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Quantitative trait loci for osteochondrosis in Hanoverian warmblood horses

Session Ph7.7

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Introduction

- Osteochondrosis (OC) is a developmental orthopedic disease in domestic animals
- Multifactorial in origin and genetic components play a central role in the pathogenesis
- Prevalence of OC is between 10 % and 30 % across a range of different horse breeds

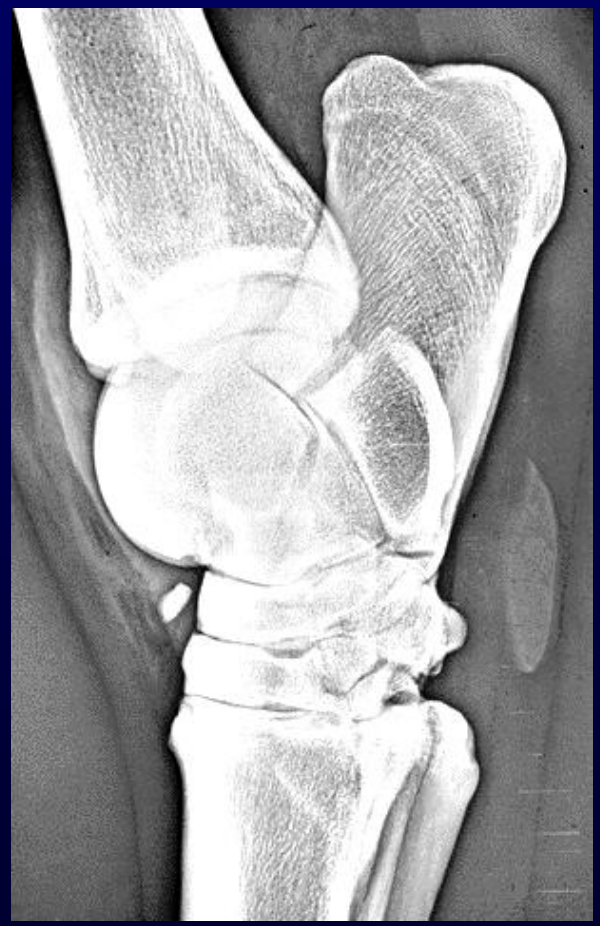
OC / OCD

- **Osteochondrosis (OC)**

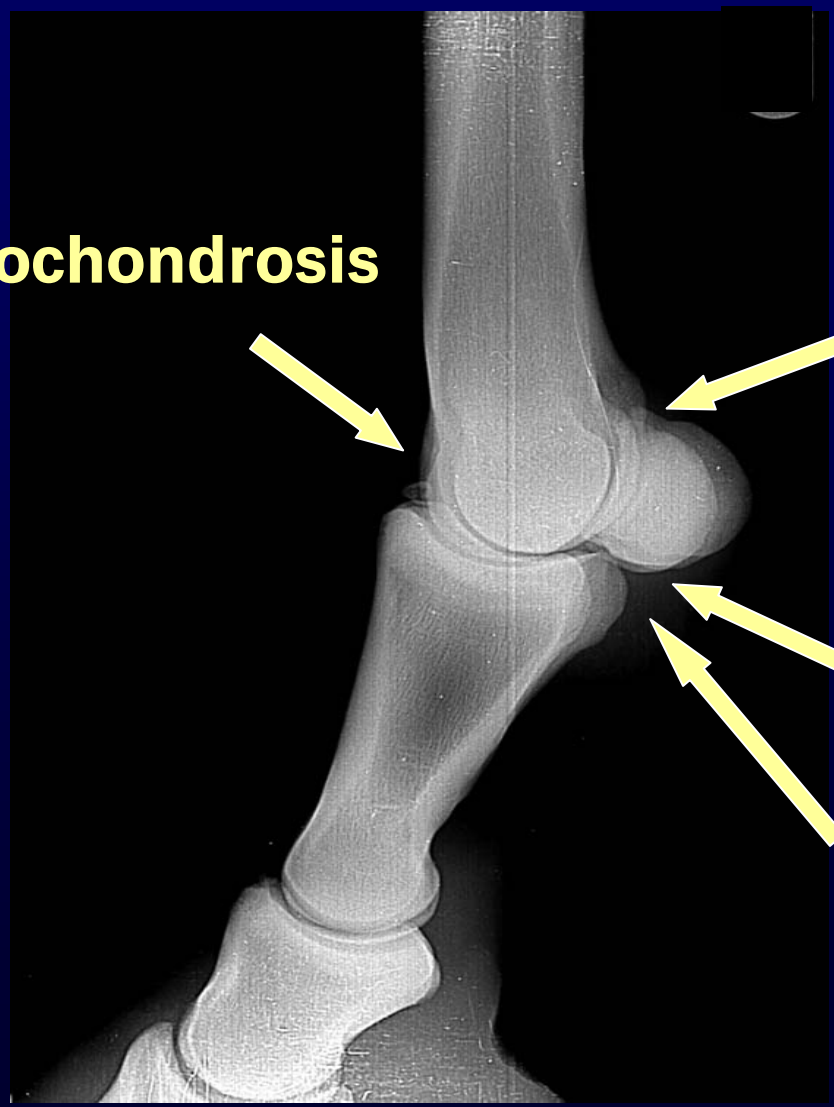
  caused by disturbed differentiation and maturation of cartilage

- **O. dissecans (OCD)**

  presence of osteochondral fragments



Osteochondrosis in fetlock joints (radiological changes)



osteochondrosis

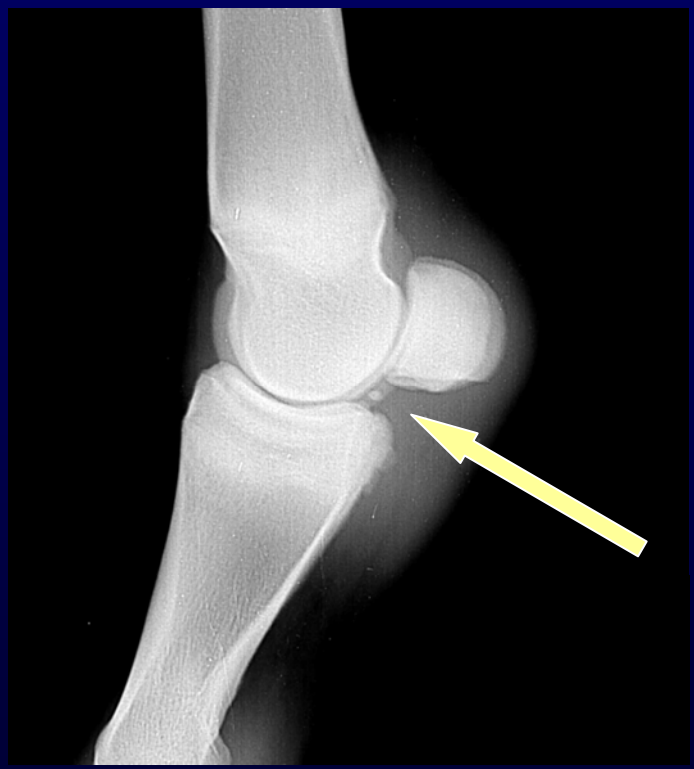
palmar/ plantar osteochondral fragments (POFs)

proximal

distal

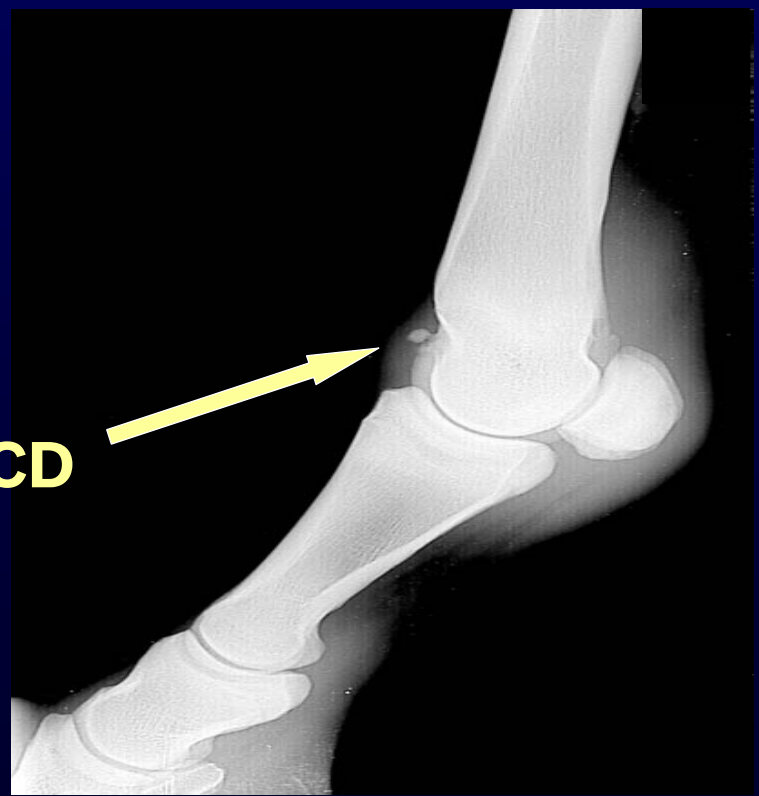
palmar/plantar

Palmar / plantar osteochondral fragments in fetlock joints and fetlock OCD



POF

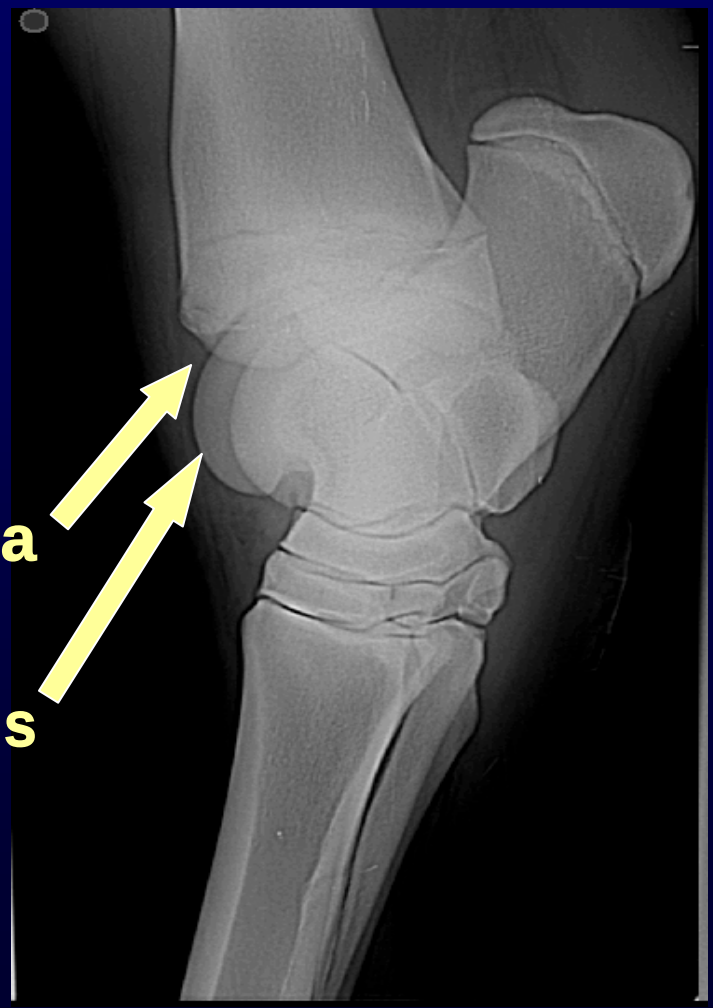
Fetlock OCD



Osteochondrosis in hock joints (radiological changes)

intermediate ridge of distal tibia

lateral trochlea of talus



Objectives

(1) Identification of quantitative trait loci (QTL) for osteochondrosis based on an affected half-sib design

(2) Development of markers in linkage disequilibrium

(3) Characterization of genes causing OC

Approach

(1) Collection of paternal half-sib families at high risk for osteochondrosis

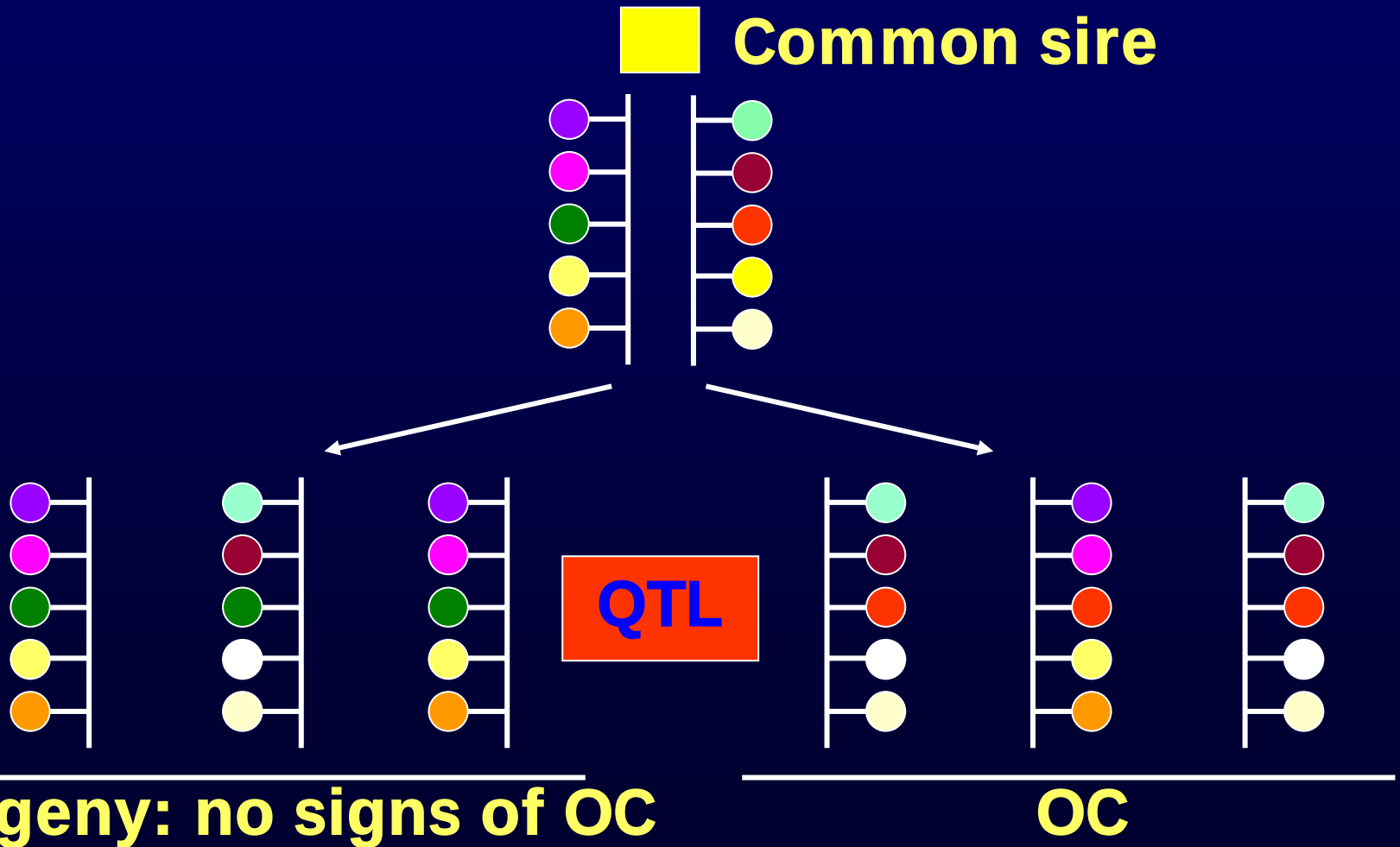
(2) Whole genome scan to identify QTL

(3) Increasing marker density in OC-QTL regions

(4) Fine mapping using linkage and association analyses

Approach

Scanning of chromosomes for genomic regions shared by all horses affected by OC: quantitative trait loci (QTL)



Data

- 629 Hanoverian warmblood foals
- Age at first radiological examination between 5-9 months
- Age at second radiological examination: 2 years (68 % of the foals sampled)
- Digital radiographs of fetlock, hock and stifle joints
- 14 paternal half-sib groups including 211 horses

Family data - phenotypic traits

Trait	Prevalence
OC fetlock joints	51.9 %
OC hock joints	32.7 %
OCD fetlock joints	25.0 %
OCD hock joints	24.0 %

Microsatellite markers

- Selection of 157 microsatellite markers
- Average marker distance less than 20 cM (Swinburne *et al.* 2006)
- Average PIC of 47.6 %
  Mean observed HET of 63.3 %
- Increasing the marker density with 61 additional markers

Whole genome scan

- 19 putative genomic regions on 17 different chromosomes
- QTL differentially distributed between fetlock and hock OC
- Several QTL are possibly involved in the development of OC

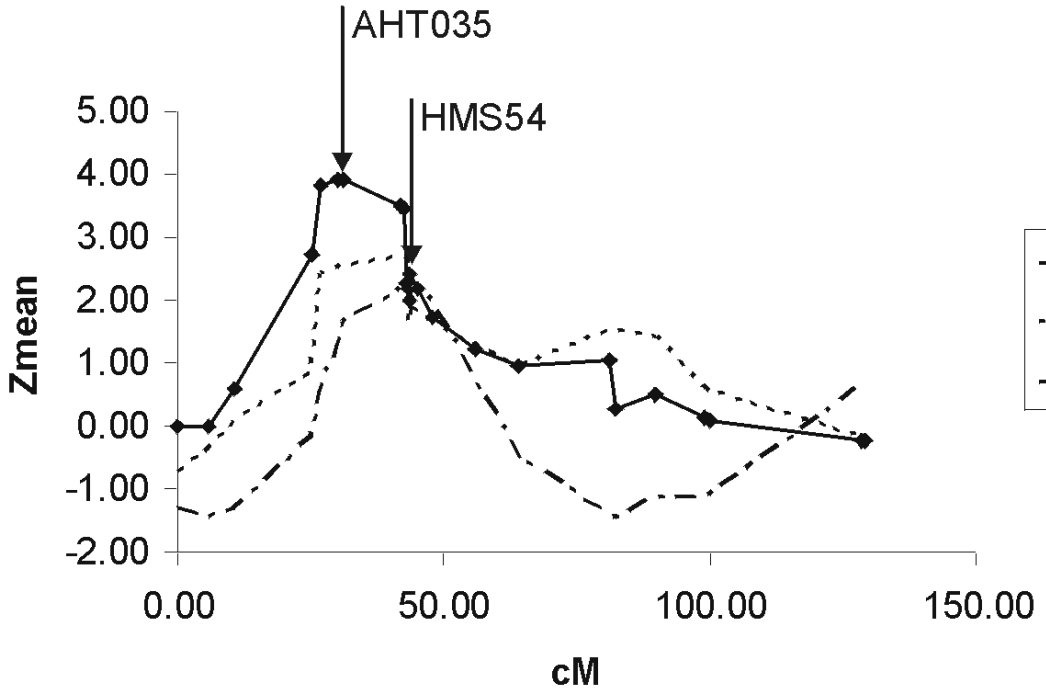
Significant QTL for OC
(nominal significance threshold for chromosome-wide multipoint analyses)

ECA	Fetlock joints		Hock joints	
	OC	OCD	OC	OCD
2	< 0.001	< 0.001	0.012	-
3	-	0.02	-	-
4	0.04	-	0.012	-
5	0.006	0.002	0.08	0.03
7	-	-	0.03	0.04
8	-	-	0.05	0.04
9	-	-	0.05	0.02

Significant QTL for OC
(nominal significance threshold for chromosome-wide multipoint analyses)

ECA	Fetlock joints		Hock joints	
	OC	OCD	OC	OCD
14	-	0.03	-	-
15	-	-	0.009	0.05
16	0.04	0.04	0.009	0.012
18	0.06	0.06	0.04	0.07
19	-	0.03	0.04	-
21	-	-	0.04	0.04
22	-	0.03	-	-
30	0.03	-	-	-

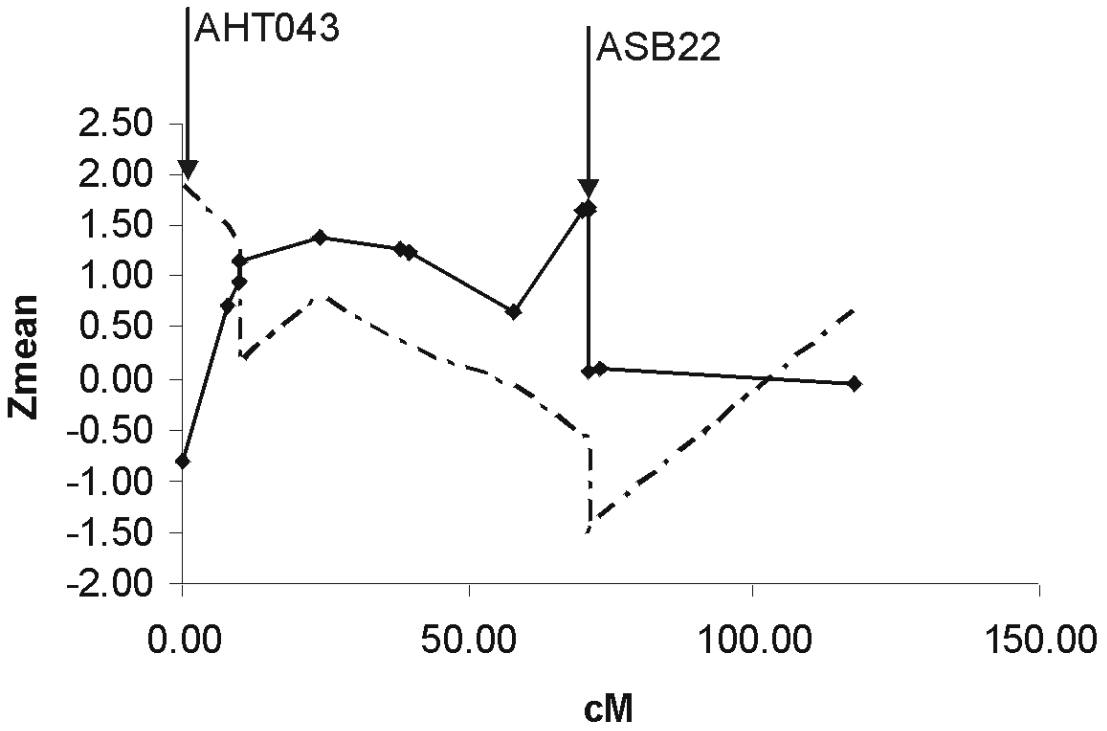
QTL on ECA2



OC-F: osteochondrosis in the fetlock joints
OCD-F: osteochondrosis dissecans in the fetlock joints
OC-H: osteochondrosis in the hock
● microsatellite markers genotyped in this study

Nominal error probabilities of the multipoint chromosome-wide tests on ECA2 for fetlock and hock OC

QTL on ECA4



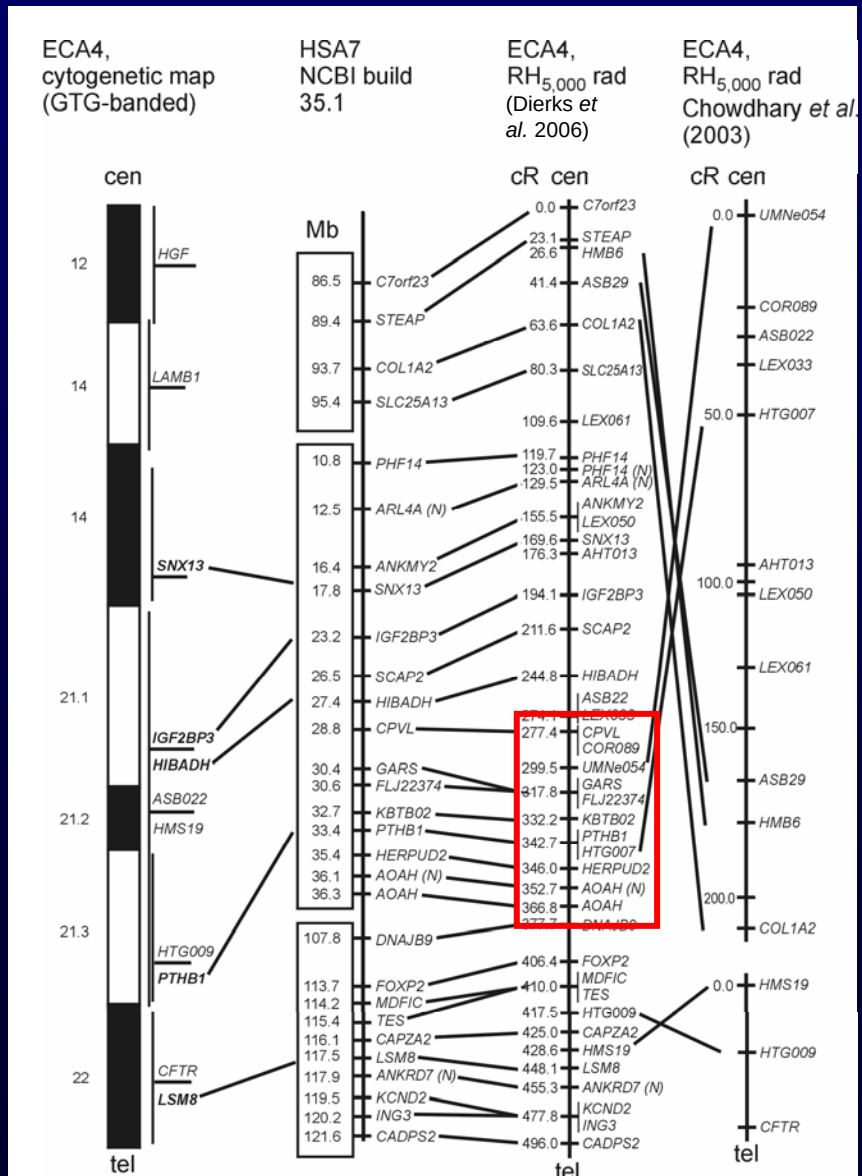
OC-F: osteochondrosis in the fetlock joints
OC-H: osteochondrosis in the hock
● microsatellite markers genotyped in this study

Nominal error probabilities of the multipoint chromosome-wide tests on ECA4 for fetlock and hock OC

Linkage and association analysis

- Development of single nucleotide polymorphisms (SNPs) in candidate genes
- Confirmation of linkage and identification of families informative for the QTL
- Linkage disequilibrium tests
- 165 horses with an age at radiological examination of about 24 months
- Additional 188 unrelated horses: 96 affected and 96 unaffected

Refinement of equine RH map and selection of candidate genes



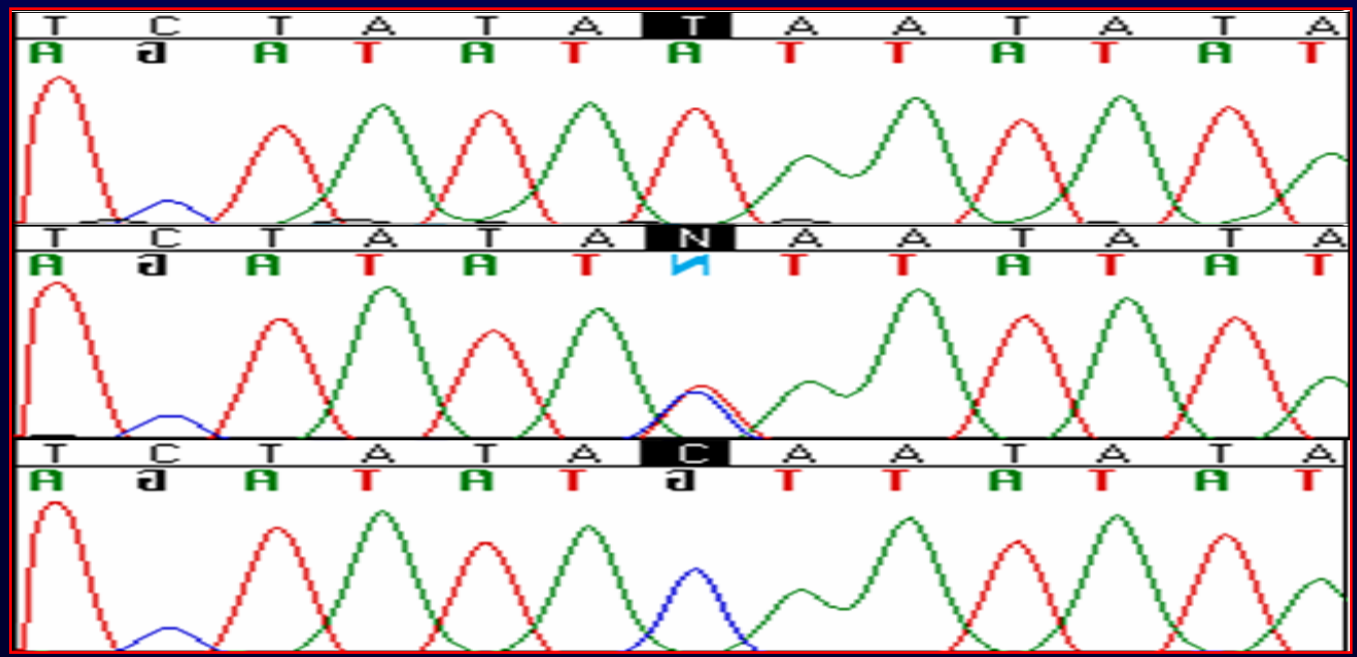
Synteny of ECA4q (70.0-73.3 cM) to HSA7 (28.8-36.3 Mb)

Development of SNPs

BAC-endsequences, ESTs, WGS sequences

PCR and subsequent sequencing of 8 stallions

SNP in PTHB1 gene

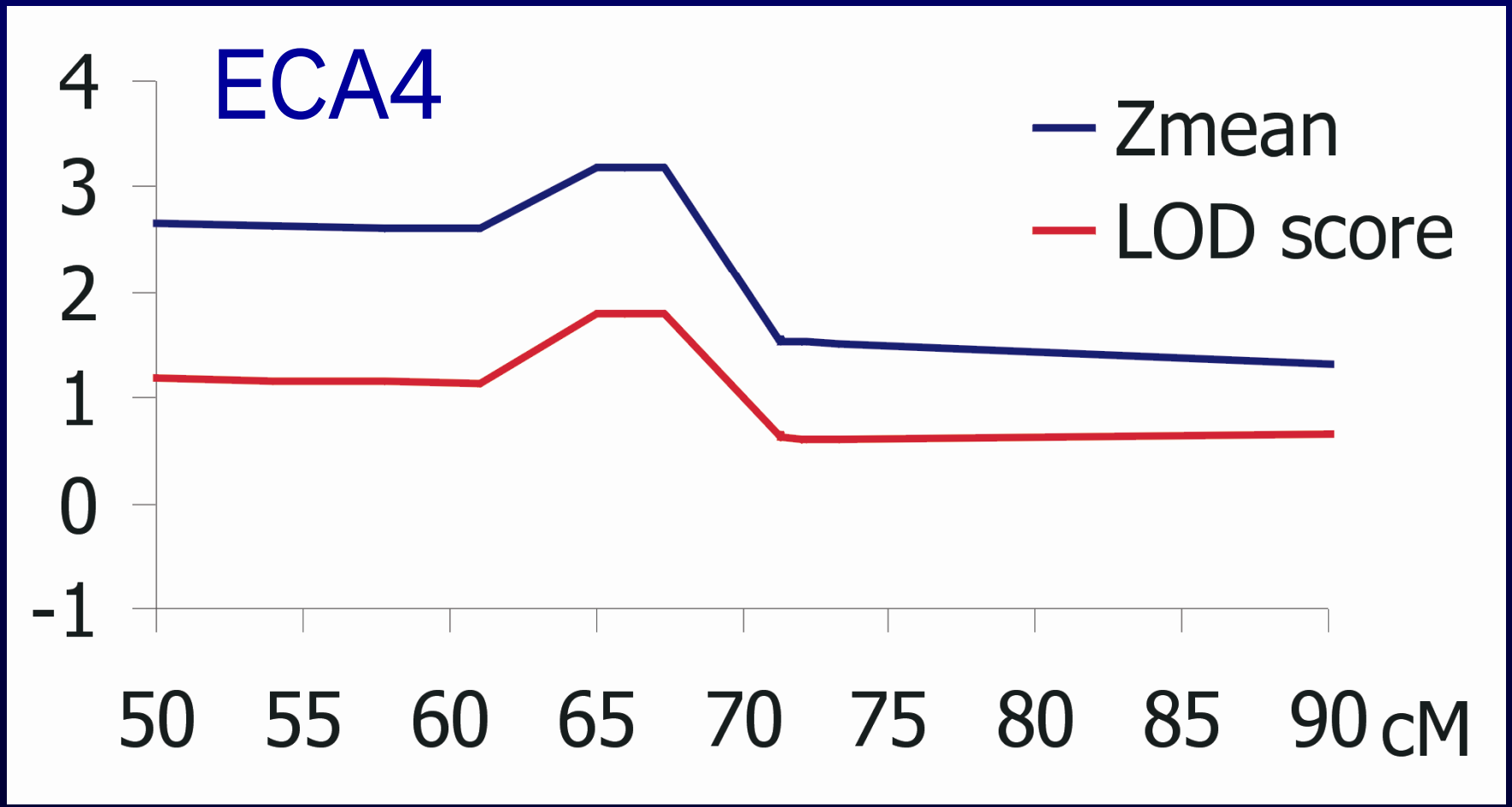


genotype TT

genotype CT

genotype CC

Linkage analysis including SNPs



Significant OC-QTL region between 54 and 71 cM

Association for hock OC and OCD

- Significant association of an intragenic SNP in PTHB1 gene within the QTL region

  $X^2 = 8.24$ and $p = 0.016$ for hock OC

  $X^2 = 12.40$ and $p = 0.002$ for hock OCD

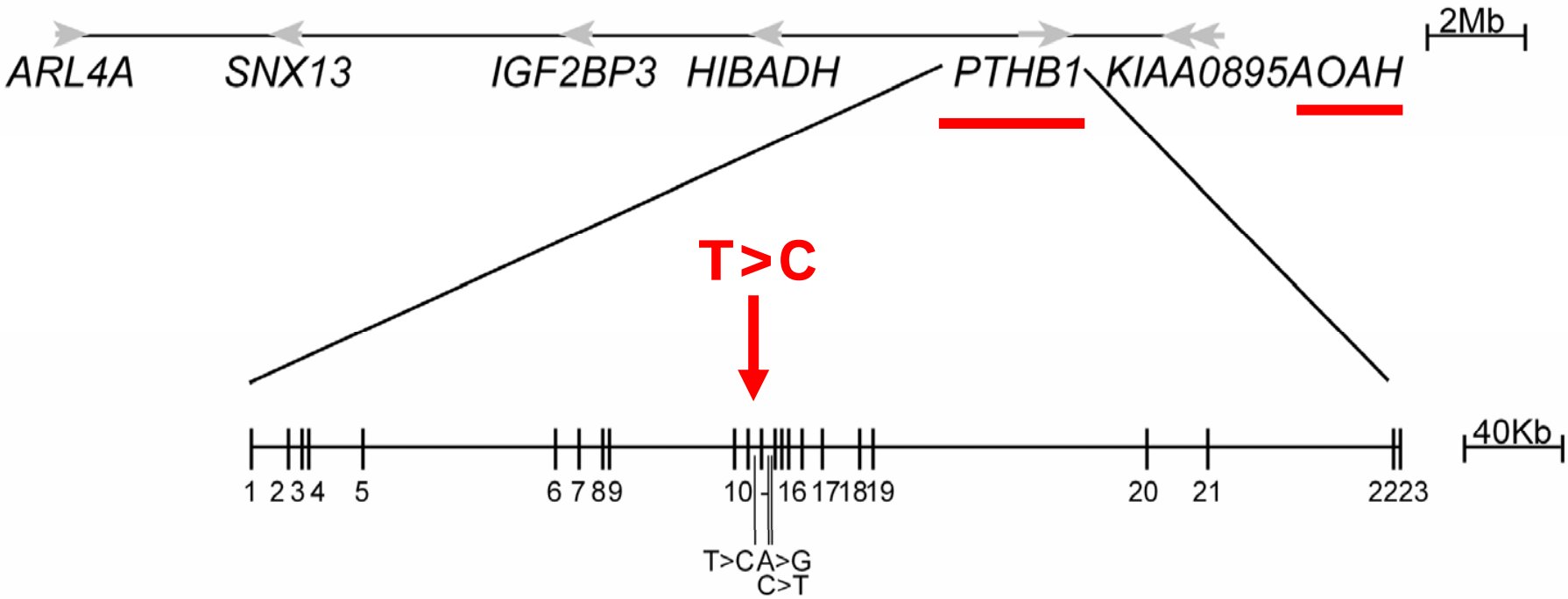
- Affirmation of association using an animal model including quantitative genetic and environmental effects

Association analysis using an animal model

Additive and dominance effects for AM072940:g.230 T>C in parathyroid hormone-responsive B1 gene (*PTHB1*) with $q_c = 0.34$

Trait	Additive effect	P	Dominance effect	P
OC hock	0.38 ± 0.16	0.018	-0.47 ± 0.18	0.009
OCD hock	0.41 ± 0.15	0.006	-0.53 ± 0.17	0.002

SNPs in PTHB1 gene



Effects of SNPs on ECA4q on hock OC in Hanoverian warmblood horses

Gene / SNP-genotype	OC-hock	Δ	OCD-hock	Δ
PTHB1 CC CT | TT	100 % 27.6 %	- 72.4 %	100 % 19.9 %	- 80.1 %
AOAH AA AG | GG	35.2 % 18.6 %	- 16.6 %	24.8 % 13.6 %	- 11.2 %
PTHB1-AOAH CC | AA others	36.2 % 14.8 %	- 21.4 %	26.7 % 11.1 %	- 15.6 %

Confirmation study in Coldblood horses

- Affirmation of the QTL on ECA4q in South German coldblood horses (Wittwer and Distl, 2006)
- Association confirmed by SNPs in the same genomic region and same gene

Outlook

- Development of SNPs for fine mapping of further OC-QTL
- Combined linkage and association analysis
- Mutation analysis of associated genes using cDNA and genomic DNA

Thanks for your attention !

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