CHARACTERIZATION OF A HUMAN PERFORMANCE GENE IN THE HORSE

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SUMMARY

Although estimates of heritability of performance in the Thoroughbred racehorse have been calculated to be between 10-40%, no genes influencing performance have been identified. Based on comparative studies with human, we identified the angiotensin-converting enzyme (ACE) gene as an equine candidate gene for performance. From characterization of nine SNPs, nine equine ACE haplotypes were identified in 34 horses. The haplotypes occurred at differing frequencies in breeds of different athletic capabilities, including endurance Arabians, Thoroughbred racehorses and Draught horses. One haplotype had a similar effect on equine ACE levels as the recognised *Alu* insertion/deletion polymorphism in the human gene. Further studies are suggested to test the association of this haplotype with a range of indices of performance in substantially larger cohorts of horses before these markers can be used for prediction of racing performance.

INTRODUCTION

The Thoroughbred racehorse has been selectively bred for its performance for hundreds of years. While it has been shown that aspects of racing performance including race times, earnings, handicaps and performance rates are up to 40% heritable (Langlois 1980; Tolley *et al*. 1983), the underlying characterization of genes contributing to this significant genetic variation is completely absent.

Angiotensin converting enzyme (ACE) is essential for the control of blood pressure. The human ACE gene contains an insertion polymorphism within intron 16, which accounts for nearly half of the variation in circulating enzyme levels (Rigat *et al.* 1990; Tiret *et al*. 1992). Associations have also been observed between the polymorphism and response to training, and elite endurance and/or sprint performance in human athletes (Gayagay *et al.* 1998; Montgomery *et al*. 1998; Myerson *et al.* 1999; Alvarez *et al*. 2000).

We present here a preliminary characterization of the equine ACE gene, whilst also demonstrating an association of equine ACE haplotypes with circulating enzyme levels.

MATERIALS AND METHODS

Sequencing the Equine ACE gene. Since the equine ACE gene sequence was not available, primers were selected based on the aligned cDNA sequences of the human (J04144), rabbit (X62551), rat (AF201332) and chicken (L40175) ACE genes. Primers were used to amplify regions of BAC clone 801F9 (supplied by Dr Francois Piumi, INRA) which was shown to contain the gene. Direct BAC sequencing was used to obtain the 5' and 3' UTRs, while exon/intron boundaries were identified by sequencing equine cDNA and alignment with human cDNA structure.

Screening for polymorphisms. Common polymorphisms were detected by sequencing three pools of DNA from 10 Thoroughbred racehorses (TB pool), 14 endurance Arabian horses (AR pool), and 10 horses of mixed breeds (MB pool). Polymorphisms were identified by comparing the chromatograms of pooled sequence with that of a single animal, and confirmed by genotyping of animals within the pool. Common SNPs (MAF > 5%) were also typed across a panel of 40 horses: 10 each of TB, AR, Standardbred (SB) and Draught (Heavy) horses (HH). RFLP or partial sequencing (when no relevant restriction enzymes were available) was used to genotype individuals.

Association Study. Racehorses (n = 203) from the UK for which circulating ACE levels were available (Coomer *et al.* 2003) were genotyped for ACE haplotypes. Initially, only those horses with ACE levels falling further than 1 SD from the mean were typed. The remaining horses were then genotyped for the SNPs comprising one haplotype that was significantly associated with enzyme level.

Statistical analysis. Haplotypes were generated from the SNP data derived from the multi-breed panel with the program PHASE version 2.0.2 (Stephens *et al*. 2001). χ^2 tests were used to assess the association between haplotypes and ACE level, while one-way ANOVA was used to determine the size of variation accounted for by haplotypes.

RESULTS and DISCUSSION

Equine ACE gene sequence, polymorphisms and haplotypes. The genomic sequence of the equine ACE gene was derived with the exception of the central region of three large introns (18, 20 and 23). The gene structure was as seen in other mammalian species and consisted of 26 exons. The sequence is 86% conserved between the horse and human. Over 10 kb of sequence, covering 73% of the cDNA, was screened for base changes. Sixteen sequence changes were identified with 11 SNPs in non-coding sequence, three silent SNPs, and one non-synonymous SNP (exon 26 G3872A). One SNP within intron 20 was triallelic. A poly-A stretch of variable length was identified in intron 14, associated with an equine repetitive element-2 (ERE-2), but proved difficult to reliably genotype and so was not further analyzed. Nine of the SNPs were found in more than one animal and were genotyped across the 40 horse panel. Inheritance of these SNPs conformed to nine predicted haplotypes (Figure 1), which differed in frequency between different breeds (Figure 2).

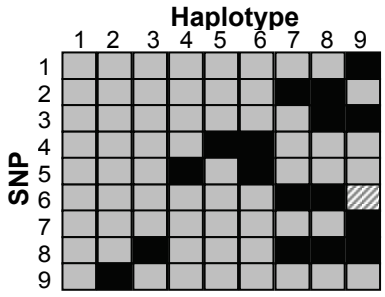

Figure 1: Nine common haplotypes found within the equine ACE gene. Grey blocks indicate the common allele, black blocks indicate the minor allele, and the striped bock in SNP 6 indicates the third allele.

Of the nine haplotypes, the haplotype represented by only common SNP alleles (haplotype 1) was the most frequent, found on 47 of the 80 possible chromosomes and in all breeds. Of the remaining haplotypes, six were observed more than once (haplotypes 2, 5, 6, 7, 8 and 9). More haplotypes with fewer representations were identified in SB and Draught horses, perhaps reflecting the higher level of genetic diversity seen in the founders and consequent generations of those populations. Haplotypes 7, 8 and 9 were seen only in the ARs and TBs, whilst haplotypes 2, 3, 4 and 5 were only found in the heavier breeds. While there appears to be significant differences between the distribution of ACE haplotypes between breeds it is difficult to say whether this is a true effect of selection, genetic drift, or sampling bias caused by the small numbers of each haplotype within each group, and the study needs to be expanded to determine this.

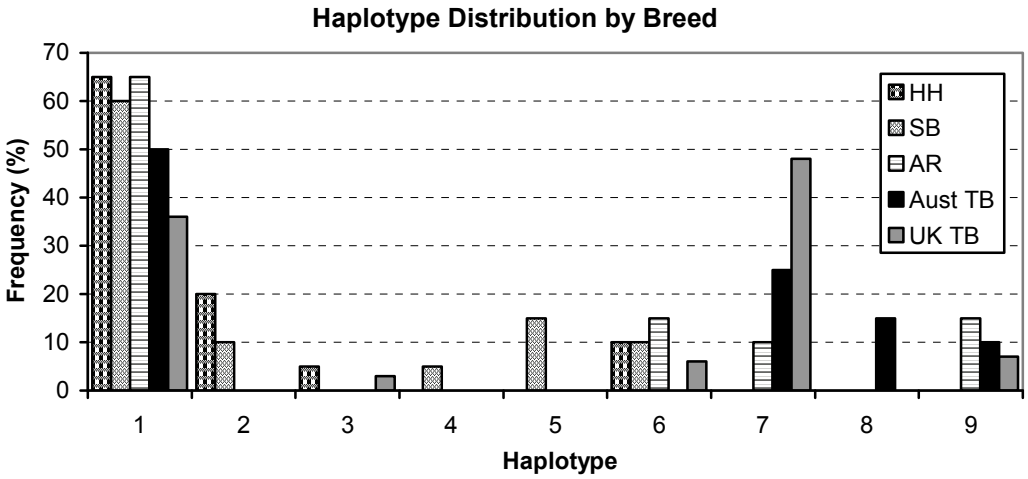

Figure 2: Distribution of ACE gene haplotypes across the UK TBs from the association study and 10 horses of different breeds: Thoroughbred, Arabian, Standardbred and Draught horses.

Association study. Sixty-two horses with circulating ACE levels representing the extreme ends of the distribution were genotyped for the nine haplotypes. Each horse had a previously determined ACE genotype: no new haplotype combinations were discovered (Figure 2). Haplotype 6 was associated with significantly lower ACE levels than the population average (P=0.007). After the remaining 141 horses in the population were genotyped for this haplotype, the association remained (P=0.000) and accounted for 10.26% of the variation in ACE concentration.

Five of the previously determined haplotypes were detected in the UK population of TBs. Haplotypes 1, 7 and 9 were common to both the Australian and UK TB populations, while 3 and 6 were found only in the UK group. This difference in haplotype distribution between the two populations may be due to local breeding trends or a simple sampling effect. It is unlikely that shuttle stallions (northern hemisphere stallions serving during the Australian breeding season) would have had the opportunity to make a large contribution to the Australian gene pool at this stage, since the majority of the horses in this study were conceived when shuttle stallions covered only 2.9 - 19% of the mare population. Local breed differences are likely to have occurred due to the popularity of certain sire lines in particular parts of the world. Again, due to the low numbers of each haplotype detected it was also impossible to determine whether the different haplotypes were associated with the different physical specialties of the breeds used, and their performances. More horses are needed to better investigate this.

The association of haplotype 6 with reduced circulating enzyme levels is similar to the documented association between the human ACE insertion allele and enzyme levels. In both cases, the polymorphisms involved are intronic and as such do not affect the coding sequence, and must influence gene expression through other methods. In both species the markers identified to date may not be responsible for the observed effects; and instead may be close to or in linkage disequilibrium with the causative polymorphisms. Alternately, the effect may be caused by changes to an unrecognized non-coding regulatory region or microRNA, either within the gene (cis acting) or elsewhere (trans acting). Further study is needed on both species to determine the mode of action of these polymorphisms.

CONCLUSION

We have characterized the equine angiotensin-converting enzyme gene, identifying nine commonly inherited haplotypes that appear to occur at different frequencies in different breeds. One haplotype was associated with circulating enzyme levels, and had a similar effect as the intronic polymorphism in the human ACE gene. Further study is needed to determine the mode of action of the equine markers, the extent of the effect on enzyme levels, and to elucidate whether there is also an association with racing performance in the horse.

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REFERENCES

Alvarez, R., Terrados, N., Ortolano, R., Iglesias-Cubero, G., Reguero, J.R., Batalla, A., Cortina, A., Fernandez-Garcia, B., Rodriguez, C., Braga, S., Alvarez, V. and Coto, E. (2000) *Eur. J. Appl. Physiol.* **82:** 117.

Coomer, R.P.C., Forhead, A.J., Bathe, A.P. and Head, M.J. (2003) *Equine Vet. J.* **35:**96.

Gayagay, G., Yu, B., Hambly, B., Boston, T., Hahn, A., Celermajer, D.S. and Trent, R.J. (1998) *Hum. Genet.* **103:**48.

Langlois, B. (1980) *Livestock Prod. Sci***. 7:**591

Montgomery, H.E., Marshall, R., Hemingway, H., Myerson, S., Clarkson, P., Dollery, C., Hayward, M., Holliman, D.E., Jubb, M., World, M., Thomas, E.L., Brynes, A.E., Saeed, N., Barnard, M., Bell, J.D., Prasad, K., Rayson, M., Talmud, P.J. and Humphries, S.E. (1998) *Nature* **393:**221.

Myerson, S., Hemingway, H., Budget, R., Martin, J., Humphries, S. and Montgomery, H. (1999) *J. Appl. Physiol.* **87:**1313.

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P. and Soubrier, F. (1990) *J. Clin. Invest.* **86:**1343.

Stephens, M., Smith, N. and Donnelly, P. (2001*) Am. J. Hum. Genet.* **68:**978.

Tiret, L., Rigat, B., Visvikis, S., Breda, C., Corvol, P., Cambien, F. and Soubrier, F. (1992). *Am. J. Hum. Genet.* **51:**197

Tolley, E.A., Notter, D.R. and Marlowe, T.J. (1983) *J. Anim. Sci.* **56:**1294