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DNA Typing of Swine Class II Major Histocompatibility Complex Polymorphisms

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Abstract

Tissue transplant experiments have revealed a cluster of genes responsible for graft rejection, the major histocompatibility complex (MHC). The MHC is a region dense in immune genes with a role in antigen presentation. Porcine MHC genes are highly polymorphic, varying amongst breeds. Unlike other species, such as mouse, cattle, and human, serological approaches to derive MHC haplotypes have been unsuccessful. To identify porcine haplotypes it is essential to utilize DNA typing approaches. In humans and swine (who have 85% homology in MHC class II), exon 2 of MHC class II genes encodes the first domain of the beta chain and this domain is in direct contact with the processed peptide and/or the T cell receptor during antigen presentation. Specific polymorphisms in these class II genes leads to an increase in susceptibility to diseases such as pneumoconiosis, autoimmune diseases, and allo/xeno graft rejection. In an effort to understand polymorphisms and tissue expression of MHC class II genes (DQB and DRB) DNA from divergent breeds of unrelated pig was analyzed. Primers targeting exon 2 of DQB and DRB were shown to be locus specific, and amplified all alleles. Expression of exon 2 PCR amplicons was verified through sequence analysis revealing over 35 different alleles among the 12 breeds with an average of 7 animals per breed represented. Preliminary data reveals breed specific polymorphisms within both genes. A subsequent analysis was done to analyze expression in various tissue samples. These tissue samples were analyzed through RT PCR to quantitate RNA expression in each.

Introduction

In the past decade much emphasis has been placed on finding an alternative donor source for tissue and organ grafting, besides that of humans. The possibility of the use of pigs as a donor appeared to be an ideal candidate due to similar anatomy and physiology. In previous tissue transplant experiments a cluster of genes appeared to be responsible for graft rejection, known as the major histocompatibility complex (MHC). In pigs the MHC gene is known as the swine leukocyte antigen (SLA), and is located on chromosome 7. This gene family is divided into two main classes based on protein structure, class I and class II. The genomic organization of SLA is in the order class II then class I divided by the centromere. This layout is similar to that of the human leukocyte antigen (HLA) and bovine leukocyte antigen (BLA). (Chardon 1999) Both of these classes are responsible for antigen peptide presentation to CD8+ and CD4+ T cells respectively.

Previous studies have demonstrated MHC class II genes to exhibit a great degree of polymorphisms especially represented in exon 2 of DRB and DQB. Many believe that some of these polymorphisms may actually trigger a stronger immune response, due to their location on the molecule. Many of these allelic polymorphisms in class II appear to influence disease resistance and other immune responses. (Hashimoto 1999) Some studies have actually demonstrated that DR and DQ of class II are essential in transplantation tolerance which is induced by activation of regulatory T cells. T cells of the immune system are able to recognize allo- and xenogeneic antigens by either direct or indirect pathways. It has been reported that both pathways could be used in the recognition of swine MHC antigens by human T cells, but most likely the indirect pathway is mainly used. These two pathways can be distinguished by the use of antigen presenting cells (APCs). In the direct pathway leukocytes of the donor often work as stimulators of T cells and as APCs. On the other hand for the indirect pathway only APCs from the recipient are functioned to respond. (Wimmers 2004)

With the ongoing interest in the field of transplant research, much work has focused on exon 2 of the class II genes because this particular section of the gene encodes the first domain of the beta-chain. The beta domain is in direct contact with the processed peptide and the T-cell receptor during antigen presentation. Due to this arrangement most of the class II beta-chains have been shown to have extensive polymorphisms. The discovery of these polymorphisms has led to the need for an accurate way to determine the haplotype, or the set of alleles which characterize an individual's entire MHC locus, of a particular donor patient by DNA typing. (The need for an accurate depiction of haplotype is crucial in the pre-screening process for transplant patients.) Finally with the ability to identify the location of particular polymorphisms, certain MHC molecular transformations may be identified that lead to T-cell activation and graft rejection over others.

Objectives

- To identify the presence of SNPs represented in MHC class II genes
- To identify the location of these SNPs on the MHC molecule
- To determine expression level of MHC class II genes in various tissues

Materials and Methods

A. PCR based sequencing of MHC class II genes

- Exon 2 of both, DQB and DRB were amplified and sequenced.
- Twelve different breeds of pigs were analyzed, with an average of seven animals per breed.
- sequences were aligned to evaluate the presence of single nucleotide repeats (SNPs)

B. SNPs placement on the MHC molcule

- Sequences were aligned and compared to that of the human
- Amino acids were compared and SNPs resulting in an amino acid change were highlighted as non synomymous
- The non synonymous SNPs were mapped onto the crystallized structure of MHC to determine their location and possible functional changes.

C. RT PCR

- Primers were designed to span from exon 3 4for both DQB and DRB.
- cDNA from 10 different tissue samples was isolated
- Sybr green RT PCR reactions were performed for all combinations of tissue samples and primers

References

Chardon P and Renard C. (1999) The Major histocompatibility complex in swine. Immunological Reviews 167.

Hashimoto K et al. (1999) Conservation of MHC class I and its related molecules in vertebrates. Immunological Reviews 167:81-100.

Wimmers K, Schellander K. et al. (2004) BF, HP, DQB, DRB are associated with hemolytic complement activity, acute phase protein reaction and antibody response in the pig. Immunology and Immunopathology 99:215-228.

Results

A. PCR based sequencing of DQB and DRB

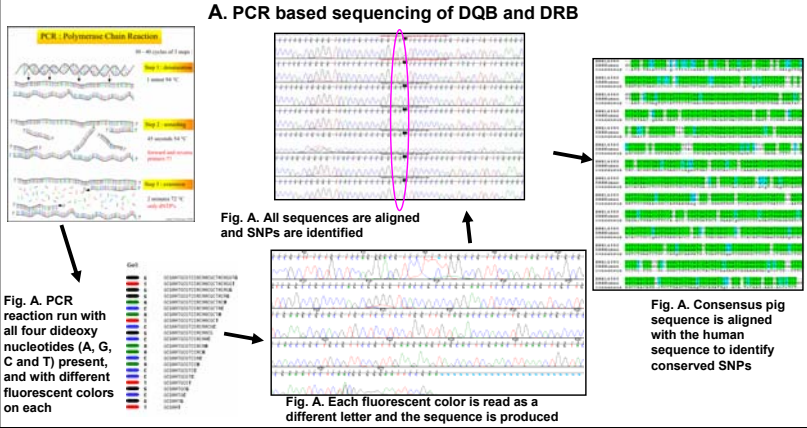

Fig. A. PCR reaction run with all four dideoxy nucleotides (A, G, C and T) present, and with different fluorescent colors on each

Fig. A. All sequences are aligned and SNPs are identified

Fig. A. Each fluorescent color is read as a different letter and the sequence is produced

Fig. A. Consensus pig sequence is aligned with the human sequence to identify conserved SNPs

B. SNP placement on DQB

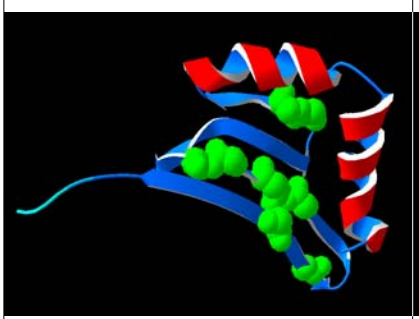

Fig. B. Crystal structure of DQB molecule with polymorphisms mapped in green.

B. SNP placement on DRB

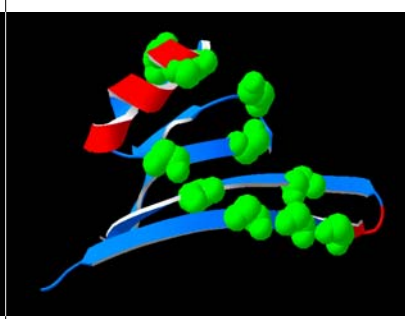

Fig. B. Crystal structure of DRB molecule with polymorphisms mapped in green.

C. RT-PCR DRB

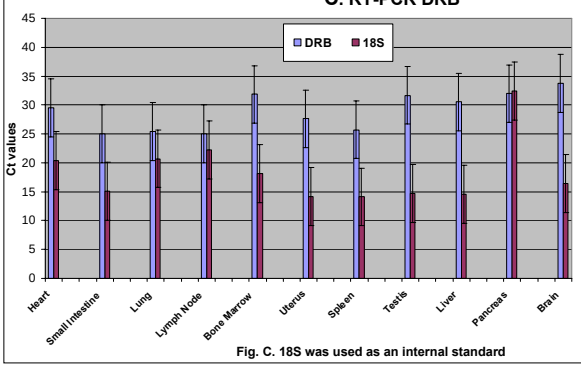

Fig. C. 18S was used as an internal standard

Fig. C. Fold changes of the target gene relative to brain tissue

Tissue	DRB
Heart	286
Small Intestine	161
Lung	6364
Lymph Node	23085
Bone Marrow	12
Uterus	15
Spleen	52
Testis	1.3
Liver	2.5
Pancreas	216691
Brain	1

*RT-PCR calculations were done using a ΔΔCt method

Conclusions

A. Expression of exon 2 PCR amplicons was verified through sequence analysis revealing over 35 different alleles among the 12 breeds
   - Data appears to reveal breed specific polymorphisms within both genes analyzed.

B. Analysis of the location of the SNPs reveals that they are located within the alpha helixes which are primarily responsible for antigen binding.
   - Further analysis may reveal functional changes in antigenic binding due to these polymorphisms.

C. When all of the tissue samples are normalized to brain tissue, it appears that lung, lymph node and pancreas have at least a 6364 fold increase.

Acknowledgements

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