Assignment¹ of the *Equus caballus* interleukin 8 gene (*IL8*) to chromosome 3q14.2→q14.3 by in situ hybridization

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¹ To our knowledge this is the first time this gene has been mapped in horse.

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Rationale and significance

Human interleukin 8 (IL-8) is an anti-inflammatory cytokine. A significant increase of the IL-8 concentration has been found in the plasma of marathon runners (Suzuki et al., 2000) and a similar upregulation of this interleukin was recently detected in lymphocytes of horses immediately after intense exercise (Cappelli, Verini-Supplizi, Capomaccio and Silvestrelli unpublished results). *Equus caballus* (ECA) *IL8* full length cDNA, isolated from peripheral blood lymphocytes of an endurance horse, was sequenced (GenBank accession number AY184956). The mRNA sequence is 1089 nt in length and contains a 306 nt region that putatively codes for a 101 amino acid protein. In the framework of studying the modulation of gene expression profiles in racing horses, we report here the chromosomal location of ECA *IL8*. This result adds a new type I marker to the FISH map of the horse.

Materials and methods

Two *Equus caballus* genomic BAC clones (63-D22 and 163-L3) containing the *IL8* gene were obtained by PCR screening of segment I of the hierarchically pooled CHORI-241 horse BAC library. Primers for PCR were designed based on the ECA *IL8* mRNA sequence AY184956; forward primer 5'-TCTTGGCCGCTCTCCTGCTTCT-3' and reverse primer 5'-CCACTCTCAATCGCTCTCATTTTC-3'. Cycling parameters were an initial denaturation cycle at 95°C for 2 min followed by 30 cycles at 94°C for 40 s, 61°C for 40 s and 72°C for 1 min and a final extension cycle at 72°C for 5 min. The PCR product was sequenced with an ABI PRISM[®] 310 Genetic Analyzer (Applied Biosystems) using the amplification primers and an ABI PRISM Big Dye sequencing kit (Applied Biosystems) according to the manufacturer's instruction. Sequences were aligned using the MultAlin software (<http://prodes.toulouse.inra.fr/multalin/multalin.html>). BAC DNAs as well as genomic DNA from horse fibroblasts were isolated by standard procedures.

Metaphase spreads were obtained from primary fibroblasts of a normal (2n = 64) female horse. The BAC DNA probes, labeled with Cy3-dUTP by nick translation, were hybridized as previously described (Azalin et al., 2001) to the metaphase chromosomes counterstained with Hoechst. Digital images were obtained using a Zeiss Axioplan Fluorescence Microscope and a cooled CCD camera (Photometrics). Cy3 and Hoechst fluorescence signals, detected with specific filters, were recorded separately as gray-scale images. Pseudo-coloring and merging of images were performed using the Adobe Photoshop software. G-banding was obtained by inverting the Hoechst staining. The identification of G-banded chromosomes was performed according to Bowling et al. (1997).

Probe names: 63-D22, 163-L3

Probe type: horse genomic BAC clones

Vector: pECBAC1

Proof of authenticity: sequencing

Gene reference: *Equus caballus* IL-8 mRNA (GenBank accession number AY184956)

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Results and discussion

Using primers deduced from the ECA *IL8* mRNA sequence, an 1150-bp genomic DNA fragment was amplified by PCR on a template of genomic DNA extracted from horse primary fibroblasts. A portion of the PCR fragment was sequenced. The sequence was 98% identical to the AY184956 GenBank sequence, except for an intron fragment. The same primers and PCR conditions were used to screen the BAC library. Two overlapping clones, 63-D22 and 163-L3, were isolated and their identity was confirmed by sequencing a portion of the PCR products obtained with the ECA *IL8* primers.

Each BAC clone was used as probe in a FISH experiment. The results showed that *IL8* is localized on ECA3q14.2→q14.3 (Fig. 1). In this region of horse chromosome 3 the following genes have been previously mapped: *PDHA2* (3q13), *ALB* (3q14.3) and *PDGFRA* (3q21) (Chowdhary et al, 2003). This gene order is conserved in the human genome in the region comprised between 4q22.3 and 4q12. In conclusion, in both horse and human the *IL8* gene is located between *PDHA2* and *ALB*.

Mapping data:

Most precise location: 3q14.2→q14.3

No. of cells examined: 10 cells hybridized with the 63-D22 probe and 10 cells hybridized with the 163-L3 probe

Number of cells with specific signal:

63-D22 probe: 2 (1), 3 (1), 4 (8) chromatids per cell

163-L3 probe: 2 (1), 3 (0), 4 (9) chromatids per cell

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Fig. 1. Chromosomal localization of the ECA *IL8* gene to horse metaphase chromosomes. **(A)** A metaphase probed with the 63-D22 BAC clone. FISH signals (upper panel), G-banding (lower panel) are shown; **(B)** a partial horse metaphase probed with the 163-L3 BAC clone. Arrowheads point to the G-banded chromosomes displaying FISH signals.