Inheritance and Genetic Mapping of the Campus Syndrome (CPS): A High-Frequency Tremor Disease in Pigs


A new progressive tremor disorder called Campus syndrome (CPS) was observed among the progeny of a normal boar of the Pietrain breed in Germany. Extensive backcross experiments indicate that CPS is inherited as an autosomal dominant trait, and the founder boar, Campus, is believed to be a gonadal mosaic. A linkage analysis of 57 animals mapped the CPS gene to a region on porcine chromosome 7 flanked by the markers SW1418 and SW352, which is homologous to a part of human chromosome (HSA) 14. Human dominant distal myopathy type 1 (MPD1) has been mapped to the homologous region of HSA14. As the myopathological findings in MDP1 show striking similarities to CPS, this porcine disorder may serve as an animal model for MPD1.

Tremor disorders in humans are a heterogeneous disease manifestation with often unknown etiopathogenesis. This heterogeneity presents a major obstacle to the direct isolation of causative genes, and well-defined animal models of tremor disorders in swine can therefore provide useful tools for the mapping and cloning of such genes.

In pigs, four inherited tremor syndromes have been characterized: a congenital sex-linked recessive form in Landrace pigs (type AIII) and a congenital autosomal recessive form in Saddlebacks (type AIV) (Done 1976; Harding et al. 1973)—both are caused by hypomyelino-genesis; an autosomal recessive primary myopathy in the Pietrain called Creeper syndrome (Wells et al. 1980), in which the muscular tremor begins at about 3 weeks of age and becomes progressively worse (Bradley and Wells 1980); and a progressive neurodegeneration with muscular tremor of the thighs, which develops 2–20 weeks postnatally (O'Toole et al. 1994).

This report describes the genetic characterization and mapping of a new hereditary tremor disorder in the Pietrain breed: Campus syndrome (CPS). CPS is characterized by a progressive high-frequency tremor and a reduced life expectancy in affected animals (Richter et al. 1995; Schulze et al. 1996; Wissel et al. 1997).

Our breeding studies indicate that CPS is inherited as an autosomal dominant trait, and the founder boar, Campus, is believed to be a germline mosaic. Initial genotyping of three animals allowed us to dismiss several chromosomal areas for linkage and thus accelerated the mapping of CPS to a region spanning less than 10 cM on pig chromosome (SSC) 7q1.5–2.3.

Materials and Methods

Origin and Phenotype

Piglets with a progressive tremor were identified among the progeny of a clinically normal boar of the Pietrain breed housed in a commercial center for artificial insemination in southern Germany. The boar Campus was transferred to the Hanover School of Veterinary Medicine to characterize the defect further. Affected piglets are indistinguishable from their littermates until the first clinical signs appear at 2–9 weeks of age. A muscular tremor of the hind and fore quarters is observed when the piglets are standing or moving. At rest, the tremor ceases immediately. Clinical examination of affected piglets revealed an elevated body temperature of 40–41°C, and increased plasma lactate levels. Affected piglets are very susceptible to stress. Their life expectancy is markedly reduced to 3–18 months, and most animals die before sexual maturity. Genotyping at the ry-
Table 1. Results of matings with the founder boar Campus (A,B), with three affected sows (C–E), and with a healthy offspring from an affected sow (F)

<table>
<thead>
<tr>
<th>Sire</th>
<th>Dam</th>
<th>No. of dams</th>
<th>No. of litters</th>
<th>No. of piglets weaned</th>
<th>No. of litters</th>
<th>Piglets weaned</th>
<th>Affected piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Campus</td>
<td>F₀</td>
<td>11</td>
<td>19</td>
<td>148</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td>Campus</td>
<td>F₁</td>
<td>14</td>
<td>19</td>
<td>152</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>A + B</td>
<td></td>
<td></td>
<td>25</td>
<td>38</td>
<td>300</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>C</td>
<td>Campus</td>
<td>AS</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>F₁</td>
<td>AS</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>F₂</td>
<td>AS</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C + D + E</td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>15</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>Junior</td>
<td>SA</td>
<td>6</td>
<td>7</td>
<td>68</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

Campus = founder boar.
F₀ = unrelated dam/sire.
F₁ = F₁ generation, healthy offspring from affected litter.
AS = affected sow.
Junior = healthy offspring of Campus × affected sows (animal 94 in Figure 1).
SA = F₀ or F₁ sow which had at least one affected litter.

Breeding Studies
In order to clarify the mode of inheritance, a total of 83 litters and 650 offspring were produced. All piglets were observed until at least 10 weeks of age to ensure identification of late-onset cases. The boar Campus was mated repeatedly to 11 sows of different breeds (F₀) [3 Pietrain (Pi), 3 Large White (DE), 2 German Landrace (DL), 1 Duroc (Du), 1 Hampshire (Ha), and 1 crossbred Pietrain × German Landrace]. A total of 19 F₁ litters were born and 148 piglets weaned (Table 1A). Healthy offspring from affected litters were reared for backcross (19 litters, 152 piglets weaned; Table 1B) and intercross matings (results not shown). With intensive medical care, three affected females (AS) could be reared until puberty and mated to Campus (one litter, two piglets weaned; Table 1C), to a normal son from an affected litter (one litter, seven piglets weaned; Table 1D), and to a normal, completely unrelated boar of the German Saddleback breed (one litter, six piglets weaned; Table 1E). The first two litters were born after foster sows after embryo transfer. The third litter was carried to term by the affected mother herself. Finally, a healthy boar (Junior) from an affected mother was mated to six sows which had had at least one affected litter (SA) (7 litters, 68 piglets weaned; Table 1F).

Genetic Mapping
To identify informative markers, a set of 254 porcine microsatellite primers covering the porcine genome (233 markers were provided by the U.S. pig genome project: http://www.public.iastate.edu/~pigmap) was tested on the founder boar and two affected offspring. As the founder boar was believed to be a germline mosaic (see results), only the founder boar Campus, affected offspring (n = 26), and their unaffected mothers; together with 10 affected and 5 normal piglets from matings with three affected F₁ sows, were selected for further genotyping, making a total of 58 animals (Figure 1). The power for linkage analysis of this pedigree was proven using

Figure 1. Campus pedigree showing allele segregation and disease haplotype for individuals included in linkage analysis. The founder boar Campus is shown as a striped box and is repeated in the pedigree. Affected offspring are symbolized by black boxes (males) or circles (females). Crossed boxes indicate members of the family for whom DNA samples were not available. The order of markers is according to Rohrer et al. (1997), as shown in Table 3. Genotypes for SW1418 are shown only for informative families. Frames indicate the haplotype cosegregating with the disease. Recombination events (R) observed in animals 47 and 56 (○) locate the CPS gene proximal to SW352 and distal to SW1418.
the simulation software SLINK (Weeks et al. 1990). This set of animals was then analyzed with informative markers from SSC 7. The PCR reaction mix contained 12.5 ng DNA, standard PCR buffer (Appligene, Heidelberg, Germany), 30 μM of each dNTP (using dUTP instead of dTTP) (Pharmacia, Freiburg, Germany), 5 pmol of each primer, and 0.25 U Taq polymerase (Appligene, Heidelberg, Germany) in a final volume of 10 μl. PCR was performed in microtiter plates with a PTC-100TM cycler (MJ Research, Inc.). After denaturation at 94°C for 3 min, 30 cycles of PCR were performed (30 s at 94°C, 30 s at the relevant annealing temperature, 30 s at 72°C) followed by a final elongation for 5 min at 72°C. PCR products were mixed with formamide loading buffer, separated on 6% or 8% denaturing polyacrylamide gels, and visualized by silver staining (Bassam et al. 1991). Genotypes were independently scored twice.

**Linkage Analysis**

Two-point and multipoint analysis were performed using CRI-MAP, version 2.4 (Green et al. 1990). According to the results of the segregation analysis, disease inheritance was assumed to be autosomal dominant. In backcrosses to unaffected males (2 Pietrain, 1 Large White, 2 German Landschwein, 1 Duroc, and 1 crossbreed) and in backcrosses of 11 healthy F1 females, respectively.

The frequency of the defect was strongly raised among offspring from affected sows. In three litters with two, seven, and six weaned piglets, one, five, and four tremor piglets were observed (Table 1C–E). This increases the segregation ratio in matings between normal and affected animals to 10/15 (67%), which is not significantly different from the 50% expected for an autosomal dominant disease. In summary, affected piglets were only observed among offspring of the founder boar Campus and among offspring of affected mothers. This indicates a dominant mode of inheritance, with Campus being a gonadal mosaic for a spontaneous mutation.

A healthy boar born in a backcross of an affected mother to Campus was mated to six different sows, which had at least one affected litter. He produced only healthy offspring (7 litters, 68 piglets weaned; Table 1F). Under a recessive mode of inheritance, the probability to observe only healthy piglets among 68 offspring from obligate carrier × carrier matings is 0.754 (0.0000003%). In addition, the segregation ratio calculated from all matings with putative carrier animals was significantly different from a recessive inheritance (data not shown). The model of monogenic recessive inheritance was therefore rejected. The observed frequency of the defect among all offspring from Campus in matings with healthy sows was 26/300 (8.7%). Therefore, only 8.7% of the sperm of Campus contain the mutation.

**Genetic Mapping**

The founder boar Campus was heterozygous for 98 and homozygous for 60 of the 254 markers tested. The remaining 96 markers allowed no definite scoring of alleles or failed to amplify a PCR product. Among the 98 informative markers, 26 markers located on 14 of the 18 autosomes were unlikely to be closely linked to CPS because the two affected offspring inherited different alleles from the boar. Of the four autosomes without “unlinked” markers, SSC7 showed an interesting region of 40 cM with six informative markers and without unlinked markers. Genotyping of the selected pedigree (n = 58; Figure 1) was therefore started with markers from this region. The initial marker tested (SW632) was linked with CPS at θ = 0.16 (LOD score = 3.31). An additional 22 loci on SSC7 were studied; 9 markers were not informative, 1 marker was informative only with part of the family, and the remaining 12 markers gave a LOD score greater than three (Table 2). The highest LOD score of 11.44 at θ = 0.00 was obtained with SW1614.

Due to the dominant germline mutation, Campus transmitted the same marker haplotype with and without the putative de novo mutation to his offspring. Therefore, linkage analysis had to be restricted to affected progeny only, and the relationship between Campus and unaffected F1 offspring (e.g., animals 4, 20, 22, 24, 38, 40, 42, 44, 53, 54, and 57 in Figure 1) was neglected.

The recombination events observed in animals 47 and 56 identified SW1418 and SW352 as flanking markers, spanning a region of about 8 cM.

**Discussion**

Both the inheritance and the clinical features of CPS differentiate the Campus syndrome from previously reported inherited tremor diseases in pigs. Our breeding studies exclude a recessive mode of inheritance and indicate a dominant disorder with a germline mutation in the healthy founder boar Campus. This explains the unusual segregation rate of 8.7% among the offspring of Campus and the marked increase in frequency of affected piglets among the offspring of affected sows. It was difficult to increase the number of offspring of affected animals for further segregation analysis, because affected pigs normally die before sexual maturity due to their susceptibility to stress. Unfortunately, our efforts to raise male affected animals for the collection of sperm failed. The expression of the disease seems to be independent from the genetic background, as affected offspring occurred in matings...
between Campus and sows from five different breeds.

From previous investigations (Richter et al. 1995; Schulze et al. 1996; Wissel et al. 1997) there was no clear indication of putative candidate genes for CPS. Therefore a linkage analysis was initiated to map the disease. As there are only one or two meiotic events between the founder boar Campus and the affected animals, the chromosomal segment containing the affected gene which is inherited from the founder is expected to be large. This makes it possible to detect linkage with a rather sparse set of markers.

For linkage analysis, the large family material available from the breeding studies had to be reduced to 57 animals (Figure 1), because unaffected offspring might have inherited the affected chromosome without inheriting the causative mutation from the founder boar (e.g., animals 4, 40, 42, 53, and 94 in Figure 1). Furthermore, in backcrosses of unaffected Campus daughters (n = 10), their ancestors had to be neglected in the linkage analysis. This reduced the number of phase-known meioses and hindered the unequivocal localization of CPS in a multipoint analysis. However, haplotype reconstruction allowed the localization of CPS between the flanking markers SW1418 and SW352 in a region of approximately 8 cM according to the map of Rohrer et al. (1997).

Within this region the porcine genome maps are sparse for putative candidate genes. However, comparative mapping allows us to derive candidate genes from the more detailed human map. Bidirectional chromosome painting of the porcine and human genome shows that SSC7 is homologous to HSA 6, 14, and 15 (Goureau et al. 1996). Although the cytogenetic localization of the markers flanking CPS is unknown, the centromeric marker SW1418 is closely linked to ANPEP, which has been physically mapped to 7q1.5 (Smith et al. 1995). In addition, the flanking marker SW352 lies proximal to SW304, which was located by fluorescence in situ hybridization to 7q2.1-2.3 (Tammen et al. 1998). Furthermore, on the pig linkage map the inhibitor of the transcription factor nuclear factor kappa B (NFκB) and the T-cell receptor alpha chain (TCRA) (Musilova et al. 1996; Rohrer et al. 1997) map to the region in which CPS is located. This defines a region of homology around HSA 14q11-q13 (Figure 2).

In humans, an unknown gene for the dominant disease distal myopathy type 1 (MPD1) is located on HSA 14q11-13 (Laing et al. 1995). MPD1 shows genetic, clinical and myopathological similarities to CPS: Both diseases are autosomal dominant, progressive neuromuscular disorders with juvenile onset, both show excessive variation in fiber size, small angulated dark fibers in NADH-TR preparations, moth-eaten fibers and nuclear clumps (Laing et al. 1995; Schulze et al. 1996). However, the predominant tremor and the stress susceptibility in CPS are not observed in MPD1. Laing et al. (1995) suggested the genes for alpha and beta heavy chains of myosin, MYH6 and MYH7, as putative candidate genes for MPD1. These genes are located on HSA 14q12 (Matsuo-ka et al. 1988). Porcine MYH7 was recently mapped between the markers ANPEP and S0029 (Davoli et al. 1998) to the same area on SSC7 in which CPS is located (Figure 2). Therefore the genes for alpha and beta heavy chains of myosin must also be considered as candidate genes for CPS. Although MYH6 and MYH7 are cardiac isoforms of the myosin heavy chain, they are also expressed in skeletal muscles (Fananapazir et al. 1993; Jendreski et al. 1987). Furthermore, Fananapazir et al. (1993) connected the central core disease of skeletal muscle (CCD) in humans to mutations in MYH7. In addition, mutations in the adult isoform MYH7 lead in humans to hypertrophic cardiomyopathy (HCM), which is a cause of sudden death in healthy young individuals (Geisterfer-Lawrance et al. 1990; Marian et al. 1995). However, an association between sudden death in HCM and the sudden death of pigs exposed to minor stress situations in CPS remains ambiguous, because affected piglets showed no macroscopic or microscopic alterations of the heart.

The identification of the CPS gene will provide insights into the pathogenesis of this disorder and might supply useful tools for the mapping and cloning of the gene causing MPD1.

References


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