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Full Length Research Paper

Comparative genome analysis of trypanotolerance QTL

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Certain breeds of domestic ruminants show remarkable resistance to the effects of African trypanosomosis. Unlike susceptible animals, trypanotolerant animals control parasitemia and do not show severe anaemia or production loss. Identification of trypanotolerance genes in cattle is hampered by cost and breeding time. Marked differences between inbred strains of mice in their response to *T. congolense* infection can be exploited in the analysis of the genetic basis of the infection. Murine trypanotolerance QTLs have been identified on chromosome 17, 5 and 1, and designated as *Tir*1, 2 and 3, respectively. *Tir*1 and 2 have been fine mapped to a confidence interval of 1 cM. In order to find the mouse homologous region on the bovine genome, nucleotide sequence across 95% CI of *Tir*2 and 3 were used in the selection of candidate genes. Homologous sequences were used in the definition of synteny relationships and subsequent identification of the shared disease response genes. The homologous genes within the human genome were then identified and aligned to the bovine radiation hybrid map in order to identify the mouse/bovine homologous regions. This revealed homology between murine and bovine QTL on *Tir*3 while the region on *Tir*2 is linked to innate immune response.

Key words: Trypanosomosis, quantitative trait loci, homology.

INTRODUCTION

The N'Dama cattle and West African shorthorn breeds are resistant to trypanosomosis (Trypanotolerance) whereas the Zebu and European cattle breeds are susceptible. Under *Trypanosoma congolense* challenge, these trypanotolerant animals are better able to control parasite proliferation, mount an efficient immune response and maintain body weight (Hanotte et al., 2003). A single inherited genetic controlling factor for this innate resistance has yet to be found since resistance appeared to be inherited as a polygenic trait. (Hanotte et al., 2003; Tabel et al., 2000). Both cattle and mice display an innate resistance to the development of trypanosomosis. Trypanotolerance appears to be related to the control of parasitemia, a capacity associated with an event that regulates parasite growth and determines how rapidly the immune response is triggered.

Determination of the genetic basis of trypanotolerance in livestock is not only expensive but also limited by time due to the long generation time especially in cattle. In mice, C57BL/6J strain is relatively resistant to this disease while BALB/c and A/J are susceptible (Morrison et al., 1978). Murine trypanotolerance QTLs have been identified on chromosome 17, 5 and 1, and designated *Tir*1, 2 and 3 respectively. The confidence intervals of these QTL have now been refined, with the resultant 1 cM *Tir*1 and *Tir*2 intervals being sufficient for positional cloning or positional candidate gene analysis (Iraqi et al., 2001). Positional candidate gene analysis within refined QTL regions has been important in dissection of complex traits in livestock (Paterson, 1995).

Following the initial trypanotolerance QTL mapping in cattle and fine mapping in mice, comparative analysis and identification of candidate genes was undertaken in this work in order to identify a common disease response pathway. Though trypanotolerance in cattle and mice may not be homologous in phenotypic expression, the mouse model may be useful to identify candidate trypanotolerance genes, which would be useful in comparative mapping of livestock trypanotolerance genes.

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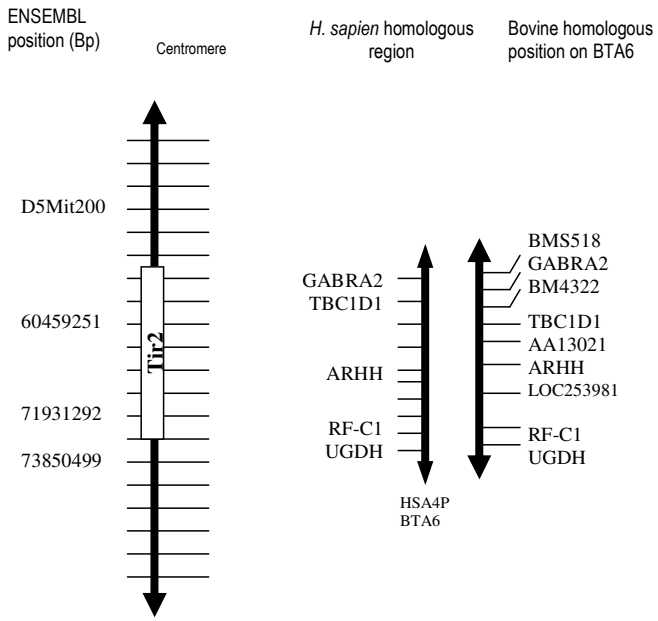

Figure 1. Human and mouse homologous map with candidate genes within *Tir2* and their location relative to the bovine genome shown. *Tir2* maps to HAS 4P and BTA6 with all the genes in the same order.

Comparative mapping takes advantage of the existence of the rich marker density maps of the human and murine genomes to determine regions of homology on their genomes. The chromosomal synteny between homologous genes of different species such as found in human, bovine and murine genome (Moore et al., 1991) facilitate application of QTLanalysis in comparative mapping. This work therefore endeavoured to identify the bovine homologous regions to *Tir*2 and 3 and hence select candidate trypanotolerance genes common to both hosts. Once the genes have been identified their markers would possibly be used in livestock breeding improvement programs. Alternatively this can be used to develop genetic markers for maker assisted introgression (MAI) breeding programs.

MATERIALS AND METHODS

*Tir*2 and 3 were fine mapped using F_{12} advanced intercross lines (AIL) developed from an F_2 cross between C57BL/6J and A/J mouse strains. The F_{12} AIL population used had the *Tir*1 locus fixed for the A/J strain. Relative positions of *Tir*3 and *Tir*2 in mice were estimated from mouse genome data base at (http://www.informatics.jax.org/), mouse sequence data base at ENSEMBL (http://www.ensembl.org/Mus_musculus/) and F_{12} mapping population. The F_{12} linkage distances in centimorgan were then converted into megabase pairs (Mbp) and aligned on the human genome at ENSEMBL. Comparative mapping of the mouse and bovine trypanotolerance QTL previously identified a region of homology between *Tir*1 and two cattle QTL on BTA16 (Hanotte et al., 2003). Each of the *Tir*2 and 3 QTL were therefore aligned on the sequenced mouse genome. Consequently the representative regions were physically assembled to the human genome and the resultant cattle region derived from the already published human/cattle radiation hybrid map. To construct mouse cattle comparative map (Figure 1), markers on the cattle radiation hybrid map (Everts-van der Wind et al., 2004) were also aligned on the 13 Mb mouse *Tir*2 and 28 Mb on *Tir*3 sequences.

Mouse genes within each of the intervals were subsequently submitted to multiple BLAST search on the human genome (Altschul et al., 1990). The corresponding regions of the human genome were likewise submitted through multiple BLAST to the cattle non redundant (nr), expressed sequence tags (EST), high throughput genome sequences (HTGS) and genome survey sequences (GSS) databases (www.genome.org). Candidate genes were selected only if they were documented as being homologous to mouse and human genes or located on cattle QTL (Hanotte et al., 2003) and/or if mapped with high confidence on the bovine radiation hybrid map. The selected mouse/human comparative regions were then assembled and their cattle homologues aligned (Figure 1). Sequenced genes were listed and possible candidate genes selected for future investigations.

RESULTS

A key strategy for the identification of genes predisposing livestock to trypanosomosis could use comparative mapping of mouse trypanotolerance genes on the bovine genome. This is then preceded by positional candidate gene analysis of the bovine locus with an aim of screening the QTL region for known genes. A comparison of murine *Tir*1 and 2 sequences to the human Gen-Bank/EMBL databases and the bovine radiation hybrid map revealed that sequences within the region were homologous. Candidate gene analysis was

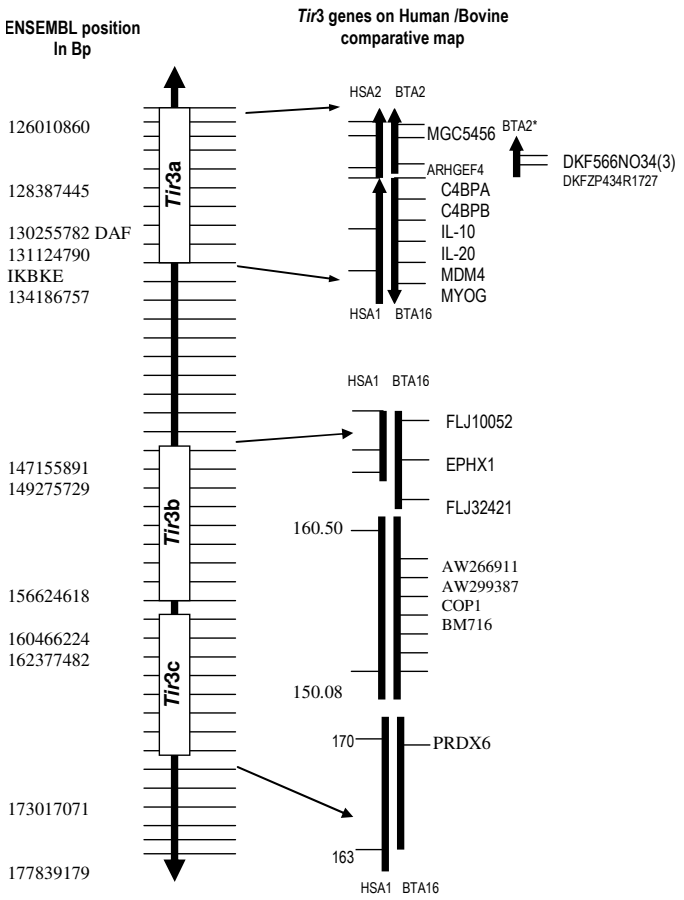

Figure 2. Human and mouse homologous maps with candidate genes within *Tir*3 and their location relative to the bovine genome shown. *Tir*3 maps to BTA1 and 16.

more precise on the mapped *Tir*2 95% CI than the *Tir*3 as the latter was still mapped to large confidence intervals (Figure 1).

*Tir*2 region spans 13391248 base pairs around 39 cM on mouse chromosome 5. The locus was comparatively mapped to chromosome 4 on the human genome (HSA4P) and chromosome 6 on the bovine genome (BTA6) (Figure 1). Cattle BTA4 region on the cR_{5000} bovine radiation hybrid map (Everts-van der Wind et al., 2004) showed homology to the mouse chromosome 5, 60459251 - 73850499 base pair region and human chromosomes 1 (HSA1) position 386204444 to 38653660. Plausible candidate genes on the loci include TLR genes which can now be treated as candidate genes for further analysis. A comparison of cattle, human and murine TLR 1 and 6 sequences available on the GenBank/ EMBL databases revealed that their sequences are homologous. Though the region is well mapped on the mouse genome, it was not well mapped on the cattle genome. This was established from the highly conserved homology between mouse/human and human/bovine regions.

DISCUSSION

Gene densities on the mouse and cattle regions were high. About 5 genes common to both mouse *Tir*2 and the homologous bovine regions were confirmed (Figure 1). The genes shown on Figure 1 correspond to exons with exact matches to known genes in mouse, human and cattle. Mouse genes sequences with no homology to cattle genomic sequence were not included. Most of the named mouse genes were present on the cattle homologous region in the same order along the chromosome. The interpretation of the mouse-cattle conserved synteny map was limited since the cattle sequences were derived from the cattle comparative radiation hybrid map described by Everts-van der Wind (2004) and the cattle sequence database at ENSEMBL (www.ensembl.org). The latter was yet to be annotated at the time of writing this report. The region of strict conserved synteny on *Tir*2 and 3 extended over at least 12 Mb which is the size of the mouse–cattle QTL.

*Tir*2 region encompass genes that regulate expression of cytokines such as TNF. Among the genes are TLR1 and 6 while *Tir*1 encompasses the TNF locus. In mice, the TNF-α gene has been shown to contribute to typanotolerance (Iraqi and Teale, 1998; Iraqi and Teale, 1997; Iraqi et al., 2001). TLR 1 and 6 as possible candidate genes on *Tir*2 were mapped to 60459251 - 73850499bp on the mouse genome while in human the homologous region was found on chromosome 4P14 (HSA4P14) which maps to chromosome 16 on the sequenced bovine genome. Disease responses in both mice and cattle may therefore depend on cytokine profiles triggered by these TLR genes.

*Tir*3 region (Figure 2) shares homology with part of the cattle trypanotolerance QTL. Homologous sequences can therefore be used to build synteny relationships and subsequently identify the shared disease response pathways common to this species. Since TLR genes were selected from *Tir*2 QTL for future analysis, the criterion for the selection of possible trypanotolerance candidate genes within *Tir*3 QTL was not only based on their involvement in various immune responses but also their linkage to the TLR pathway. Candidate genes within these loci include TLR5, interleukin 24, Interleukin-20, Interleukin 19, Interleukin-10 precursor (IL-10), MAPK-activated protein kinase 2 and inhibitor of nuclear factor kappa-B kinase (Ikk). IL-10 has been implicated in the pathogenesis of trypanosomosis (Baetselier et al., 2001). These results provide essential mapping information for the identification of candidate trypanotolerance genes in mice and are useful for the identification of the homologous genes in livestock. The genes and genetic markers can be used to enhance the genetic improvement of breeding stock through marker-assisted selection. This will in turn lead to the identification of the homologous genes in other livestock. Such information is necessary for the design of sustainable management of trypanoso-

mosis in livestock.

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