Genetics of Autoimmune Diseases: A Multistep Process

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Abstract

It has so far been difficult to identify genes behind polygenic autoimmune diseases such
as rheumatoid arthritis (RA), multiple sclerosis (MS), and type I diabetes (T1D). With
proper animal models, some of the complexity behind these diseases can be reduced.
The use of linkage analysis and positional cloning of genes in animal models for RA
resulted in the identification of one of the genes regulating severity of arthritis in rats and
mice, the *Ncf1* gene. The *Ncf1* gene encodes for the Ncf1 protein that is involved in
production of free oxygen radicals through the NADPH oxidase complex, which opens up
a new pathway for therapeutic treatment of inflammatory diseases. In most cases,
however, a QTL is the sum effect of several genes within and outside the QTL, which
make positional cloning difficult. Here we will discuss the possibilities and difficulties of
gene identification in animal models of autoimmune disorders.

Introduction

The major autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis,
(MS) and type I diabetes (T1D) as well as related inflammatory disorders such as asthma,
psoriasis, and atherosclerosis are complex, and it has been difficult to understand their
fundamental genetic and environmental causes. With proper animal models genetic and
environmental factors can be better controlled and also manipulated. This enables
genetic approaches that lead to an insight not possible to achieve with direct human

studies only. The possibility of combining the genetic knowledge with direct animal

studies will enhance the understanding of these diseases and shorten the time needed to

develop new therapies with higher efficiency and limited side-effects. We will discuss

these possibilities, mainly using examples from recent studies of models for RA.

Definition of Autoimmune Disease: Physiology Versus Pathology

Identification of genes controlling autoimmune diseases requires that we have a clear

definition of what an autoimmune disease is. Over the years, this definition has been

shifting and also varies depending on whether it is seen from a clinical point of view or

from a basic science point of view.

From a clinical point of view, these diseases are inflammatory diseases of unknown

origin, which cannot be explained by any obvious cause such as infection or allergen

exposure. To strengthen the classification they should be chronic, i.e., they should last for

a period that exceeds a normal acute inflammatory phase of usually 3– 4 weeks. The

occurrence of autoantibodies targeting some relevant tissue antigen is often, but not

always, included as an additional criteria . However, the pathogenic relevance of such

antibodies is not always clear. These definitions have led us to classify a number of

commonly occurring chronic diseases as classical autoimmune, for example MS, RA,

T1D, and systemic lupus erythematosus (SLE). A borderline group is made up of

inflammatory diseases with a possible but not proven infectious cause such as

spondyloarthropathies and inflammatory bowel diseases. Another borderline group

comprises diseases in which an inflammatory component has emerged as one of the

major driving factors, for example, psoriasis and atherosclerosis. It is obvious that from

this perspective autoimmune diseases compose a highly heterogeneous group, even

within each disease classification, as there are not yet means to link diagnosis with

disease mechanisms. Rather we are bound to description of the more obvious pathologic

end-result, such as the lack of insulin in T1D or the neurological deficit in MS.

From the basic science standpoint, autoimmune recognition is often the phenomenon of interest for definition of autoimmune diseases. It stems from a tradition of viewing autoimmunity as forbidden self or "horror autotoxicus," as Burnett described it. The view has been broadened over the years with inclusion of innate immune recognition, or more colorfully expressed as "danger," as a factor not only in combat of infections but also in playing a role in the development of autoimmunity. The immune recognition is seen as the essential key for understanding both regulatory/suppressor and helper/effector mechanisms. Autoimmunity is defined through autoimmune recognition by lymphocytes, an event that is physiologic but results in an autoimmune disease when such lymphocytes are pathogenic. Proof for autoimmunity can be tested in animal models but not in humans, which has led to the development of animal models for autoimmune diseases. The models are mainly of two types: one that is induced and one where the disease develops spontaneously. Of the induced models, many are induced through immunization with an autoantigen, and the pathogenicity of autoreactive lymphocytes can be clearly shown. In general, only the acute phase of the first inflammatory response is studied. Examples of induced models are collagen-induced arthritis (CIA) as a model for RA [1, 2] and experimental autoimmune encephalomyelitis (EAE) as a model for MS [3]. Other models develop "spontaneously" due to genetic aberrations. In the spontaneous models, as in humans, the pathogenicity of specific autoimmune recognition is often difficult to document and the disease course is often chronic. But in contrast to humans, these models are often monogenic, highly penetrant and less influenced by environmental factors. Examples of spontaneous models are the transgenic models for arthritis (TNFα [4], IL1Rα deficiency [5], anti-glucose-6-phosphoisomeras T cell receptor [6]), various spontaneous lupus models (NZB xNZW, MRL/lpr, BXSB) [7] and diabetes in the NOD mouse [8].

Clearly, discrepancies between results from studies of autoimmune diseases between

human and mouse are not only influenced by the species differences but are in most cases due to different disease definitions and methods. A better definition of the human diseases and also more appropriate, well-characterized animal models are therefore needed.

From Genes to Disease: The Basic Science Approach

The weakness of the basic science approach is the difficulties to clearly define the pathology of the phenomenon. In search of the pathologic effect of the mechanisms, numerous experiments has been carried out in animal models for human diseases where basically any disturbance of the physiology after knocking out a hypothesized gene is described as pathological. Clearly, in a complex system such as the immune system, the result will be pathologic in most cases, but the question remains, what is relevant for a naturally selected disease as occurring in humans? And how do we know that the postulated genes, proteins, mechanisms, or pathways are indeed relevant and essential for an autoimmune disease in humans? One way to find out is to first identify the genes involved in the disease development. Then, when we have defined the most critical genes and thereby the most critical pathways, the basic approaches to studying the molecular interacting pathways will be crucial for understanding the disease.

From Disease to Genes: The Monogenic Success and the Polygenic Failure

The cause of autoimmune diseases seems to be easily delivered by genetics. Why not just define the genes carried by diseased individuals and then determine their functional genetics? Is this not just a question of adding resources into the human genetic project and its prolongations in the form of various strategic research platforms? The human genome project has indeed been fruitful and we have had enormous success with hundreds of monogenic diseases. One example in the autoimmune field is the

identification of the *AIRE* (autoimmune regulator) gene causing the APECED syndrome [9, 10]. The APECED syndrome is a familiar disease characterized by a combination of fungal infections and autoimmune inflammation of various endocrine organs. It is a monogenic disease with high penetrance and caused by mutations in the *AIRE* gene. This discovery did not only identify the gene behind APECED but also led to a renewed understanding of autoimmunity as the *AIRE* gene plays an important role in the induction of central tolerance through its expression in the thymic epithelial cells [11]. However, polymorphism in AIRE could not explain the major autoimmune diseases that are more complex and polygenic in nature. Genetic linkage analysis of large familiar cohorts has been relatively unsuccessful. A few, relatively highly penetrant genes, such as *NOD2* [12] and *PTPN22* [13], have been replicated in several association studies, but these are likely to reflect only the tip of the iceberg. What we need is a more complete picture of the genetic architecture, with all the genes involved, high- as well as low-penetrant, and to know how they interact with each other and with the environment. However, even when the association with a gene or haplotype is known, it can still be difficult to find the actual function. One example of the difficulties is the MHC complex. Most autoimmune diseases have been known for decades to be associated with various strongly conserved MHC haplotypes. However, it has not been possible in any disease to clearly identify the gene(s) within the haplotypes, although there is circumstantial evidence for the importance of certain class II genes (DR4/DR1 with the shared epitope) in RA [14] and class I genes (B27) in spondyloarthropathies [15].

It is likely that more of the genetic secrets in humans will be revealed by adding resources, as done through the HapMap project and other efforts. However, the approach requires substantial resources and the question is whether we can make shortcuts and what complementary approaches we can use. The use of improved animal models for human diseases would be a major advantage in several aspects. The environmental factors could be controlled and we have inbred strains simplifying the genetics and the

linkage analysis, strains that can be genetically manipulated and thereby allow us to test our hypotheses (Fig. 1).

The Use of Animal Models in a Disease-to-Gene Approach

Experience from studies on animal models of autoimmune diseases show that they are much more efficient and appropriate for linkage analysis than human cohorts. The process of positional cloning involves five generic steps that intend to lead from phenotype to gene discovery (described in Fig. 2). In general, an F2 intercross of 100–400 animals will reveal loci explaining 10%– 50% of a given disease trait and also allows for studying some of the genetic interactions involved. Thus, the power is much higher compared to human studies. What we have learned from animal models for autoimmune diseases so far is that there are a multitude of genes of variable penetrance controlling a disease, and in addition, there are complex genetic interactions and also interactions with the environment. It is important to remember that, depending on the phenotype, the number of loci, their relative effects, and how they interact with each other and the environment varies considerably. Most likely, the complexity revealed in the genetic studies of animal models will also apply for humans. Therefore, the definition of both genetic and environmental interactions are critical for finding the underlying genes causing and controlling autoimmune diseases, a task that is also difficult in animal models, here illustrated by two examples: the positional cloning of the *Pia4* gene *Ncf1* and the complex structure and interactions of the *Eae3* and *Eae2* loci.

Position Cloning of *Ncf1*, a Genetic Polymorphism Explaining a Major Quantitative Trait Locus Controlling Chronic Inflammation

Pristane-induced arthritis (PIA) in rats is a model mimicking RA. Arthritis is induced by a subcutaneous injection of 100– 200 µl of the adjuvant oil pristane at the base of the tail and signs of arthritis appear after about 14 days. The subsequent development of arthritis is chronic and relapsing in the susceptible DA strain, with an erosive and symmetric

destruction of peripheral joints and the disease fulfils the classical RA criteria [16, 17]. PIA is T cell-dependent and there is no evidence for an influence of B cells or arthritogenic antibodies in contrast to CIA, the primarily used arthritis model in mice, in which arthritogenic antibodies to CII play a significant role [18].

To identify the genes contributing to the disease, the arthritis-susceptible DA rat was crossed with the arthritis-resistant E3 rat [19, 20]. In a number of both intercrosses and backcrosses involving more than 1,000 rats, the major quantitative trait loci (QTLs) could be identified (Fig. 3). These loci could clearly be separated to control specific phases of the disease course, i.e., onset, severity, and chronicity (Fig. 4). One of the QTLs, *Pia4*, located on chromosome 12, was found to be associated with severity of PIA. By insertion of the genetic fragment of interest from the resistant strain (in this case E3) into the genome of the susceptible DA strain through conventional backcross breeding (more than ten generations), we created a congenic strain. At this stage, the congenic fragment was 20 cM and we found that the congenic rat developed much milder arthritis than the parental DA rat. In fact, the difference was significant using fewer than ten rats in each group, which is a criterion for going further to clone the underlying gene/s. In other words, the prerequisite was that we had transformed the trait to be more Mendelian. On this basis, we started a process of collecting and testing small cohorts of animals having recombinations within this fragment in order to establish subcongenic strains [21]. Each new subcongenic strain was screened for the presence of the disease-associated genes by testing them for PIA susceptibility. With this method, the fragment could be reduced to contain only two genes, *Ncf1* and *Gtf2i*. No differential expression of either Ncf1 or Gtf2i could be detected and only *Ncf1* (alias *p47phox*) had polymorphism alterations in the coding sequence leading to changes in amino acid sequence in the translated protein, suggesting that Ncf1 was essential for arthritis susceptibility. One of the alterations was at position 106 ATG/GTG and resulted in a Met/Val alteration, the other one was at position 153 ATG/ACG and resulted in a Met/Thr alteration. Brown Norway (BN) rats, which are

resistant to arthritis, share all genotypes but one with DA, the alteration in position 153, suggesting that this alteration is important for the Ncf1 function. Consequently, this approach allowed us to identify a causative gene, which could now be subjected for further functional analysis and studies of this new pathogenic pathway associated with arthritis severity.

The Ncf1 Protein and the NADPH Complex

How could the *Ncf1* gene be involved in the development of arthritis? To our advantage, a huge effort in investigation and characterization had already been made regarding the *Ncf1* gene and the encoded Ncf1 protein. The fact that a lot of information was already available simplified the process of finding phenotypes associated with the genotype. The Ncf1 protein is part of the NADPH oxidase complex expressed in phagocytic cells. The complex is composed of five subunits, of which two, Cyba (alias gp91phox) and Cybb (alias alias p22 phox), resides in the membrane where they form a heterodimeric flavohemoprotein—cytochrome b_{558}. The other three subunits—Ncf1, Ncf2 (alias p67phox) and Ncf4 (alias p40phox)—are in the resting state forming a complex in the cytosol, but when activated by any of a wide variety of stimuli, the Ncf1 protein gets heavily phosphorylated and the complex migrates to the membrane to form the activated complex [22]. The Ncf1 protein is the subunit responsible for transporting the cytosolic complex to the endosomal membrane or the cell membrane during activation [23]. The NADPH oxidase complex functions as an electron donor in the oxidative burst process activated in response to pathogenic invaders, ultimately generating reactive oxygen species (ROS) [24]. To investigate the functional consequences of the identified *Ncf1* polymorphism, we tested the ability of rat peritoneal cells to produce ROS. The surprising results showed that the arthritis-susceptible DA rat had a *lower* burst than the resistant DA. Ncf^{E3} congenic rat [21]. One allele containing the functional *Ncf1* was enough to correct for the deficiency in oxidative burst and protect from disease since the congenic

heterozygous for the Ncf^{E3} *allele* had a milder disease than the homozygous DA rat.

These results contradict the general belief that oxidative burst promotes inflammation and

suggest that the oxidative burst is involved in a previously unknown pathway controlling

inflammation. In fact, transfer of spleen-derived T cells from the congenic DA. Ncf^{E3} rat to

the parental DA and vice versa showed that the *Ncf1* polymorphism operated before

activation of the T cells, indicating that it plays a role in the control of autoimmune

activation.

Pathway Analysis

Natural selection does not operate on single genes but rather on molecular pathways.

Thus, the discovery of a genetic polymorphism, like in *Ncf1*, regulating a disease trait,

opens up the possibility for the discovery of a new pathway leading to arthritis. The

pathways are likely to be shared between different species; however, the exact nucleotide

variant in the rat *Ncf1* gene is probably specific. Thus, for further studies it can be

assumed that the pathway will operate similarly in rats, mice, and humans.

To investigate this possibility we looked to the mouse. In the mouse there is an arthritis

QTL identified that contains the *Ncf1* gene. However, we found that *Ncf1* was not

polymorphic and we did not find an effect on the oxidative burst using a congenic strain

with a fragment derived from C3H on a B10.Q background (P. Olofsson et al., unpublished

data). Another mouse that was made deficient for *Ncf1* was not possible to use for our

purpose, as the mutation results in a complete destruction of the gene. Moreover, the

Ncf1-targeted mutation also contains a genetically linked 129-derived fragment [25],

known to contain other polymorphic genes of importance for arthritis [26]. However, a

worldwide search through available mouse strains identified a variant of a C57B1/6 strain

with mutations in both the leptin receptor and the *Ncf1* gene [27]. The point mutation in

the *Ncf1* gene is located at position – 2 in exon 8 and results in an aberrant splicing of

the *Ncf1* mRNA transcripts. One of the transcripts resulting from this mutation give rise to

low levels of a protein that is truncated, lacking eight amino acids in the second SH3 domain that are important for interaction with the Ncf2 protein, thus resulting in absence of detectable NADPH oxidase activity. Consequently, we now had another mutation that presumably should affect the same pathway as was discovered in the rat. The C57Bl/6 mouse with the mutation in the *Ncf1* gene was therefore backcrossed to the arthritis-susceptible B10.Q strain. The mice were tested for susceptibility to CIA, where arthritis is induced by an intradermal injection of rat collagen type II (CII) emulsified in complete Freund's adjuvant. The B10.Q mice homozygous for the mutation in *Ncf1* developed severe arthritis with chronic development, i.e., similar to the low oxidative burst responder DA rat strain [28]. Mice heterozygous for the mutation were found to develop a less severe arthritis than *Ncf1*-mutated mice resembling the situation in rats where one functional *Ncf1* allele is enough to correct both oxidative burst and disease severity. Interestingly, both the delayed type hypersensitivity (DTH) and antibody response to CII were higher in the *Ncf1* mutated mice, indicating an increased activity of CII reactive T cells. We also found that some female *Ncf1* mutated mice in the breeding colony spontaneously developed arthritis. In an experiment, 3 out of 11 female mice developed severe arthritis a few days after partus, confirming earlier findings that there is a high risk for arthritis susceptibility during the postpartum period [29]. The arthritis was severe and chronic, as in CIA, and the mice with spontaneous arthritis also had a higher level of antibodies against CII with the same fine-specificity as in CIA [28].

The overall data clearly suggest another role of NADPH oxidase-derived ROS than just elimination of pathogens, adding a complexity to the redox status of the cell. ROS response has been reported not only in phagocytes but also in antigen-presenting cells (APCs), such as dendritic cells (DCs) [30]. Also, T cells have been reported to express Ncf1 during antigen presentation and activation [31], adding further complexity to the process of finding the mechanisms behind the phenotype.

The immune regulatory effect of ROS could be involved in one or more of the different

stages of the interactions of the immune system. There are a number of pathways that are proposed to be affected by free radicals and state of oxidation. On the antigen-presenting level, it has been shown that by inhibiting ROS production, the proliferation of T cells in response to antigen presentation of DCs is prevented [30], suggesting that the burst response in an APC could modulate the antigen presentation capacity of that cell. The level of radicals could also directly act on T cells. ROS could possibly be transferred over the synapse from APC to T cell and thereby alter the oxidation state of the recipient cell. It has been suggested that the level of oxidation on membranes of cells, influenced by interacting cells, are important for function [32]. Extracellular ROS has also been shown to act as an immune regulator through its interaction with the T cell proliferation regulator NO [33]. The oxidation could also possibly act by affecting other important structures on the cell surface and thereby alter their status. It has been shown that oxidation on T cell receptor signaling pathways, such as the linker for activation of T cells (LAT) molecule, modulates the function of T cells [34]. It is also possible that ROS by itself acts as a modulator of the system. It is known that ROS in low concentrations serves as a second messenger in the initiation and amplification of signaling at the antigen receptor in lymphocyte activation (reviewed in [32]). Another possibility is that ROS is involved in feedback mechanisms. Direct cell– cell contact between an activated T cell and human PMN has been shown to induce intracellular ROS production in the PMN, which then could act as an intracellular messenger [35]. Further studies are needed in order to clarify which effect is of importance for the development of arthritis.

Therapeutic Possibilities Can Be Immediately Explored

Taken together these data show that it is possible to identify the underlying genes of a QTL. It also shows that the importance of this finding is the identification of new pathogenic pathways and that these pathways are conserved between species. The NADPH oxidase complex proved to be an important regulator of the immune system and

the pathway appears to involve autoreactive T cells, but it is not yet fully understood. This new pathway for modulating inflammatory diseases is, however, expected to open up new possibilities for generating drugs to target chronic inflammatory autoimmune diseases such as arthritis. Most importantly, using the animal model platform including the congenic strains, methods are then already prepared for testing proof of concepts of the different therapeutic possibilities. This will contribute to shorten the time from the target identification to validation.

Analyses of a QTL Containing Interacting Genes

Several factors affected the successes of the positional cloning of *Ncf1*. First of all, it is a gene with a large effect size, accounting for about 20% of the total phenotypic variation [19]. Moreover, the mutation has a dominant effect on a well-characterized phenotype and also, the rest of the genome in the DA background allows the functional expression of the allele. However, in many cases a QTL effect is the sum effect of several positionally linked genes and their interactions with other genes both within and outside the QTL. This certainly makes positional cloning more difficult.

One such example is two of the first QTLs reported to control an animal model of an autoimmune disease: the *Eae2* and *Eae3* loci associated with experimental autoimmune encephalomyelitis [36]. *Eae2* and *Eae3* are located on chromosomes 3 and 15, respectively, and these loci have an interacting effect on the disease [36]. We made congenic strains with these fragments from the resistant RIIIS/J strain on the susceptible B10.RIII background. The effect on disease in these mice was mild, far from being Mendelian and therefore not suitable for positional cloning of the underlying genes. Clearly both the environmental and genetic conditions needed to be better defined in order to increase the effect of the RIIIS/J alleles [37, 38]. Firstly we found that both loci also controlled collagen-induced arthritis. Moreover, studying environmental factors affecting the arthritis phenotype we found that inducing the disease without tubercle

antigens in the adjuvant increased the penetrance of the congenic effect in both

chromosome 15 and chromosome 3 congenics. We also utilized the observation that

these loci interacted and developed a strategy to define the genetic interactions. The

congenics were intercrossed for more than eight generations, resulting in a partial

advanced intercross cohort of roughly 1,000 mice. This accumulated both the

recombination density and statistical power to dissect the loci into several subloci and to

identify the interactions between them. Within the previous QTL on chromosome 3

(*Eae3*), we were able to identify three separate loci (*Cia5*, *Cia21*, and *Cia22*) and within

the previous QTL on chromosome 15 (*Eae2*) we found four loci (*Cia26*, *Cia30*, *Cia31*, and

Cia32). Most of these loci interacted with each other (Fig. 5) and RIIIS/J alleles at the

different loci could be either disease-promoting or disease-decreasing. For example; *Cia5*

affected the onset and early severity of arthritis in additive interaction with *Cia30* on

chromosome 15, whereas the *Cia21* and *Cia22* affected severity during the chronic phase

of the disease in epistatic interaction with *Cia31* on chromosome 15. The definition of the

environmental conditions and the genetic interactions was a prerequisite to dissect the

Eae2 and *Eae3* QTLs. Human studies will have the same problem, but they are more

readily seen and analyzed in animal model studies. In the case of the *Eae2/Eae3* project,

the interactions between the QTLs could be detected in the F2 intercross with no more

than approximately 100 animals. However, the breakdown of the two QTLs into seven

and detecting the specific interactions among them was only possible by collecting a

larger number of recombinations in approximately 1,000 mice. What we learned was that

by analyzing the genetic interactions in the F2 intercross we could get information on how

to design congenic strains with the optimal effect on the phenotype. The knowledge of the

genetic interactions is then not only useful in the positional cloning process but also once

the gene is identified and studied in humans.

The interactions within a QTL are another case and they pose both new problems and

possibilities. The problems are the difficulties in splitting up a tightly linked haplotype. This

is an apparent problem in human studies, for example in addressing conserved haplotypes within the MHC region. In animal models, haplotypes can be intentionally broken up through the search of recombinations. Such recombinations will often lead to new and changed effects on the phenotype, possibly caused by a balancing interaction between closely positioned genes. It will be a challenging task to investigate in detail such clusters but it is also important to remember that the minimal congenic fragment, containing such conserved haplotypes, are likely to have been selected for a balanced control of a specific pathway. Such congenic strains will therefore be of value for studies of pathophysiologic pathways and thereby for understanding the fundamental laws determining the possibility of developing drugs with efficiency and with limited side-effects.

The possibility of genetically modifying mouse strains through transgene and embryonic stem cell-based technologies is a major advantage in the further dissection of the critical genes and haplotypes. Studies on the role of MHC genes are an excellent example of this. The establishment of H-2 (i.e., mouse MHC) congenic strains was the basis for the identification of the MHC regions and such strains are still useful for studies of the role of MHC. It has, however, been difficult to split this region in order to isolate each gene and thereby understand its function. Instead, transgenic experiments have been conducted with which it has been possible to insert both specific murine class II genes [39, 40] and their human counterparts [41– 44], and thereby prove the role of these genes for the development of autoimmune disease such as RA and MS. These studies also show the risk in transgenic technology as overexpression of class II genes may also lead to an artifactual toxicity in B cells [45, 46], which is the most likely explanation for a suppressive effect by transgenic class II genes, as has been reported in diabetes in the NOD mouse.

Conclusions

It is essential to backtrack the work of nature, and disease-causing factors need to be

searched for in the balance between a naturally but historically selected genome and newly introduced environmental factors. Due to the complexity of the major diseases, it has been difficult to follow an approach to make a direct search for the genetic and environmental factors causing autoimmune diseases. We have been arguing for the usefulness of proper animal models, with which it is possible to make a shortcut for the identifications of the major genetically selected pathways leading to autoimmune disease. Once identified the strength of the well-developed tools for experimental studies can be used. In combination this will shorten the time and efforts to develop new types of treatment addressing the disease-causing mechanisms.

Acknowledgements

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Figure legends

Fig. 1 The advantages of using animal models for genetic studies

Fig. 2 The five generic steps in positional cloning of quantitative trait genes. The first step is to map QTLs to chromosomal segments, usually in an F2 intercross or N2 backcross. This also involves the selection of inbred strains different for the phenotype studied. The number of F2 or N2 progeny genotyped and phenotyped determines the size of the linked regions and also sets the detection limits for how weak QTLs that can be found. The second step is to genetically isolate single QTLs and to measure the phenotype effect of each. This is usually done in a congenic strain. If the effect is reproduced in the congenic strain, then the third step is to reduce the size of the critical region as much as possible.

Before molecular studies can be undertaken for gene identification, the linked region must

be reduced to a few Mb, preferably less. The fourth step is then to identify and evaluate

candidate genes with different methods, all dependent on the identity of the genes. The

fifth and final step is to establish proof of identity of the candidate genes with, for

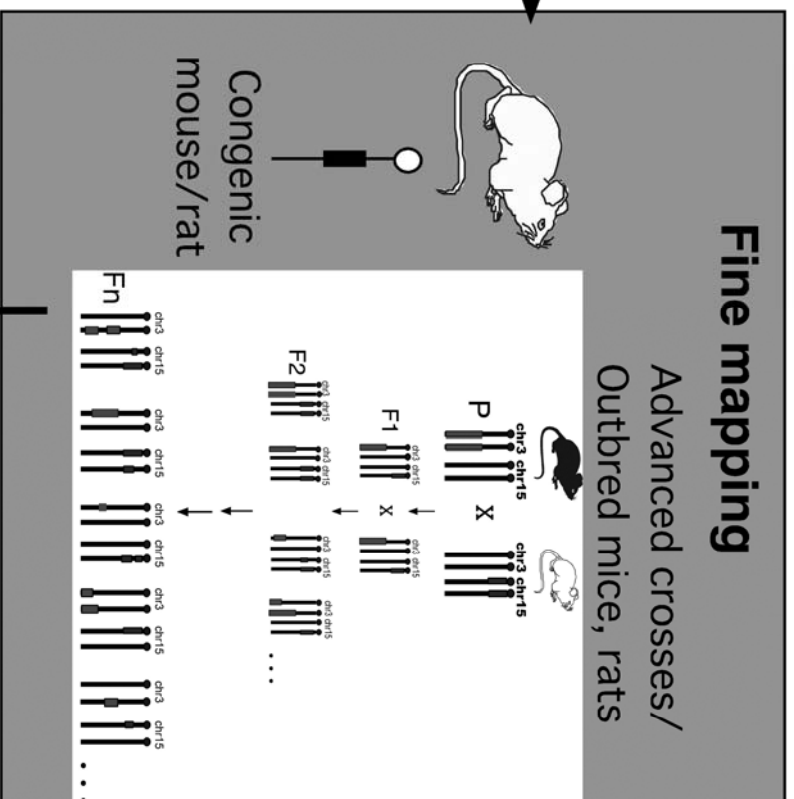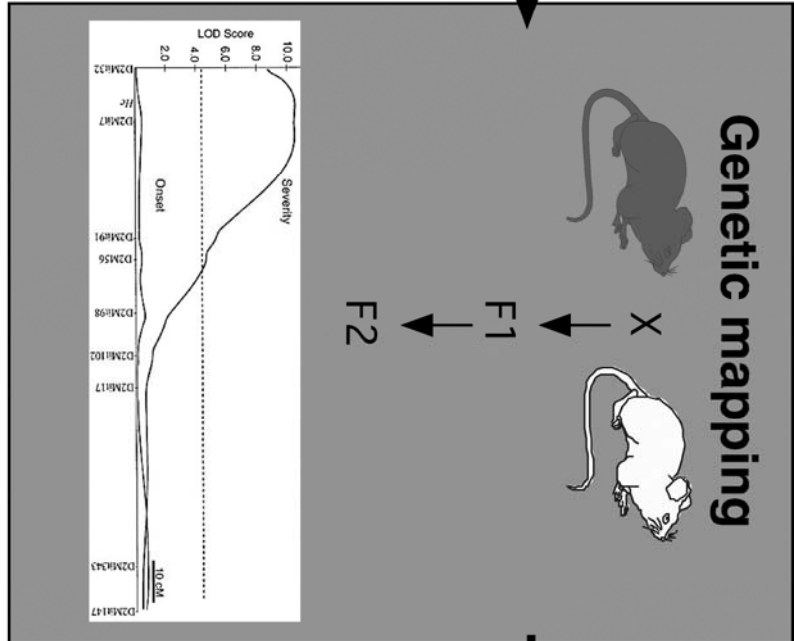example, gene targeting, transgenic mice, or other functional tests

Fig. 3 Known regions in the rat genome linked to PIA

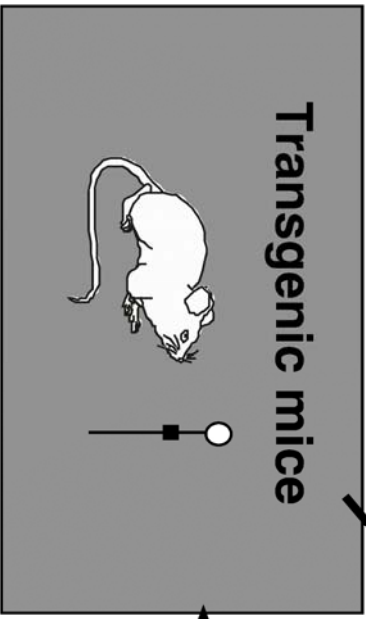**Fig. 4** Different loci control separate phases of PIA

Fig. 5 a A schematic view of the genetic interactions between the *Eae2* and *Eae3* loci

affecting arthritis. **b** The sum of arthritis score in female mice depending on their

genotypes at *Cia21* and *Cia32*

The advantages of using animal models are mainly;

- The animals can be genetically controlled. Laboratory mouse and rat strains are inbred which dramatically facilitate genetic studies.

- The environment can be better controlled for animals than for humans. The influence of environment on disease phenotype can be studied i.e. immunization, infection or stress.

- Manipulative experiments can be made. The genome of inbred strains can be changed by mutations, insertions and deletions.

- It is more ethical to use animals instead of humans for experimental research purpose.

Phenotype

Comparative analysis of human disease

Transgenic mice

"The Gene"

Genetic mapping

X → F1 → F2

LOD Score

Fine mapping

Congenic mouse/rat
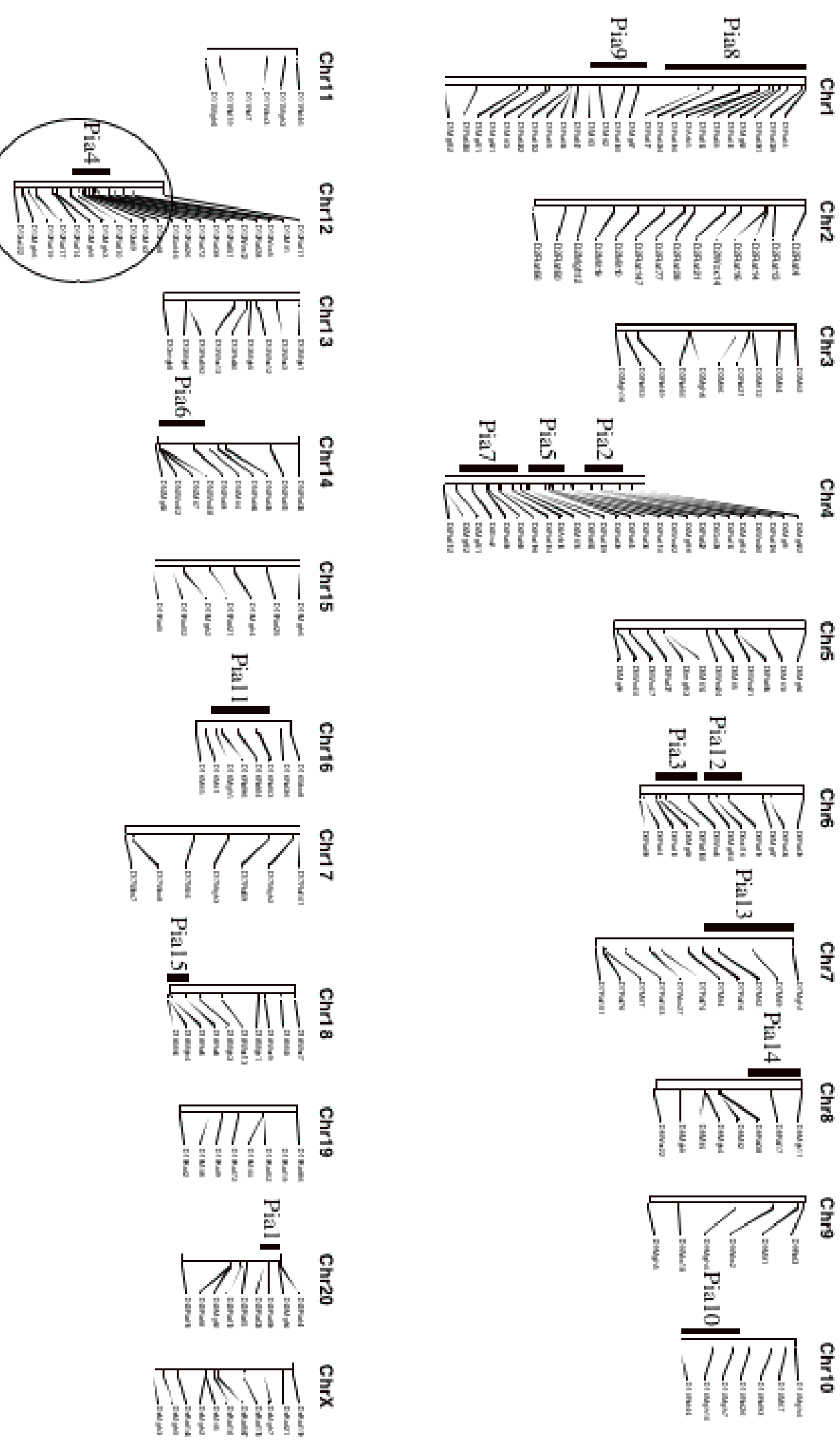
Advanced crosses/ Outbred mice, rats

Candidate gene evaluation

Polymorphisms?
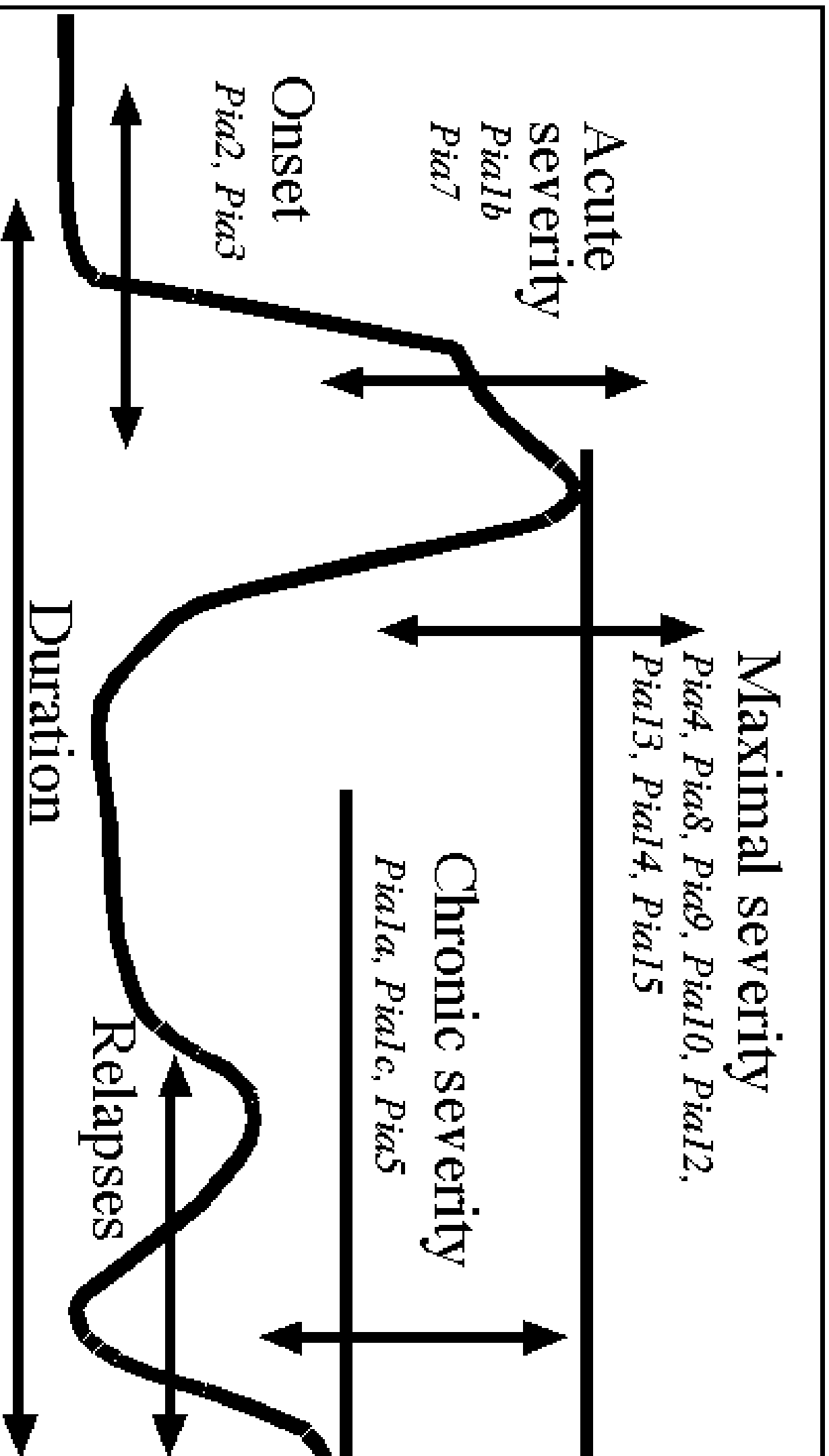aat gcg tta cag gtt
aat gcg ttc cag gtt

Function?

T cell → T cell

Expression?

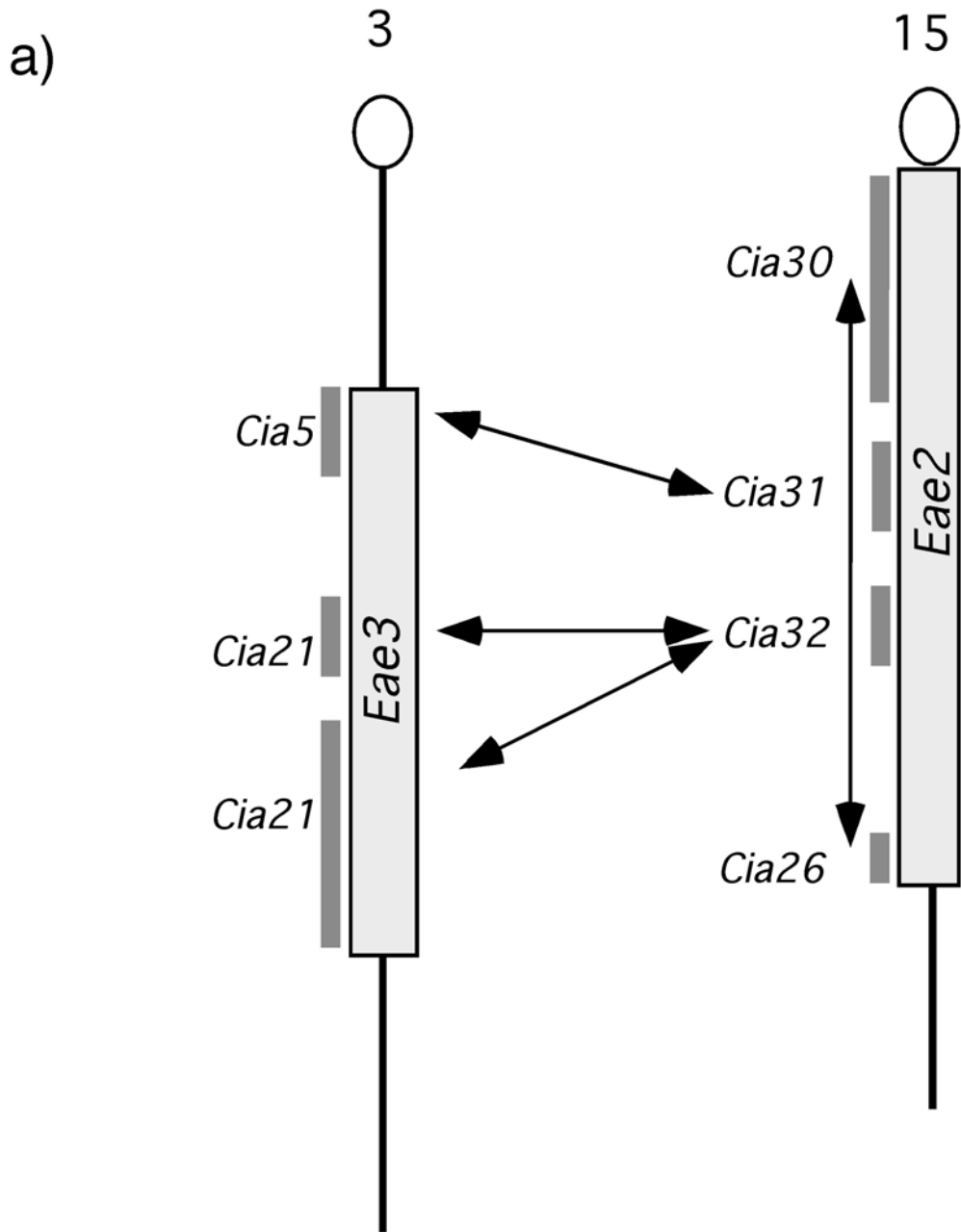# Identified quantitative trait loci for PIA

QTLs controlling specific subtraits of arthritis in E3xDA rat crosses

Duration

Onset
Pia2, Pia3

Acute
severity
Pia1b
Pia7

Relapses

Chronic severity
Pia1a, Pia1c, Pia5

Maximal severity
Pia4, Pia8, Pia9, Pia10, Pia12,
Pia13, Pia14, Pia15

a)

3   15

Cia30

Cia5

Cia31

Eae2

Cia21   Cia32

Eae3

Cia21

Cia26

b)

Arhtritis severity

	Cia21		
	b	h	a
b	82	64	37
Cia32 h	229	92	49
a	74	58	56