A nonsense mutation in *B3GALNT2* is concordant with hydrocephalus in Friesian horses

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KONINKLIJK FRIESCH PAARDEN-STAMBOEK

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Introduction

Friesian horse breed

- Inbreeding rate >1%
- Higher incidence of genetic defects

Hydrocephalus

- Communicative – external – impaired absorption
- Often stillborn, dystocia in dams

Incidence expected to be a few percent!

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Introduction

In humans, ~40% cases known genetic cause

- X-linked recessive: *L1CAM*
- Autosomal recessive: *CCDC88C* (HSA14) and *MPDZ* (HSA9)

Our aims

- Investigate mode of inheritance
- Develop DNA assay
- Identify causal mutation

Materials and Methods

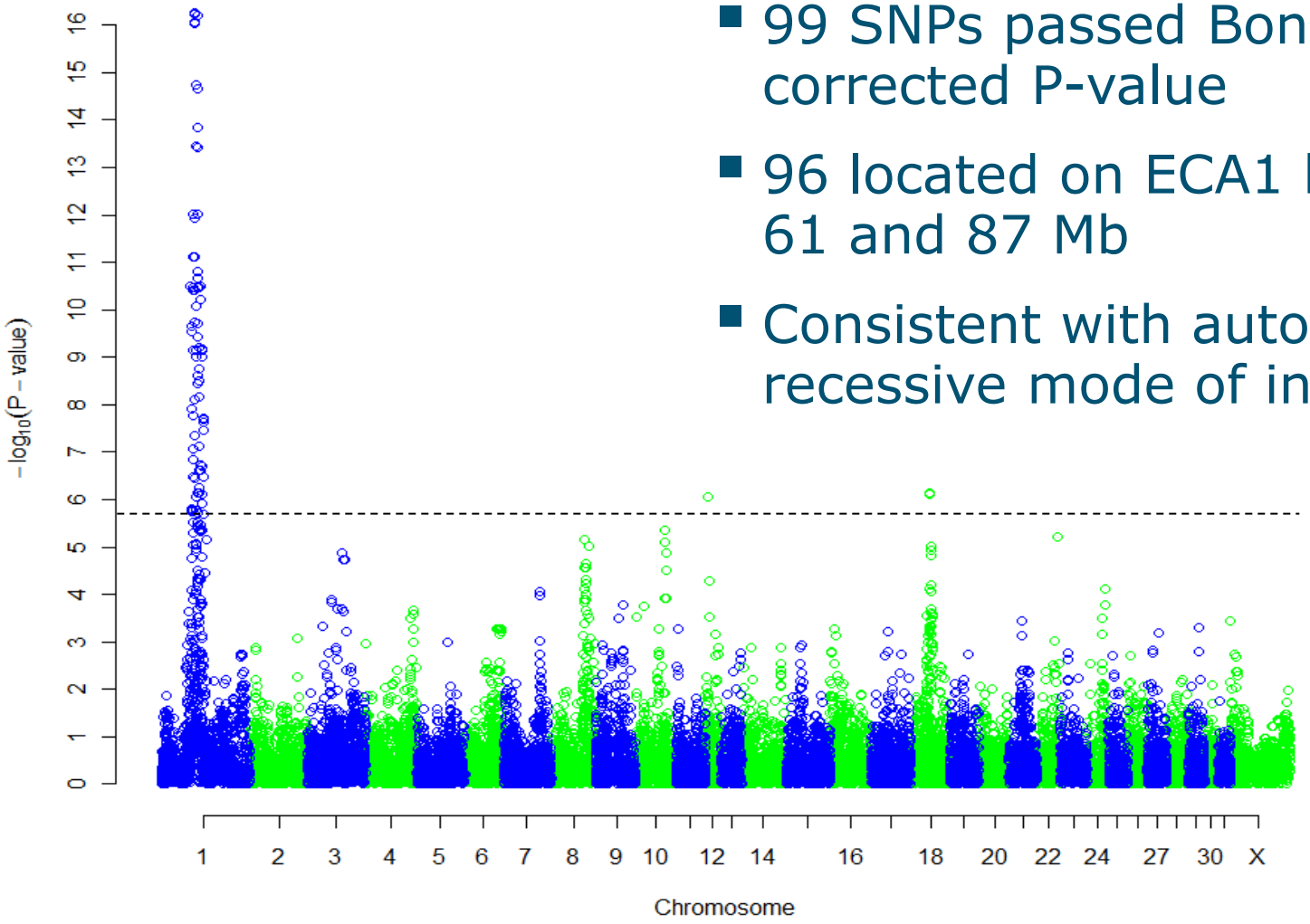
Materials

- 13 cases and 69 controls
- Illumina® EquineSNP50 Genotyping BeadChip

Methods

- After QC: 29,720 SNPs
- Single SNP association
- χ^2 test 2 × 3 (genotypic) tables
- Bonferroni corrected significance level: 1.68×10^{-6}
- Regions of homozygosity

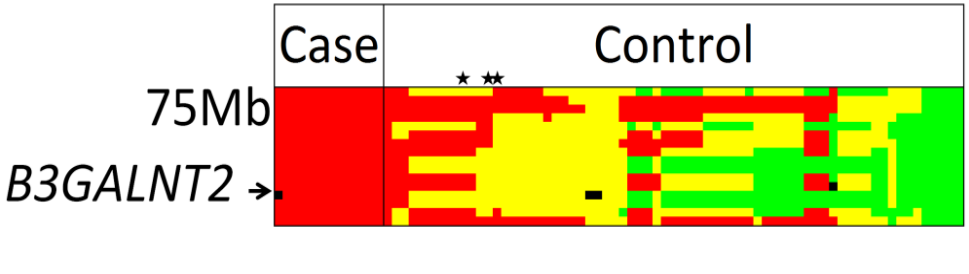
Results – GWAS

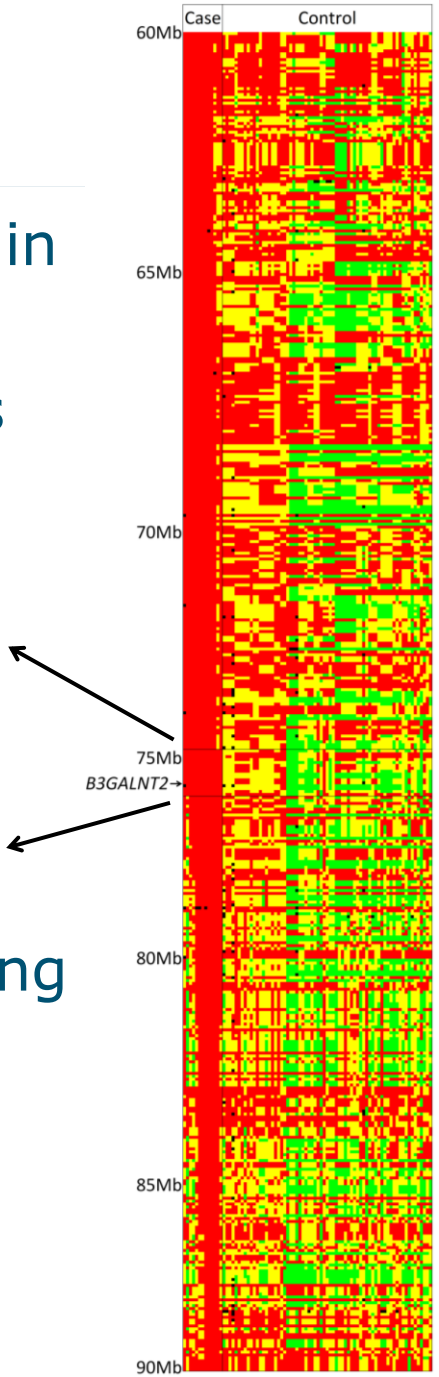


- 99 SNPs passed Bonferroni corrected P-value
- 96 located on ECA1 between 61 and 87 Mb
- Consistent with autosomal recessive mode of inheritance

Results – homozygosity

- Homozygosity extended for at least 10.5Mb in 12 out of 13 cases
- All 13 cases homozygous with shared alleles and 1.47Mb in length





- Sequence sufficiently large region surrounding associated SNPs

Methods and Results – NGS

NGS of 4 cases and 6 controls

- Exons and adjacent 20bp of genes in region of interest

3 variants fitted case-control model recessive disorder

- 2 known (missense) variants in *LYST* and *NID1*
- variant in *B3GALNT2* very likely candidate

Mutation in *B3GALNT2*

- Nonsense allele T (c.1423C>T)
- ECA1:75,907,505 (exon 12)
- Effect on protein (p.Gln475*)

	TT	TC	CC
Cases	4	0	0
Controls	0	1	5

Methods and Results – validation

Validation: genotyping mutation Sanger sequencing

Population screening: Friesian horses DNA tested mutation

	TT	TC	CC	Total	
Validation					
Cases	16	0	0	16	
Controls	0	32	36	68	all 17 dams of cases TC
Population screening					
Controls ♂	0	8	52	60	13% carrier
Controls ♀	0	139	666	805	17% carrier

Estimate frequency T allele is 0.085

Discussion

- Hydrocephalus recognized in many species: livestock, laboratory animals and pets
- In humans, genetic heterogeneity

  → the American Journal of Human Genetics (2013)

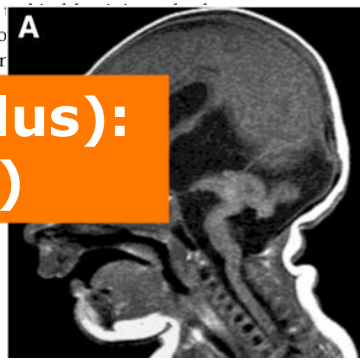
B3GALNT2

MDDGA11: muscular dystrophy-dystroglycanopathy with brain and eye anomalies, type A, 11

***B3GALNT2* knockdown zebrafish embryos: hydrocephalus phenotype**

4 out of 6 patients including hydrocephalus

mutation patient 7 (with hydrocephalus): exon 12 c.1423C>T (homozygous)



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Discussion – muscular dystrophy

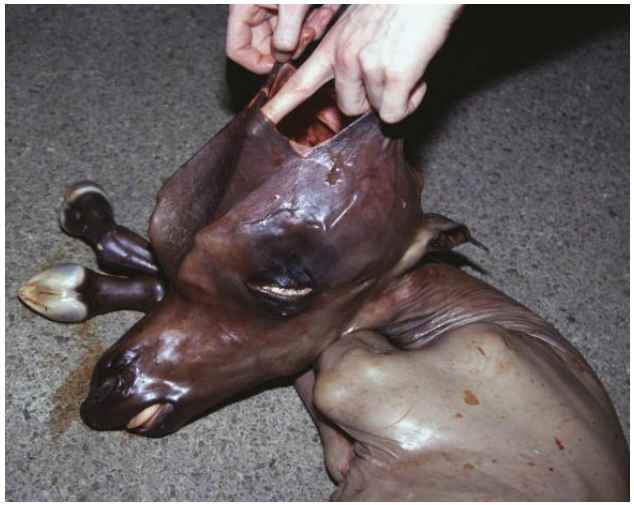
In Friesian horses muscular dystrophy never considered

- Affected foals stillborn, no clinical observation

Exact same nonsense mutation as in humans

- Same complex might underlie the phenotype observed in Friesian horses

Subsequent immunohistochemical examination of muscle biopsies



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With an application in practice! A direct DNA assay for hydrocephalus in Friesian horses is now available.

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