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Forskningscentralen för jordbruk och livsmedelsekonomi

Abnormal gene expression caused by retroviral insertion results in the immotile short tail sperm defect in the Finnish Large White

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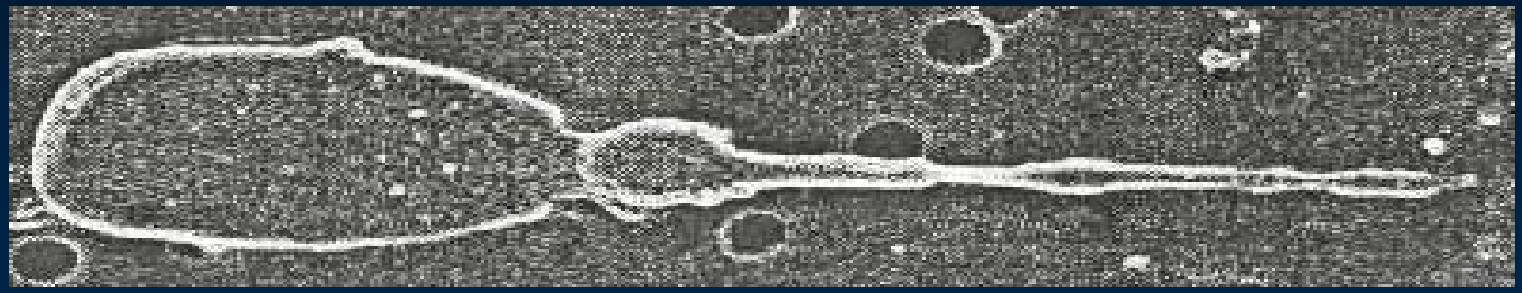
Immotile short tail sperm (ISTS)

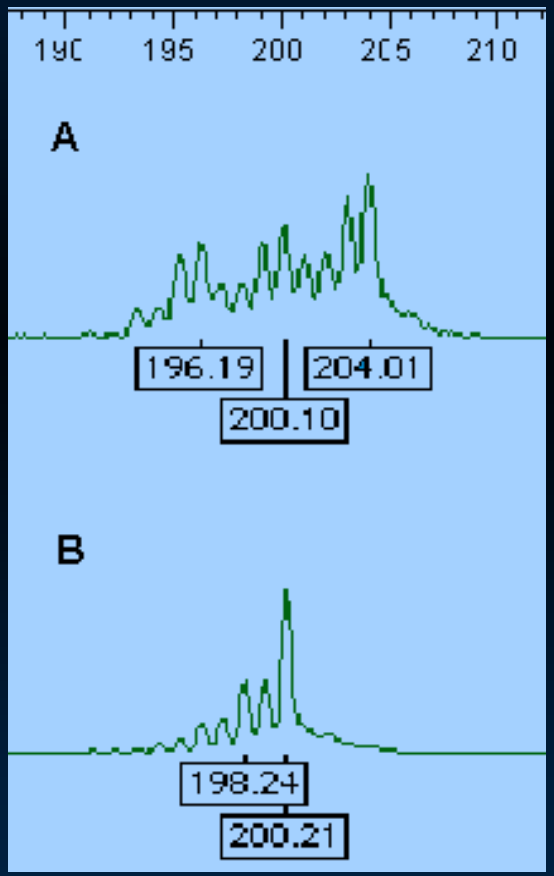
- First case in the Finnish Yorkshire in 1987
- Became common at the end of 1990
- Presently 85 boars identified with ISTS
- Autosomal recessive inheritance
- Similar symptoms have been described in other species (Kartagener syndrome)

The ISTS phenotype

- Immotile sperm
- Shortened sperm tail
- Oligospermia
- The 9+2 axonemal structure is severely altered
- No effect on respiratory function or female reproduction ➤ seems to only affect sperm flagella



Genome mapping



- Homozygosity mapping and DNA pooling used for genome scan
- 228 autosomal microsatellite markers (The U.S. pig genome project)
- One marker revealed a significant difference in allele distribution between affected and control boars

Haplotypes

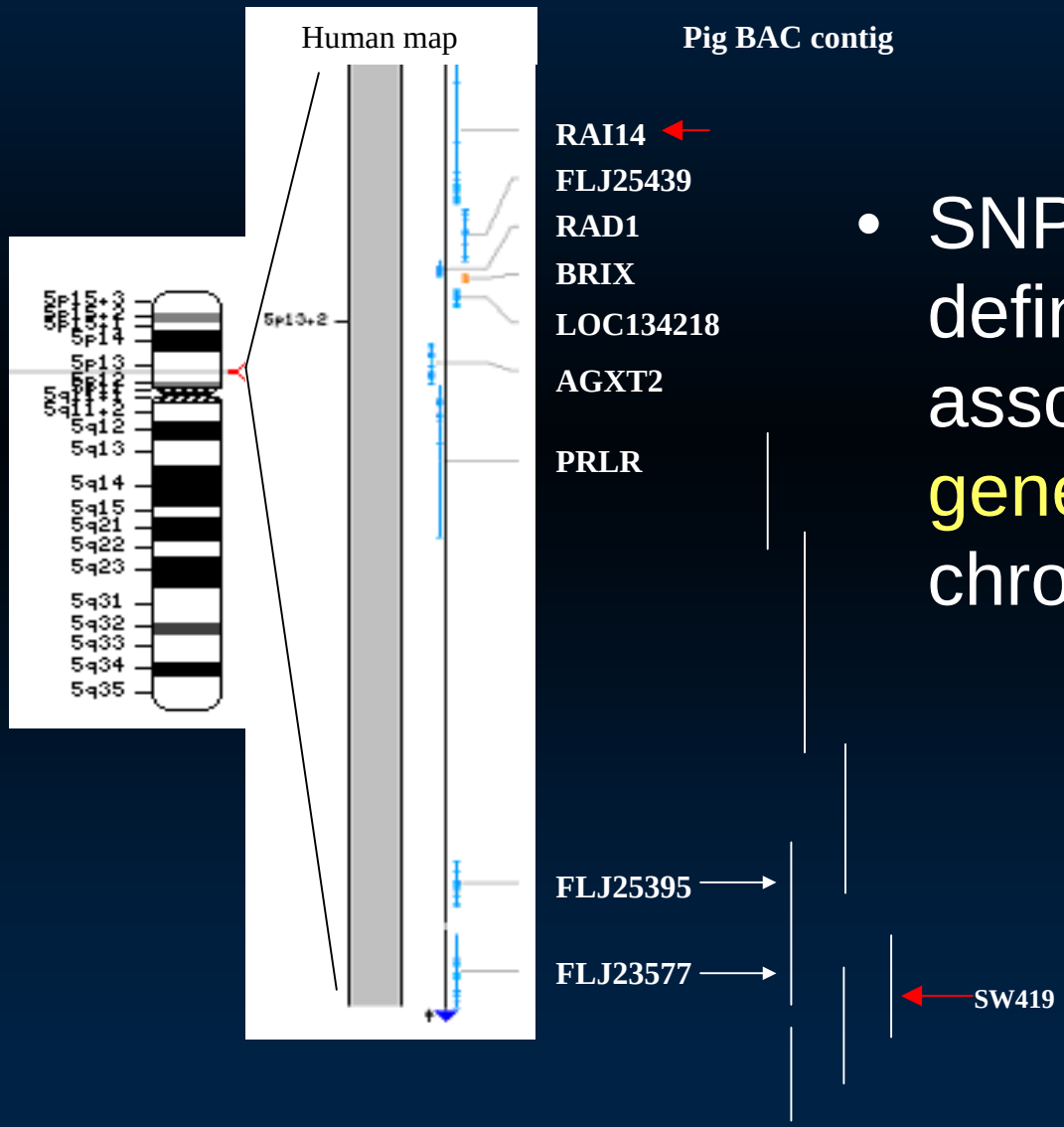
- Affected chromosome haplotypes indicate that mutation maps between markers SW2411 and SW419 → markers for MAS

SW1035	SW2411	ISTS	SW419	Total
2	3	-	4	1
2	3	-	1	57
1	3	-	1	1
2	1	-	1	1
3	4	-	1	2
1	4	-	1	8

Fine-mapping

- BAC-clones were picked up with two disease-associated markers
- Chromosome walking with end sequences
- Comparative mapping (DIAS) defined the disease associated region to 2 Mbp on human chromosome 5
- Sequence analysis of porcine orthologs revealed several SNPs

Comparative mapping



- SNPs within RAI14 defined the disease-associated region to 8 genes on human chromosome 5

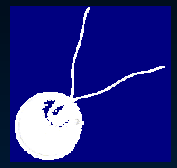
Candidate gene

- Expressed in the rat testis and during cilia development (tissue specific)

  -In seminiferous tubules stage-specific expression

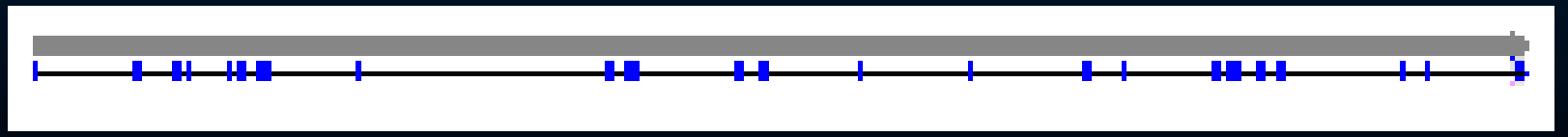
  -In tracheal epithelial cells the expression closely paralleled with axonemal dynein

- Similarity to CPC1 in the *Chlamydomonas* (unicellular green alga)



- CPC1 is essential for the assembly of the central pair structure

Candidate gene

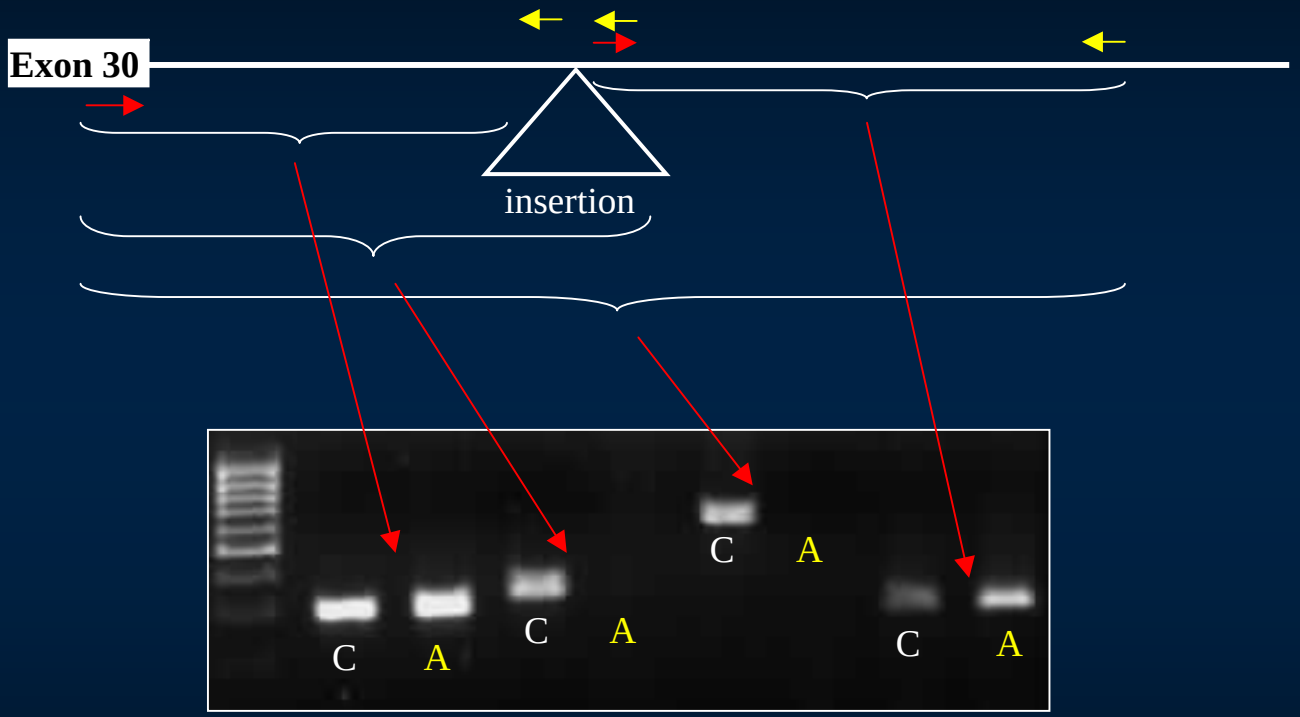


Genomic sequence of the human gene 150 000 bp

→ mRNA 5580 bp 43 exons

- mRNA from the pig testis isolated and sequenced
- Exon 30 missing in affected boars

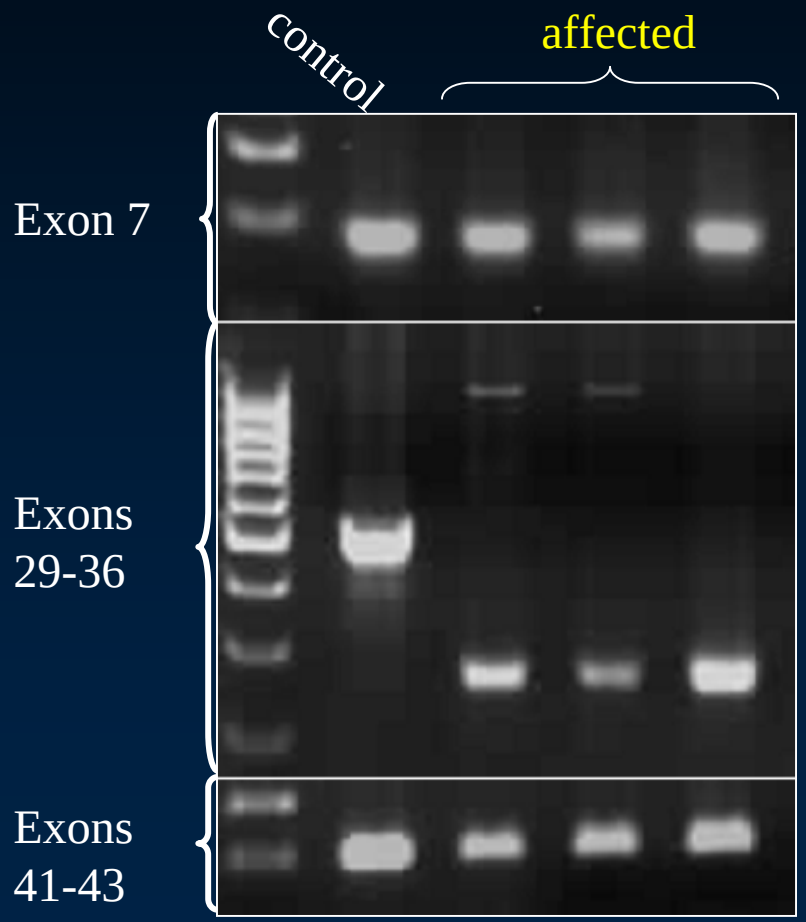
Genomic mutation



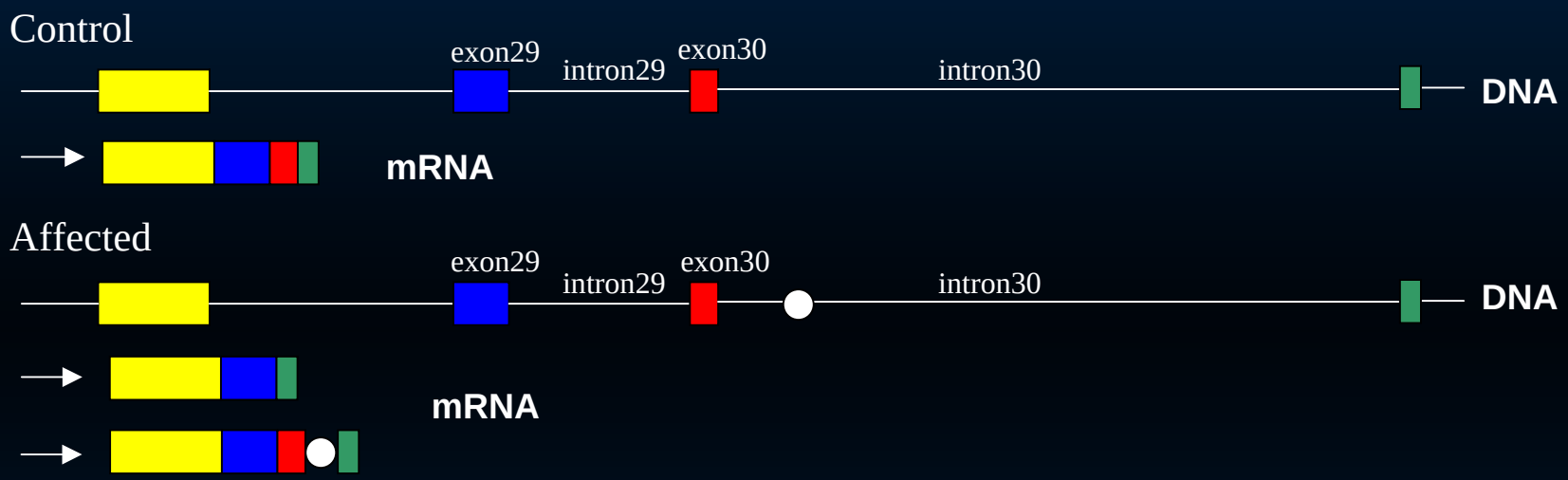
C=control A=affected

- Sequencing of the fragment revealed an inserted porcine endogenous retrovirus

RT-PCR for gene expression in the testis



Insertion affects splicing



- In most affected transcripts exon 30 is skipped
- In a few cases, exon 30 is present together with part of the insertion sequence
- Translation stop codons are created in both cases

Conclusions

- ISTS locus mapped to porcine chromosome 16 and the causal gene for the defect identified
- At the RNA level, ISTS appears to be due to premature translation stop codons in the testis specific transcripts created by abnormal splicing
- A retroviral insertion within intron 30 seems to be the cause for the altered splicing pattern

Thank you for your attention!