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Replication and fine-mapping of a QTL for recurrent airway obstruction (RAO) in European Warmblood horses

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Overview

Introduction

Narrowing down the QTL in the family

Replication and fine-mapping in independent animals

Positional candidate gene analysis results

Conclusion

Recurrent airway obstruction (RAO)

RAO is one of the most common airway diseases of mature horses.

Following exposure to hay dust, …

reversible bronchoconstriction,

increased mucus production

and neutrophilic inflammation in small airways.

It has a familial basis with a complex mode of inheritance

Hypersensitivity 1 in RAO!

↑ IgE in BALF

X strong early-phase response

Late-phase response 6-9 h later

Recruitment of neutrophils and airway obstruction

Formerly Mast cells

Now $CD4^+$ T-cells

Th2-type cytokines (IL4, IL13,…)

Phenotyping system

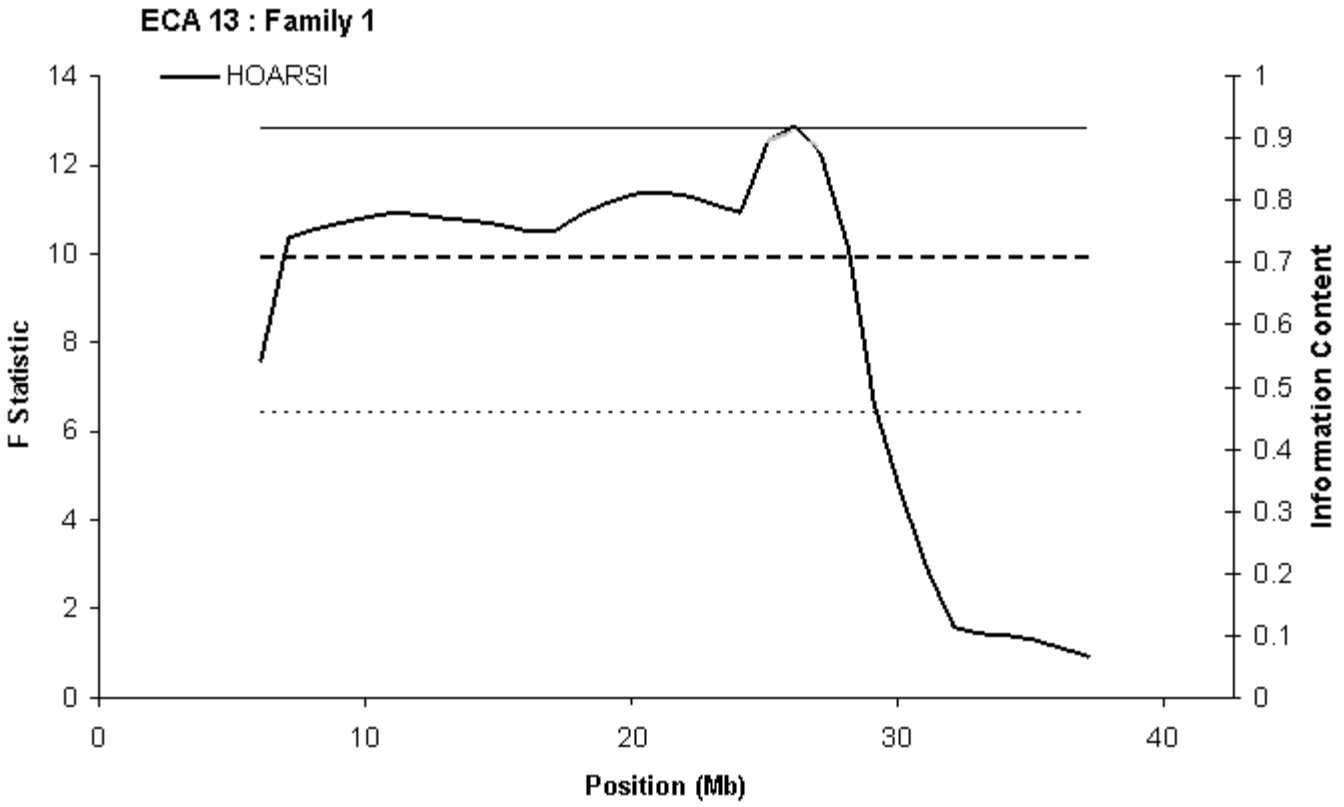
“Horse Owner Assessed Respiratory Signs Index” (HOARSI).

HOARSI 1.....2 ..... 3 4
healthy..... mild..... moderate..... severe

>5 years
clinical signs for at least 2 months

History of:
hay-feeding..... nasal discharge,
↑ breathing effort ....., coughing, ...

Whole-Genome scan and QTL detection on ECA13



Fine-mapping of the QTL in the original family

Affected sire and
his offspring:

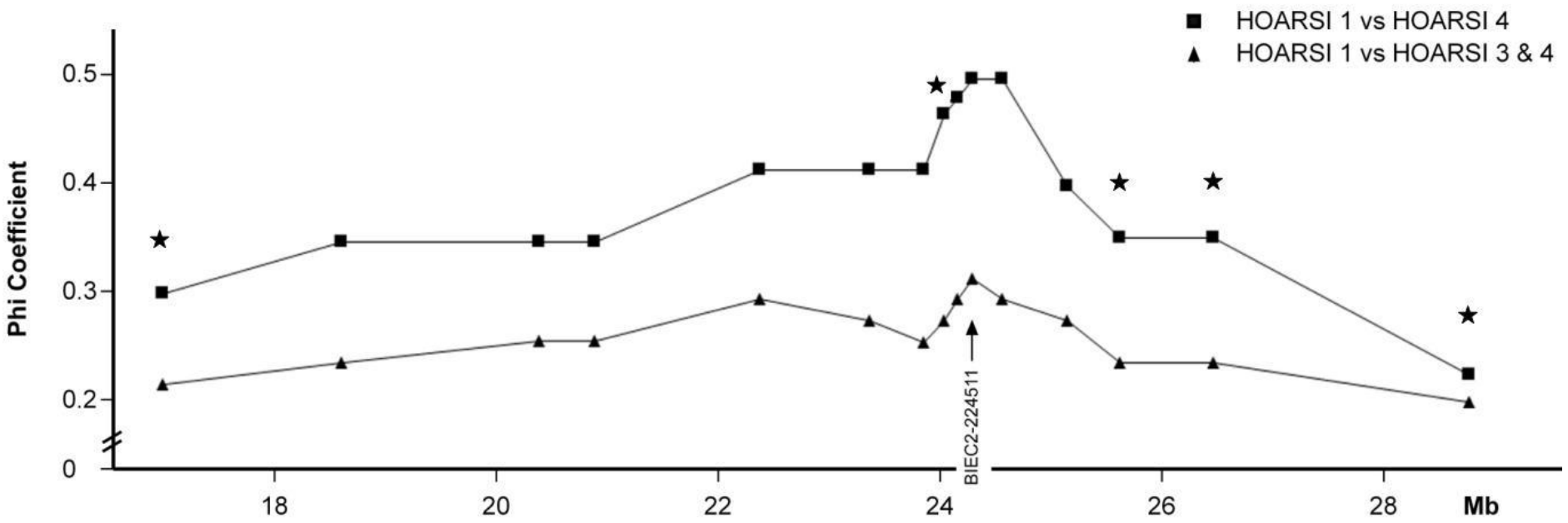
50	HOARSI 1	⟶	Controls
34	HOARSI 3	}	Cases
18	HOARSI 4	}	

↑ marker density

Genotyping 12 recombinant offspring in the QTL region

Association by estimating the phi-coefficient

Fine-mapping in the original family



Replication of this associated SNP

in unrelated horses

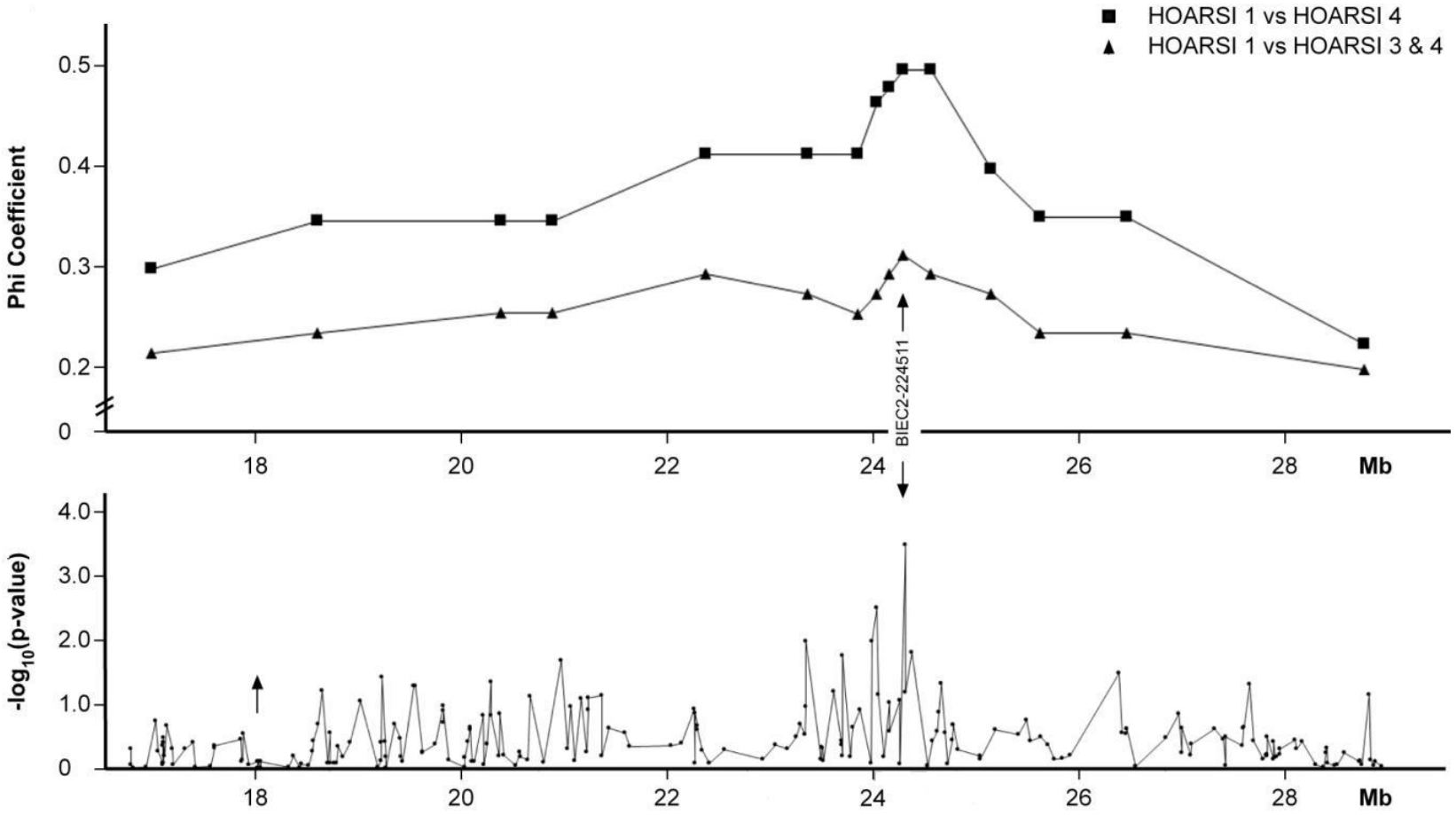
Sample population for replication

Independent cohort of 646 unrelated Warmblood horses

340 cases (HOARSI 3 and 4)
306 controls (HOARSI 1)

Allelic Association $P_{raw}=0.004664$
Genotypic Association $P_{raw}=0.00037$

Replication in unrelated horses



Replication in unrelated horses

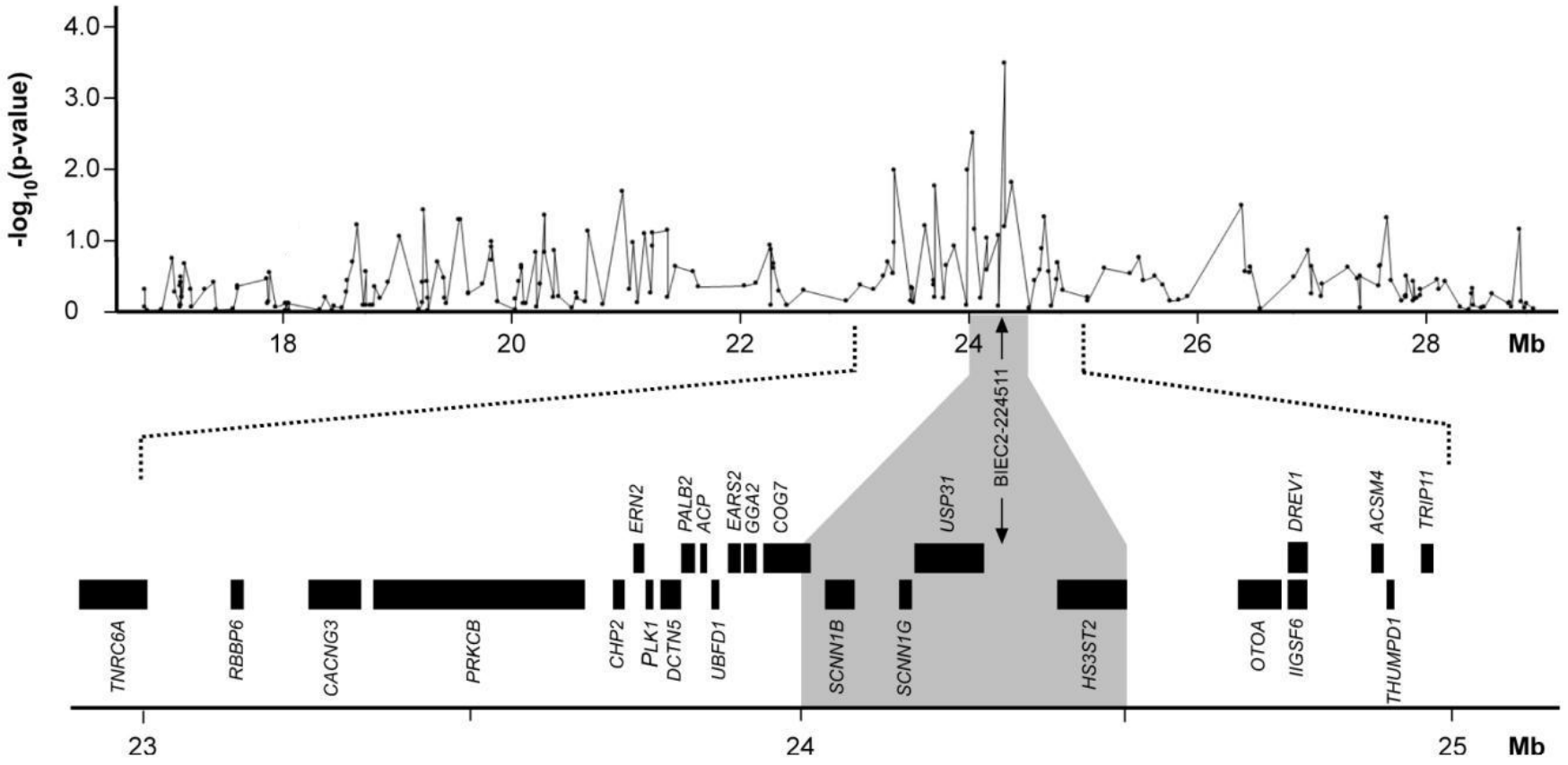
The T-allele at this SNP was associated with RAO both in the family and the unrelated horses.

The mutant T-allele increases the RAO risk.

OR = 1.14 - 1.82 (95% CI).

Independent support for the previously detected QTL

New Positional Candidate Genes



Positional Candidate Genes

Sodium channel subunits genes: SCNN1B & SCNN1B

alter physical properties of mucus

low *SCNN1B* impairs lung fluid clearance in the mouse

defects in *SCNN1G* implicated in bronchiectasis

Positional Candidate Genes

USP31 ⟶ the best SNP ⟵ *HS3ST2*

HS3ST2 ⟶ heparan sulfate 3-o-sulfate formation

specific binding sites for a variety of proteins, including chemokines

USP31 is part of the general protein degradation machinery.

↓ USP31 ⇨ ↑ TNFα mediated NF-kappaB activation

Mutation analysis of all four candidate genes

Amplification of all coding parts from 2 cases and 2 controls

Sequencing the PCR products on an ABI 3730

114 variants
Including:
4 missense mutations
2 variable coding tandem repeats

Association Study

Genotyping the promising mutations in 465 horses

Illumina Golden Gate assay on a BeadXpress station
or direct sequencing

no stronger association than previous marker BIEC2-224511

Take Home Message

The T-allele at SNP BIEC2-224511 had the best association with RAO both in the family and the unrelated horses.

The association study allowed further narrowing of the QTL interval to about 500 Kb (24.0-24.5 Mb).

No associated coding variants implies that the causative variant underlying this QTL is most likely a regulatory mutation.

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