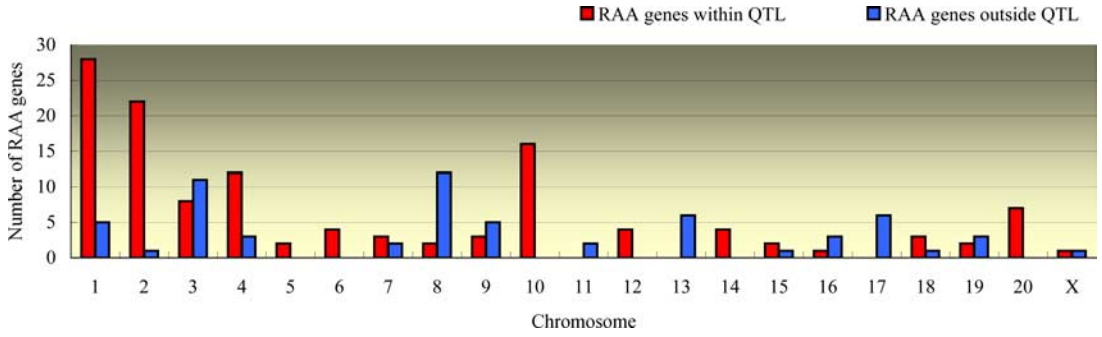

FIGURE 1. Distribution of RA-associated (RAA) genes between QTL and non-QTL regions on each chromosome.

whereas in the genome region outside of the arthritis QTL regions there is about one gene per 112,145 bp. Overall, the density of genes in QTL regions is higher than that of non-QTL regions; however, not every QTL has a high gene density. QTL located in gene-rich regions include *Cia2*, *Ciaa6*, *Ciaa5*, *Apr2*, *Cia5*, *Cia16*, *Oia3*, *Pia10*, *Cia12*, *Pia4*, *Cia14*, *Aia1*, *Cia1*, *Pia1*, and *Ciaa1*. There is a gene in every 52,795, bp in those genome regions. We also found some QTL located in gene-poor regions, including *Cia7*, *Cia11*, *Pia2*, *Pia7*, *Cia4*, *Cia8*, *Pia14*, *Cia26*, *Pia15*, and *Cia19*. In those regions, there is a gene in every 160,471 bp. These data suggest that there is no important difference between QTL regions and non-QTL regions relative to gene density.

Are genes associated with RA more likely to occur in QTL regions?

We conducted a whole genome scan to find the genes that regulate RA in rat genome. First, we obtained the genes for every chromosome and QTL from the Ensembl database (release 48) (www.ensembl.org/index.html), and then we searched PubMed (www.ensembl.org/index.html) and OMIM (Online Mendelian Inheritance in Man; www.ncbi.nlm.nih.gov/sites/entrez) to get a preliminary list of candidate genes associated with arthritis. The search terms were the combination of the symbol of the gene and arthritis. We performed the searching using PGMapper, software newly developed by us and available online at www.genediscovery.org/pgmapper/index.jsp (24). Then, we read the associated literature to determine the connection between those preliminary candidate genes and RA. A gene is considered to be a RA-associated gene if it was associated with RA in at least one of the following studies: 1) functional studies such as knockouts, transgenics, mutagenesis, RNA interference, etc.; 2) association studies; and 3) clinical studies. Through this method, we identified 185 RA-associated genes in the whole rat genome; among them, 124 are located in QTL regions. The catalogue of all RA-associated genes and the relevant literature indicating their candidacy can be found in supplemental table I.[4] In total, there are significantly more RA-associated genes in QTL regions than in non-QTL regions. To investigate whether this is also true at the chromosomal level, we examined the distribution of RA-associated genes for every chromosome. Fig. 1 shows the distribution of those genes between QTL and non-QTL regions for each chromosome. We found that the chromosomal distribution of RA-associated genes is complex. The QTL on Chr5, Chr6, Chr10, Chr12, Chr14, and Chr20 cover all RA-associated genes. There are more RA-associated genes in QTL regions than in non-QTL regions on six chromosomes, including Chr1, Chr2, Chr4, Chr7, Chr15, and Chr18. However, fewer RA-associated genes were found in QTL regions than non-QTL regions on Chr3, Chr8, Chr9, Chr16, and Chr19. There is no difference for ChrX. Surprisingly, no QTL have been identified on Chr11, Chr13, and Chr17, although obvious candidate genes exist. The information of genes on ChrY is still not available in the Ensembl database, so all QTL and genes on ChrY are excluded from our analysis. We also found that most RA-associated genes are located in QTL regions on Chr1, Chr2, Chr4, and Chr10. Similarly, there are more QTL identified on these chromosomes, suggesting that these chromosomes may play an important role in the regulation of arthritis. In addition, we also performed a two-tailed paired-sample t test using standard statistical software (SPSS) after excluding the chromosomes without arthritis QTL and the p value was 0.049, indicating that there is a statistically significant difference between the number of RA-associated genes within and outside QTL.

Polymorphisms of genes associated with RA in both QTL and non-QTL regions

By comparing the number of genes that contain one or more RA-associated polymorphisms either in coding regions or in regulatory regions between QTL regions and non-QTL regions, we found that most of those genes fall into QTL regions. Among 52 genes containing RA-associated polymorphisms, 35 are located in QTL regions. We also detected the frequency of these genes for every chromosome. Fig. 2 shows the distribution of these genes between QTL and non-QTL regions for each chromosome. The catalogue of all genes containing RA-associated polymorphisms and the references establishing their candidacy can be found in supplemental table I. There was a noticeable difference in the chromosomal distribution of these RA-associated polymorphisms, although the difference between the number of genes containing RA-associated polymorphisms within and outside arthritis QTL did not reach the statistically significant level ($p = 0.062$). The QTL regions cover all RA-associated polymorphisms on 11 chromosomes, including Chr2, Chr5, Chr6, Chr7, Chr10, Chr12, Chr14, Chr15, Chr18, Chr20, and ChrX. Chr1 has more RA-associated polymorphisms in QTL regions than in non-QTL regions. Excluding ChrY and Chr19, which has no known RA-associated polymorphisms, as well as those three chromosomes that have no known arthritis QTL, only five chromosomes, namely Chr3, Chr4, Chr8, Chr9, and Chr16, have fewer RA-associated polymorphisms in QTL regions than in non-QTL regions. Our

[4] The online version of this article contains supplemental material.

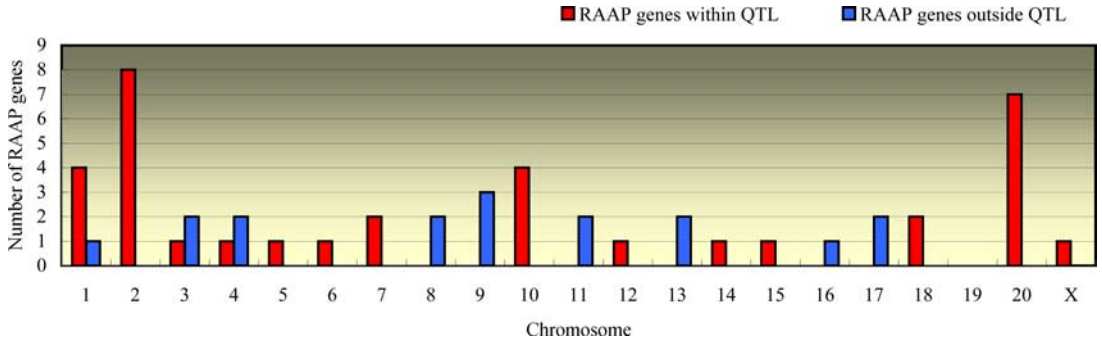

FIGURE 2. Distribution of genes containing RA-associated polymorphisms (RAAP), either in a coding region or a regulatory region, between QTL and non-QTL regions on each chromosome.

data may indicate that the genetic basis of QTL is based on polymorphisms.

Conclusions

The direct identification of causative genes underlying QTL has been slow and remains a major bottleneck in fully understanding the genetic contribution to rheumatoid arthritis. Using currently available genome data and gene annotation information, we systematically examined RA-associated genes and polymorphisms within and outside QTL over the whole rat genome. By the whole genome analysis of genes and polymorphisms, we found that there are significantly more RA-associated genes or polymorphisms in QTL regions as contrasted with non-QTL regions. However, a substantial number are not in QTL regions. Because some QTL have only a small effect on disease and because QTL identified by linkage analysis usually cover large regions of genomic sequence and include hundreds of genes, identification of specific genes remains difficult. One interesting question raised by our investigation is whether those known RA-associated genes or polymorphisms within QTL regions constitute the genetic basis of the QTL effect. Candidates must be carefully investigated in more directed experiments to establish their roles in the regulation of QTL. In general, we found multiple RA-associated genes or polymorphisms existing in a single QTL. Thus, another important question is whether those QTL are caused by a single, large-effect gene or by a series of genes, each of small effect. We should be able to answer this question with the advance of fine mapping technologies that can effectively refine the map location of identified QTL. Actually, some QTL have been dissected into subregions by fine mapping, such as *Cia5* (25) and *Oia3* (22) on chromosome 10, indicating that it is possible that some of those QTL are composed of several linked subloci.

Our analysis indicated that chromosomal distribution of RA-associated genes or polymorphisms is complex. Although for most of chromosomes there are more RA-associated genes or polymorphisms in QTL regions than in non-QTL regions, some chromosomes still have fewer RA-associated genes or polymorphisms in QTL regions than in non-QTL regions. There were even no QTL detected in Chr11, Chr13, and Chr17 despite the existence of obvious candidate genes. This may be because of the following. First, methods for QTL mapping cannot detect all QTL, especially some small-effect QTL, because of small sample size, small phenotypic variance, sparse marker coverage. Second, gene annotation is still in progress. The map location and/or function of many genes is currently unknown in the rat. For example, two well-known RA-associated genes in human, *FCRL3* (26) and *SLC22A4* (27), are still not mapped to the current Ensembl rat assembly and thus we cannot obtain the exact location of homologues of these two genes in the rat genome. Third, because direct effects on arthritis regulation may not yet be recognized for many genes, some arthritis-associated genes may be missing from the gene list collected by our investigation. There may be unknown RA-causative genes or polymorphisms in the QTL regions. Fourth, the association of some genes or polymorphisms with RA was detected in human or other species. This may not be true for the rat. The same gene or polymorphism may have different influence on the same phenotype in different ethnic groups or species. Some RA-associated genes or polymorphisms identified by human studies may have no role in the regulation of arthritis in the rat and cannot be a cause of rat QTL. Fifth, the putative contribution of some RA-associated genes or polymorphisms in the regulation of arthritis may not be true, as it cannot be replicated or confirmed by other independent studies. In addition, some genes may only have an indirect role in the pathogenesis of arthritis and cannot be considered as genetic factors underlying the QTL effect.

This investigation is the first attempt to explore the genetic and molecular basis of QTL of experimental autoimmune arthritis at a genome-wide scale. Our data indicated that the nature of QTL may be based on polymorphisms either in coding regions or in regulatory regions of genes. Those polymorphisms may cause the changes in expression and/or function of relevant genes that can account for the QTL effect. Moreover, some rare alleles and/or haplotypes appear to play important roles in the initiation and/or development of RA or other autoimmune disorders and may be important candidates underlying the QTL (28–33). In addition, some RA-associated genes identified from knockouts or transgenics may have important roles in the pathogenesis of RA but have only little opportunity to be the genetic factors causing the QTL effect. Those genes are not polymorphic in the population under investigation, so they may not be able to contribute to the phenotypic variance in quantitative traits.

Disclosures

The authors have no financial conflict of interest.

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