

GGA4q11–q24 region.^{1,8} In mice, markers orthologous to those in the GGA4q11–q24 to HSA4p16–q28 region map to four chromosomes: MMU3, MMU5, MMU8 and MMU13.²

QTL mapping: The AW33 QTL was shown to be linked to microsatellite *MCW0170*, and the ST53 QTL was tightly linked to *MCW0114*, both markers located on GGA4.¹ Our results demonstrate that these markers and therefore the associated QTLs, map within the GGA4q21–q22 and GGA4q11–12 regions, respectively. These results provide multiple entry points into the physical map and sequence of the chicken genome for identification of genes that might underlie QTLs. For example, *MCW0114* maps near *IDS*, the iduronate-2-sulfatase gene, whose product is involved in heparin sulphate metabolism, and *MCW0170* maps to a region containing well-known regulatory genes (*KIT*, *CLOCK*, *EGF* and two GABA receptor genes) and *UGDH*, also involved in glycosaminoglycan and heparin sulphate metabolism. Variation in these genes could possibly affect egg size and/or shell characteristics.

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Linkage mapping of the porcine myelin basic protein gene to chromosome 1¹

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¹Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

Source/description of primers: Myelin basic protein (MBP) constitutes 30% of the total myelin protein in the central nervous system.¹ In addition, MBP is present in various different cell types including lymphoid cells² and all types of myeloid lineage cells (i.e. macrophages, dendritic cells, granulocytes, megakaryocytes and erythroblasts).³ As foetal erythropoiesis influences uterine capacity in pigs⁴ and MBP is expressed in the haematopoietic progenitors,³ we hypothesize that MBP may influence early foetal development. A cDNA clone of the porcine *MBP* (GenBank accession number AY603684) was isolated from the 'Meat Animal Research Center (MARC) 2 PIG' expressed sequence tag (EST) library.⁵ The putative porcine MBP amino acid sequence shares 91% identity with the human MBP. This clone lacked a region corresponding to human exon 2. Clones lacking exon 2, due to differential splicing, have been identified in both mouse and human.¹ Primers were designed to amplify a 598-bp product in the 3'-untranslated region (UTR) of the cDNA. The forward (F) and reverse (R) primers correspond to bases 1251–1270 and 1848–1828 of the porcine *MBP* cDNA.

PCR primer sequences and flanking sequence of a single nucleotide polymorphism: *MBP* F: AGGTAACAGGTGGCGTGCTC

MBP R: TCAAGAACAGGGCGTATTTC

MBP Fi: ATCCCGGTCTAATTCTGAAGGT

MBP Ri: CCGTGAGAGATCACTCCATCA

Sequence flanking polymorphism: TTGCATCGTG(G/C)AGCAGATCGC

MBP probe primer: GACACACACTTTGCATCGTG

PCR conditions: Polymerase chain reactions (PCR) were carried out in a 25- μ l volume containing 100 ng genomic DNA, 1.5 mM MgCl₂, 20 pmol of each primer, 100 μ M dNTP and 0.35 U HotStarTM *Taq* polymerase (Qiagen Inc., Valencia, CA, USA). Amplification was performed under the following PCR conditions: 15 min at 94°C; 40 cycles of 30 s at 94°C, annealing for 1 min at 58°C, 1 min at 72°C and a final extension of 5 min at 72°C. Both strands of the amplified genomic DNA of parents from the MARC Swine Reference Population⁶ were sequenced and evaluated for polymorphisms.⁷

Polymorphism and chromosomal location: Two single nucleotide polymorphism (G/C at nucleotide 1757 and G/A at nucleotide 1766 in GenBank accession number AY603684) were detected in the 3'-UTR of *MBP*. Both polymorphisms were heterozygous in three of the seven F₁ sows from the MARC Swine Reference Population.⁶ An assay was designed to genotype the G/C polymorphism at nucleotide 1757 by primer extension with analyte detection on a MALDI-TOF mass spectrometer (Sequenom Inc., San Diego, CA, USA) using a pair of internal primers. The internal forward (Fi) and tailed reverse (Ri) primers correspond to bases 1612–1633 and 1788–1768 of the porcine *MBP* cDNA (GenBank accession number AY603684). This marker generated 39 informative meioses in the MARC swine reference population. The *MBP* gene was mapped using *CRI-MAP* to chromosome 1 relative position 82.9 cM, which is the same position as microsatellite markers *RLN1*, *S0082*, *S0357*, *SW80*, *SW780*, *SW1020*, *SW1619*, *SW1621*, *SW1902*, *SW2073*, *SW2416*, *SWR1427*, *SWR2182* and *SWR337* on the current MARC swine chromosome 1 linkage map (<http://www.marc.usda.gov/>).

The most significant two-point linkage of *MBP* was with *SW80* and *SW780* (LOD = 10.84) at 0.0% recombination. The *MBP* gene is located on human chromosome 18q23, which shares homology with swine chromosome 1.

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Characterization and localization of 17 microsatellites derived from BACs in the horse

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Sources/description: A series of BAC clones containing identified genes or not (sequence tagged sites, STS) were isolated from the INRA Horse BAC library¹ and screened for microsatellites. The BAC clones containing genes or STS were localized by fluorescence *in situ* hybridization (FISH). Eleven dinucleotide repeats were characterized and located by linkage analysis in addition to a set of another six microsatellites associated with genes.^{1,2} The BAC clones were digested by *Sau3A* (Promega, Madison, WI, USA) and ligated into the *BamHI* site of a pGEM-4Z vector. Clones containing a potential microsatellite were identified by screening subclones with non-radioactively labelled (TG)₁₀ and (TC)₁₀ oligonucleotides using 3'-DIG labelling kit (Boehringer Ingelheim GmbH, Germany) as described elsewhere.³ Positive

clones were sequenced using universal and reverse primers with an ABI 377 DNA Analyser (Applied Biosystems, Foster City, CA, USA). Primer pairs were designed to amplify each of the new microsatellites. Details of the microsatellite characterization are provided in Table 1.

PCR conditions: Polymorphism of the isolated microsatellites was determined by polymerase chain reaction (PCR) performed in a 10 µl final volume in the presence of 1 µCi of α -[³²P] dATP, approximately 40 ng of genomic DNA, 2 mM MgCl₂, 1 pmol of each primer (Eurogentec, Liège, Belgium) and 0.05 units of GoldStar *Taq* polymerase (Eurogentec). The concentration of unlabelled deoxynucleotides was 25 µM for dCTP, dGTP and dTTP with 2.5 mM for dATP. The PCR were performed in an MJ Research PTC100 thermocycler (Bio-Rad Laboratories, Hercules, CA, USA). The PCR conditions were an initial 5 min denaturation at 92 °C, followed by 35 cycles of 92 °C for 30 s, annealing temperature ranging from 55 to 60 °C for 30 s and 72 °C for 30 s. The PCR products were analysed by electrophoresis at 1600 V in 6% polyacrylamide/7.5 M urea gels. Gels were dried and autoradiographed overnight. The PCR conditions for genotyping and linkage analysis are described elsewhere.⁴

Allele frequencies and size range: Polymorphism and allele frequencies of the microsatellites were determined on a panel of 24 unrelated animals of four horse breeds (six Selle Français, six Arab, six Trotteur Français, six Thoroughbred). The number of alleles observed in this sample ranged from 2 to 6 (Table 1). The size allele range was determined during genotyping for linkage analysis.

Linkage and FISH mapping: Microsatellites were typed on the Newmarket three generation full-sibling reference family. Localizations were determined by two-point linkage analysis using CRIMAP software.⁵ Chromosome assignments and the two nearest linked markers, recombination frequency and LOD scores are provided in Table 2. Chromosome localizations of the microsatellites by linkage mapping were in accordance with the cytogenetic location of the corresponding BAC clones.^{1,2} These markers will help the construction of an integrated horse map.

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