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Molecular characterization of the porcine *DNAL4* gene

Abstract
The *DNAL4* (dynein, axonemal, light polypeptide 4) gene encodes a light chain of dynein. Dyneins are motor proteins that contribute to axonal transport. Cloning and characterization of the porcine *DNAL4* revealed a conserved organization with respect to the human ortholog. The porcine *DNAL4* gene consists of 4 exons and codes for a peptide of 105 amino acids. The porcine *DNAL4* gene is located on SSC5p15. Analysis of the naturally occurring variation of the *DNAL4* gene in pigs from the Piétrain und Duroc breeds revealed five SNPs in non-coding regions of the gene.

Key Words: DNAL4, dynein, sequence, polymorphism, pig

Zusammenfassung
Titel der Arbeit: **Molekulare Charakterisierung des porcinen *DNAL4* Gens**
Das *DNAL4* (dynein, axonemal, light polypeptide 4) Gen kodiert für eine kleine Untereinheit des Dyneins. Dyneine sind Motorproteine, die den axonemalen Transport vermitteln. Die Klonierung und Charakterisierung des porcinen *DNAL4* Gens zeigte eine konservierte Genstruktur im Vergleich zum humanen Ortholog. Das porcine *DNAL4* Gen besteht aus 4 Exons und kodiert für ein Peptid von 105 Aminosäuren. Das porcine *DNAL4* Gen ist auf SSC5p15 lokalisiert. Die Analyse der natürlich vorkommenden Variabilität des *DNAL4* Gens bei Schweinen der Rassen Piétrain und Duroc lieferte fünf SNPs in nicht-kodierenden Regionen des Gens.

Schlüsselwörter: *DNAL4*, Dynein, Sequenz, Polymorphismus, Schwein

Introduction
Dyneins and kinesins are microtubule motor proteins and transport their cargoes in opposite directions along microtubules. Dyneins generate force toward the minus end of microtubules and have ATPase activity. Dyneins are classified according to their form in cytoplasmic and axonemal dyneins. Most are axonemal, referring to their role in ciliary and flagellar movement (VALLEE et al., 2003). Dyneins contain as their largest subunit a heavy chain (HC) polypeptide of more than 500 kDa, which is responsible for ATPase and motor activities. The dyneins also contain a variety of accessory subunits, termed intermediate, light intermediate and light chains, some of which are common to cytoplasmic and axonemal dyneins, whereas others are specific to dynein subclasses (VALLEE et al., 2003).
We are currently characterizing a QTL region on SSC5 for drip loss in pork. In order to generate gene-associated markers for this region we provide here the genomic organization, DNA sequence, and polymorphisms of the porcine *DNAL4* gene.

Materials and Methods
In silico identification of a BAC clone and sequencing of the porcine *DNAL4* gene
The pig BAC end sequence information displayed at the ensemble browser v37 was used to identify a porcine BAC clone corresponding to the region of the human

DNAL4 gene which is located on HSA 22 (http://www.ensembl.org). Clone RP44-469M15 of the RPCI-44 male porcine BAC library (http://bacpac.chori.org) provided full coverage for the *DNAL4* gene and was therefore selected for further analysis.
DNA from the clone RP44-469M15 was isolated using the Qiagen plasmid midi kit (Qiagen, Hilden, Germany). Primers for DNA sequencing were designed on the available EST sequences for the porcine *DNAL4* gene. The complete DNA sequence of the exons and flanking regions of the porcine *DNAL4* gene was then determined on both DNA strands by a primer walking strategy using BAC DNA as sequencing template. Sequencing reactions were performed with the BigDye v3.1 kit and separated on an ABI 3730 capillary sequencer (Applied Biosystems, Rotkreuz, Switzerland).
Porcine ESTs corresponding to the *DNAL4* gene were identified by BLASTN searches with the human *DNAL4* cDNA sequence (NM_005740). Clustering of these ESTs revealed the putative porcine *DNAL4* cDNA sequence, which was used to define the exact intron/exon boundaries in the genomic sequence.

Mutation analysis

To identify variations within the porcine *DNAL4* sequence, exons with their flanking regions were PCR amplified from 9 Piétrain and 9 Duroc pigs. PCR primers are given in Table 1. All PCR products were amplified with hot start Polymerase AmpliTaq Gold (Applied Biosystems, Rotkreuz, Switzerland). PCR products were treated with shrimp alkaline phosphatase and endonuclease I (Roche, Rotkreuz, Switzerland). PCR products were directly sequenced using the sequencing chemistry described above.

Table 1
PCR primers for the amplification of *DNAL4* exons (PCR Primer zur Amplifikation der *DNAL4* Exons)

Exon	Primer	Sequence (5' – 3')	T_M [°C]	Product length [bp]
exon 1	Ex1_FA	ATG AGA ACT TCT GCG TGT GG	58	414
	Ex1_RA	AAC AGG CAG GAG ACT GAG GA		
exon 2	Ex2_F	ACG TGT GCT GGG AAG TAA GG	60	885
	Ex2_R	CCC TGC ACC TGC TTT ATT TG		
exon 3	Ex3_FA	CCT GTC AGG AGA TGG GTC AT	60	358
	Ex3_RA	TTC CAA GGA AGG AAG GCT CT		
exon 4	Ex4_FA	GTG CGG AAA CCC TCT GAC	60	536
	Ex4_RA	GAG AGG GCA GTT CCA CGT C		

Radiation hybrid mapping

Forward (5'-ACGTGTGCTGGGAAGTAAGG-3') and reverse PCR primer (5'-CCCTGCACCTGCTTTATTTG-3'), both flanking exon 2 of the porcine *DNAL4* gene, were used for the analysis of the INRA-Minnesota Porcine Radiation Hybrid (IMpRH) panel (YERLE et al., 1998). These primers generated a PCR product of 885 bp on pig genomic DNA. PCR reactions were performed using 25 ng of RH cell line DNA and PCR products were separated on 1 % agarose gels. The analysis was done in duplicate and each experiment was scored independently by two investigators. The RH results were submitted to the IMpRH database. The mapping tool at the IMpRH Server (MILAN et al., 2000) was used for the mapping of this new marker.

Fluorescence *in situ* hybridization (FISH) analysis

The porcine genomic BAC clone RP44-469M15 containing the porcine *DNAL4* gene was labeled with digoxigenin by nick translation using a Nick-Translations-Mix (Boehringer Mannheim, Mannheim, Germany). FISH on GTG-banded pig chromosomes was performed using 750 ng of digoxigenin labeled BAC DNA. In this experiment 24 µg sheared total porcine DNA and 10 µg salmon sperm were used as competitors. After hybridization over night, signal detection was performed using a Digoxigenin-Rhodamin Detection Kit (Quantum Appligene, Heidelberg, Germany). The chromosomes were counterstained with DAPI and embedded in antifade. Thirty metaphases that were previously photographed were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Results and discussion

Genomic organization of the porcine *DNAL4* gene

In order to isolate a genomic clone with the porcine *DNAL4* gene, the publicly available BAC end sequence information was utilized. BLAST analysis of the BAC clone end sequences with respect to the human genome established that the clone RP44-469M15 contained the entire *DNAL4* gene. The BAC clone RP44-469M15 was selected for sequencing and the sequence of the coding parts of the *DNAL4* gene was submitted to the EMBL nucleotide databases (Accession AM284969).

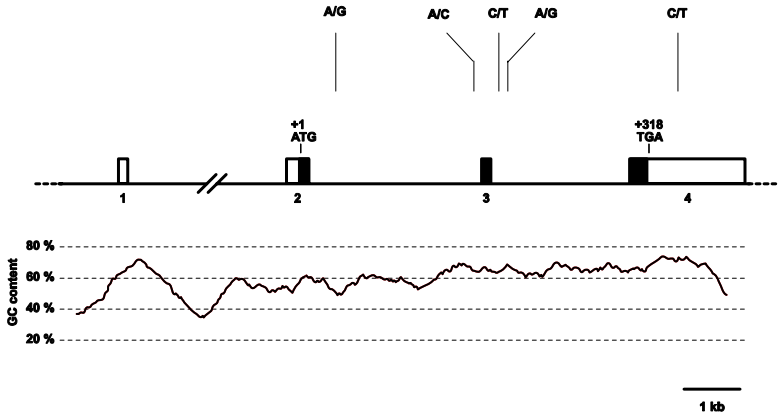

Fig. 1: Genomic structure of the porcine *DNAL4* gene. Translated exons are shown as solid boxes. Untranslated regions of exons are shown as open boxes. The position of the five newly described SNPs are indicated at the top. In the lower part, the GC content is shown. For the calculation of the GC content a 300 bp window was used (Genomstruktur des porcinen *DNAL4* Gens. Translatierte Exons sind als ausgefüllte Rechtecke, untranslatierte Exonbereiche als offene Rechtecke dargestellt. Die Position der fünf neu beschriebenen SNPs ist oberhalb des Gens angedeutet. Im unteren Teil der Abbildung ist der GC-Gehalt gezeigt. Für die Berechnung des GC-Gehalts wurden jeweils Abschnitte von 300 bp analysiert)

The exon/intron structure of the porcine *DNAL4* gene was determined by comparing the genomic sequence to available porcine EST sequences as well as to the human *DNAL4* sequence (Fig. 1). Our analysis of the genomic structure revealed that the porcine *DNAL4* gene contains 4 exons with exon/intron boundaries that conform perfectly to the GT/AG rule (Table 2). The genomic structure between the porcine and the human *DNAL4* genes is conserved. Similar to the human *DNAL4* gene the porcine *DNAL4* gene contains a CpG island at its 5'-end. At the 3'-end of the porcine *DNAL4*

gene a variant polyadenylation signal ATTAAA is located 842 bp downstream of the stop codon.

Table 2
Exon/intron boundaries of the porcine *DNAL4* gene (Exon/Intron Grenzen des porcinen *DNAL4* Gens)

3'-Splice site	Exon	5'-Splice site	Intron phase	Intron size
		-138		
	... (exon 1, >81 bp) ...	*GAGCAG**gt**gaggcctcggggc*		>1300 bp
-139		+69		
*tcctgttttctac**ag**CAACCC*	... (exon 2, 208 bp) ...	GTCAGG**gt**aaggcccagggga	0	1525 bp
+70		+153		
ccgccctgtcccc**ag**CACTCG	... (exon 3, 84 bp) ...	AACGAG**gt**actggcgccagtg	0	1210 bp
+154		+1167		
ctcctctccttcc**ag**AGCGCC	... (exon 4, >1028 bp) ...	ACAATTAAAATAACCAAAAAC		

Exon sequences are shown in uppercase letters, and intron sequences in lowercase letters. Untranslated regions are shown in italics. The conserved GT/AG exon/intron junctions are shown in boldface type. For the last exon the polyadenylation signal is shown underlined instead of an exon/intron junction. +1 corresponds to the adenine of the translation initiation codon ATG.

Analysis of the porcine *DNAL4* cDNA sequence

The *DNAL4* open reading frame is contained in exons 2 to 4 and consists of 318 nt encoding a protein of 105 amino acids that shows 100 % identity to the human Dnal4 protein and 99 % identity to the mouse Dnal4 protein.

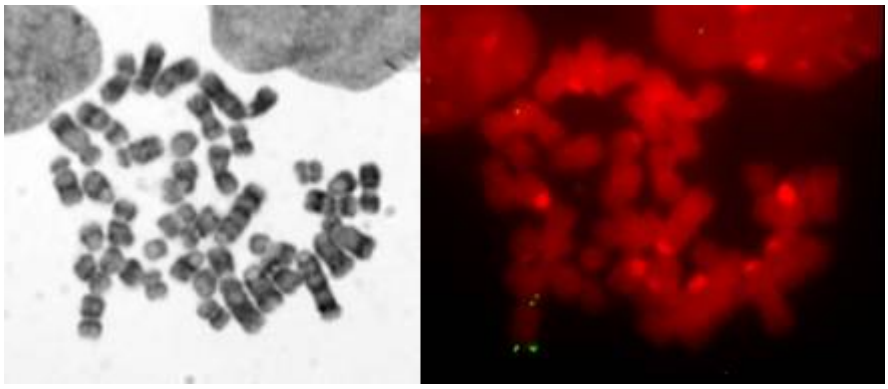

Fig. 2: Chromosome assignment of the *DNAL4* gene by FISH analysis on a porcine metaphase spread. The digoxigenin labeled BAC clone RP44-469M15 containing the porcine *DNAL4* gene was hybridized to GTG-banded metaphase chromosomes of a normal pig. Double signals are visible on both chromosomes 5p15. The chromosomes were subsequently identified by DAPI staining (Chromosomale Zuordnung des *DNAL4* Gens durch FISH Analyse auf porcinen Metaphasechromosomen. Der Digoxigenin-markierte BAC Klon RP44-469M15 mit dem porcinen *DNAL4* Gen wurde mit GTG-gebänderten Metaphasechromosomen eines normalen Schweins hybridisiert. Doppelte Signale sind auf beiden homologen Chromosomen 5p15 zu erkennen. Die Chromosomen wurden anschliessend durch DAPI-Färbung identifiziert)

Chromosomal localization of the porcine *DNAL4* gene

The BAC clone containing the porcine *DNAL4* gene was fluorescently labeled and hybridized to metaphase chromosomes. Specific hybridization signals were obtained at SSC 5p15 (Fig. 2). To confirm the localization of the porcine *DNAL4* gene, the IMpRH porcine radiation panel was analyzed. Two-point analysis revealed close linkage of *DNAL4* to the microsatellite markers AC02 at a distance of 33 cR (LOD

score 11.50) and to SW1482 (distance 41 cR, LOD score 9.85) both located on SSC 5. Multipoint analysis placed the *DNAL4* gene between AC02 and the more distal microsatellite SW1482 by minimization of breakage number. The chromosomal localization of the *DNAL4* gene at SSC 5p15 is in agreement with the known pig-human comparative map (RINK et al., 2002).

Polymorphisms of the porcine *DNAL4* gene

In order to investigate whether different alleles of the porcine *DNAL4* gene exist, four PCR products spanning the first three *DNAL4* exons with flanking regions and the coding part of the last exon, respectively, were amplified from 18 different pigs and sequenced. The animals were taken from the two genetically diverse Piétrain and Duroc pig breeds. Comparison of the individual DNA sequences revealed 5 polymorphic sites (Table 3). All of these 5 polymorphic sites are located in non-coding regions. The last 4 polymorphic sites were in perfect linkage disequilibrium in the limited sample. It is unknown whether those polymorphisms have any functional consequences, e.g. on the *DNAL4* expression.

Table 3
Polymorphisms within the porcine *DNAL4* gene (Polymorphismen im porcinen *DNAL4* Gen)

SNP position	SNP position[1]	Breed	Genotypes			Allele frequencies
IVS2+167	2383		AA	AG	GG	
		Duroc	0	1	8	0.06 / 0.94
		Piétrain	3	3	3	0.50 / 0.50
IVS2+1444	3660		CC	AC	AA	
		Duroc	0	0	9	0.00 / 1.00
		Piétrain	0	3	6	0.17 / 0.83
IVS3+4	3829		TT	CT	CC	
		Duroc	0	0	9	0.00 / 1.00
		Piétrain	0	3	6	0.17 / 0.83
IVS3+37	3862		AA	AG	GG	
		Duroc	0	0	9	0.00 / 1.00
		Piétrain	0	3	6	0.17 / 0.83
+439 (exon 4)	5321		TT	CT	CC	
(3'-UTR)		Duroc	0	0	9	0.00 / 1.00
		Piétrain	0	3	6	0.17 / 0.83

[1] positions refer to the genomic sequence of 6097bp (EMBL: AM284969)

In conclusion, this study describes genomic organization and sequence of the porcine *DNAL4* gene along with several polymorphism that are present in the pig population and can be used for linkage or association studies.

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References

MILAN. D.; HAWKEN, R.; CABAU, C.; LEROUX, S.; GENET, C.; LAHBIB, Y.; TOSSER, G.; ROBIC, A.; HATEY, T.; ALEXANDER, L.; BEATTIE, C.; SCHOOK, L.; YERLE, M.; GELLIN, J.:
IMpRH Server: an RH mapping server available on the Web. Bioinformatics **16** (2000), 558-559

RINK, A.; SANTSCHI, E.M.; EYER, K.M.; ROELOFS, B.; HESS, M.; GODFREY, M.; KARAJUSUF, E.K.; YERLE, M.; MILAN, D.; BEATTIE, C.W.:
A first-generation EST RH comparative map of the porcine and human genome. Mamm. Genome **13** (2002), 578-587

VALLEE, R.; WILLIAMS, J.; VARMA, D.; BARNHART, L.:
Dynein: An ancient protein involved in multiple modes of transport. J. Neurobiology **58** (2003), 189-200

YERLE, M.; PINTON, P.; ROBIC, A.; ALFONSO, A.; PALVADEAU, Y.; DELCROS. C.; HAWKEN, R.; ALEXANDER, L.; BEATTIE, L.B.; MILAN, D.; GELLIN, J.:
Construction of a whole genome radiation hybrid panel for high-resolution gene mapping in pigs. Cytogenet. Cell Genet. **82** (1998), 182-188

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