# CHARACTERIZATION OF THE EQUINE ANGIOTENSIN-CONVERTING ENZYME (ACE) GENE

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## INTRODUCTION

The genetic basis underlying most complex traits such as disease, conformation, reproductive ability and soundness are largely unknown in the horse. In racehorses athletic performance is of particular interest. Although genetic variation in athletic performance (racing ability) has been described, (Tolley *et al.,* 1985 ; Williamson 1998) the identity of the genes contributing to this variation is largely unknown.

Angiotensin converting enzyme (*ACE*) plays an important role in the renin-angiotensin system that is primarily responsible for blood pressure homeostasis. The enzyme is specifically involved in increasing blood pressure during conditions of physiological demand. The discovery of an intronic insertion/deletion (I/D) polymorphism in the gene encoding for this enzyme has prompted many association studies. These studies have indicated a relationship between the polymorphism and differences in susceptibility to a number of conditions such as hypertension, cardiac disease, myocardial infarction, left ventricular hypertrophy and renal disease (Schunkert 1997). Furthermore, three groups have also reported on an association of the insertion allele with elite endurance performance in human athletes. Athletes, including Olympic rowers (Gayagay *et al*., 1998), mountaineers (Montgomery *et al*., 1998), cyclists and long distance runners (Alvarez *et al*., 2000) were all selected due to the superior oxygen metabolism required for their sport. The insertion allele was seen in much higher frequency in these athletes than in the normal population. Further studies have since shown an additional association of the insertion allele with an improved anabolic response to physical training (Montgomery *et al*., 1999 ; Williams *et al*., 2000).

The insertion, an *alu* repeat, occurs in intron 16 of the angiotensin converting enzyme gene. Since *alu* repeats are repetitive DNA sequences and the polymorphism is within an intron, it is unlikely that the insertion/deletion polymorphism is the causative mutation in the human *ACE* gene. It is for this reason that it is thought to be a marker for as yet unidentified mutations that cause the differences observed. From human association and physiological studies the equine homologue of *ACE* was therefore identified as a candidate gene for contributing to genetic variation in racing performance. The purpose of this study is to characterize the equine angiotensin-converting enzyme gene and perform an association study to investigate links between this gene and equine athletic performance.

## MATERIAL AND METHODS

**Sequencing the equine angiotensin converting enzyme gene**. Alignment of cDNA sequences of several species (Accession Nos: J04144, AF201332, L40175 and X62551) obtained through Genbank (Benson *et al.,* 2000) gave conserved regions from which primers to amplify the equine *ACE* gene could be designed. Most primers were placed so the polymerase chain reaction (PCR) product spanned introns to increase the likelihood of polymorphism discovery. Specific PCR product of the correct size was sequenced on an automated sequencer (LiCor, Lincoln, USA). Homology of the obtained sequence to the human *ACE* gene was confirmed by BLAST search (Altschul *et al*., 1997).

**BAC Clone**. The INRA BAC library of approximately 40 000 clones has been created for the use of participants in the International Equine Gene Mapping Workshop. This library has an average insert size of 110 kb (varying from 80 to 160 kb) and 1.5x coverage of the equine genome (Godard *et al*., 1998). Since publication, the capacity of the library has now been increased to around 100 000 clones (F. Piumi, pers comm). Following the generation of equine ACE sequence, horse specific primers were designed. The equine specific product was used to identify a BAC clone containing the gene. DNA from the BAC clone was extracted from 100mL of culture using a QIAGEN Plasmid Midi Kit. This DNA provided the most reliable template for PCR to cover intronic regions, and was also utilized for PCR amplifications of genomic DNA that failed to amplify.

**Mapping**. The horse specific primers were used to map the equine *ACE* gene in co-operation with the International Equine Gene Mapping Workshop. The primers were used on a somatic cell hybrid panel (Shiue *et al*., 1999), a radiation hybrid panel (Chowdhary *et al.*, in press) and (as discussed above) to identify a BAC clone (Godard *et al*., 1998). In addition to facilitating sequencing, the BAC clone was also used to physically map the gene by fluorescence *in situ* hybridization.

## RESULTS AND DISCUSSION

Fifteen of the twenty-six exons and eleven introns of the gene have been sequenced from genomic DNA or an equine BAC clone as illustrated in Figure 1. No polymorphisms have been found at this stage.

Utilizing the equine specific primers, the *ACE* gene has been physically mapped to horse chromosome 11 (ECA11) on the UC Davis somatic cell hybrid panel (L Millon, pers comm), and on a radiation hybrid panel (Chowdhary *et al.,* in press). Fluorescence *in situ* hybridization (FISH) was used to map the BAC clone to ECA 11p13 (D. Milenkovic, submitted). The human *ACE* gene is assigned to human chromosome (HSA) 17q23 (Mattei *et al*., 1989). This assignment was not unexpected, as comparative mapping has shown these two regions to be homologous (Raudsepp *et al*., 1996).

**Polymorphism Detection**. Two pools of horse DNA are currently being created for screening for polymorphisms. These will consist of equal amounts of DNA from 10 unrelated thoroughbred horses, and 10 horses of mixed breeds. The mixed pool will consist of two clydesdales, two standardbreds, two quarterhorses, two warmbloods and two ponies.

**Association Study**. Polymorphisms discovered will be used in an association study. Phenotypic data will be collected in collaboration with the Sydney University Equine Performance Laboratory. The laboratory has facilities for the measurement of physiological parameters of racing performance, including the use of a treadmill to measure traits such as $VO_{2max}$. Other performance data such as racing records will also be included in the analysis.

The pools of DNA will be sequenced to detect polymorphisms for characterization across different breeds of horse. Pools were selected for sequencing as a more cost effective alternative to screening large numbers of animals (Blackhall *et al.,* 2000). An added advantage to this method is that it will only detect the more common polymorphisms (ie those with a frequency greater than 20%). Although this will reduce the number of rare mutations detected this is acceptable since it is only the more frequent variants that will be of use for association studies. The thoroughbred pool was chosen to investigate differences within the most common racing breed in Australia. The breeds chosen to create the mixed pool were selected to maximize the likelihood of single nucleotide polymorphism discovery.

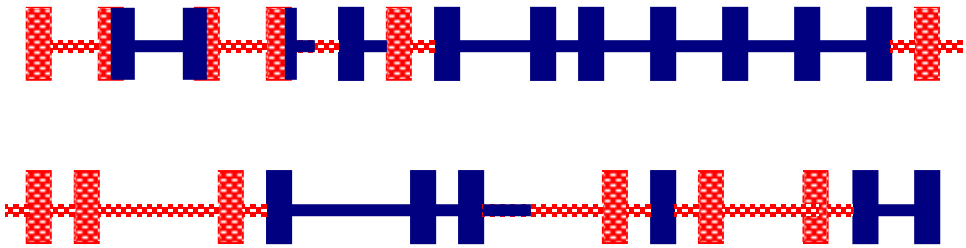

**Figure 1. Schematic representation of progress in sequencing of the equine angiotensin-converting enzyme gene overlaid on the human exon structure**

The vertical bars represent the 26 exons, and the horizontal bars the introns. Those in solid colour have been sequenced.

Future work will include more in depth analysis of associations with performance, with the aim of identifying DNA markers for athletic ability in race horses.

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