

## Bovine Spastic Paresis: A Genome-Wide Study in Fleckvieh

H. Schwarzenbacher<sup>1</sup>, H. Pausch<sup>2</sup>

<sup>1</sup>ZuchtData EDV-Dienstleistungen GmbH, 1200 Wien, Austria,

<sup>2</sup>Chair of Animal Breeding, Technische Universitaet Muenchen, 85354 Freising, Germany

**ABSTRACT:** Bovine Spastic Paresis is a neuromuscular disorder seen sporadically in many cattle breeds. It is characterized by a progressive unilateral or bilateral hyperextension of the rear limbs. A genetic background of the trait appears unquestionable and most authors postulate recessive inheritance with incomplete penetrance. In this study we carried out a genome-wide association study including 96 AI bulls that developed spastic paresis and 4,335 matched controls. Pedigree analysis suggested a dominant mode of inheritance with incomplete penetrance. Ten SNPs showed suggestive association although not genome-wide significant.

**Keywords:** cattle; spastic paresis; genome-wide association; Fleckvieh; Simmental

### Introduction

Bovine Spastic Paresis (BSP) is a neuromuscular disorder seen sporadically in many cattle breeds. It is characterized by a progressive unilateral or bilateral hyperextension of the rear limbs (“strait hock”). The onset of the disease is typically between age 3 to 8 months, although BSP may also be found in newborn calves and adult animals (Gentile and Testoni (2006), Barazzoni et al. (2003)).

First signs are hyperextension of the hock with an increase of the tibiotarsal angle as a consequence of spastic contraction of muscles which form the Achilles tendon, namely the musculus gastrocnemius and the superficial digital flexor. In progressed unilateral BSP cases the leg is held so that the foot just touches the ground or raised completely from the ground. Notably, the spasm is absent in a lying position. Affected animals walk in short pendulum-like steps. If both rear legs are affected, animals may attempt to bear more weight on the fore legs by holding them back and arching the back (Greenough (2012)).

BSP is believed to be attributed to hyperactivity of the stretch reflex, because of a dysfunction in the gamma-pathway. This defect in the action of the gamma-motor neurons could be either due to overstimulation of the gamma-pathway or lack of inhibitory mechanisms. A genetic background of the trait appears unquestionable and most authors postulate recessive inheritance with incomplete penetrance. However, up to now neither the mode of inheritance nor causal variants affecting the trait are known (Gentile and Testoni (2006)).

Unlike BSP the Bovine Spastic Syndrome (BSS) occurs in adult animals only. Characteristic are episodic, involuntary muscle contractions or spasms involving the rear limbs. This is observed particularly in adult bulls kept on artificial insemination (AI) centers although it occurs in both sexes. Affected animals show bilateral spasms of the skeletal muscles of the pelvic belt including the rump muscles. Spasms are accompanied by kyphosis. Both intensity and duration of spasms increase over time. A genetical background is assumed although unknown to date (Sponenberg et al. (1985), Tenzsen (1998)).

Cases of BSP are occasionally reported in Fleckvieh cattle, especially in bulls kept at AI centers. The aim of this study was to use genome-wide SNP data to identify genetic variants associated with BSP in Fleckvieh (dual purpose Simmental).

### Materials and Methods

**Animals.** AI centers in Austria and Germany were contacted to report BSP cases in Fleckvieh bulls. In total 96 cases from four different AI centers were reported together with the age of onset and the culling reasons for the bulls. To avoid potential population stratification matched controls were selected among a pool of nearly 30,000 animals with SNP chip genotypes available: For each of the 96 cases, controls with an Identical by State (IBS) coefficient larger than 0.125 were preselected. Among those up to 75 controls per case were sampled at random choosing 4,341 controls in total.

**Genotypes and quality control.** Illumina BovineSNP50 Bead chip genotypes (version 1 and version 2) comprising ~54,000 SNPs were available for 4,372 animals, while for 65 bulls Illumina BovineHD genotypes could be obtained. In total 43,852 common SNP across chip types were retained. SNPs with unknown chromosomal position according to UMD 3.1 genome assembly (Zimin et al. (2009)) were excluded from further analyses.

Animals and SNPs with a call-rate <0.95 and <0.90 were excluded, respectively, as well as markers with a minor allele frequency <0.5% and SNPs with >0.5% Mendelian errors. Ignoring these SNPs, animals with more than 200 Mendelian conflicts with their sires were excluded from further analyses. After quality control, the dataset contained 96 cases, 4,335 controls and 36,364 autosomal SNPs with an average per-individual call-rate of 99.63%. Processing of data and quality control was carried out with

R (<http://www.r-project.org>) and PLINK v1.07 (Purcell et al. (2007)).

**Pedigree analysis.** Pedigree records on 13,246,712 animals were used to test, among 96 cases, dominant and recessive mode of BSP inheritance by identifying common ancestors in one or both parents of BSP cases, respectively. Frequency of identified common ancestors in BSP cases was compared with those in 4,335 matched controls. Analysis was carried out using R.

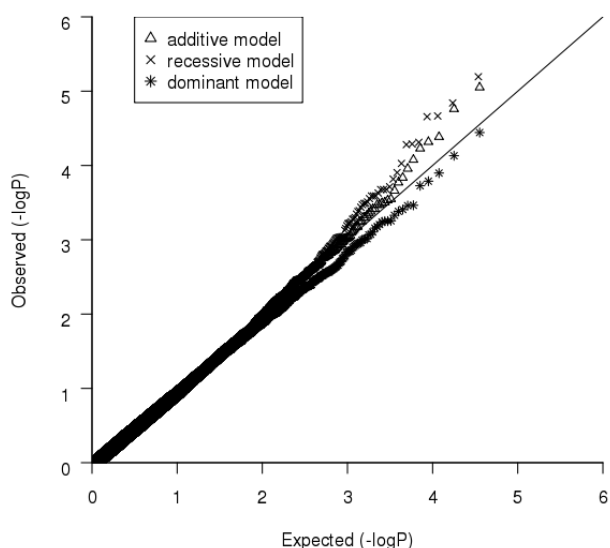
**Genome-wide association study.** Logistic regression using an additive, dominant and recessive model was used to test association of single SNPs with BSP status (1-free, 2-affected) in PLINK. Genomic inflation factors were calculated (Devlin and Roeder (1999)) and quantile-quantile were inspected to quantify the extent of false positive association signals (Voorman et al. (2011)).

## Results and Discussion

**Pedigree Analysis.** While a monogenic recessive model could be excluded, 95 out of 96 BSP cases (98.96%) shared a single ancestor in their paternal or maternal pedigrees. The same ancestor appeared only in 76.2% of pedigrees in the control animals. This indicates that a dominant-inherited variant could be a major factor predisposing to BSP in the Fleckvieh breed.

**Genome-wide association study.** Genomic inflation factors were 1, 1 and 0.927 for additive, dominant and recessive models (see also quantile-quantile plots in Figure 1). This indicates the absence of population stratification which was therefore not accounted for in the subsequent statistical analyses. None of the loci in the three different models were significant after correction for multiple testing using a FDR threshold of 0.1 (Benjamini and Hochberg (1995)). However, 11 tests in 10 different SNP had P values  $<1 \times 10^{-4}$ , indicating suggestive associations.

SNP Hapmap42187-BTA-34463 on BTA 14, is of special interest because it is located  $\sim 1.12$  Mb proximal of *CYP7B1*, a gene with a known recessive mutation leading to the phenotype spastic paraplegia-5A (SPG5A) in humans (Tsaousidou et al. (2008)). Hereditary spastic paraplegias (HSPs) are a group of monogenic disorders with a surprisingly similar appearance compared to BSP. The main clinical feature of all HSPs is a bilateral, symmetrical, slowly progressive spastic paraparesis, predominantly of the lower extremities (Harding (1983)). The age of onset, rate of progression and degree of disability are often variable between genetic types of HSP as well as within the same genetic types (Fink, (2003)). HSP is genetically heterogeneous with autosomal dominant, autosomal recessive and X-linked forms being reported. Today, more than 55 different chromosomal HSP loci are known (Boukhris et al. (2013)).



**Figure 1.** Quantile-quantile plot for genome-wide association tests using three different models.

**Table 1.** Associated SNPs from a genome-wide study on Bovine Spastic Paresis using 3 different models.

additive model <sup>†</sup>						
SNP	BTA	BP	F_A <sup>#</sup>	F_U <sup>#</sup>	OR*	P-Value
ARS-BFGL-NGS-54531	3	105277008	0.26	0.15	2.07	8.895e-06
ARS-BFGL-NGS-14293	5	109229225	0.46	0.32	1.86	1.736e-05
BTB-00285741	8	29831209	0.02	0.005	5.63	4.819e-05
Hapmap42187-BTA-34463	14	29856945	0.04	0.01	4.14	4.127e-05
recessive model <sup>†</sup>						
ARS-BFGL-NGS-42498	10	4968122	0.13	0.07	11.02	2.204e-05
ARS-BFGL-NGS-101609	11	100573430	0.35	0.27	2.85	9.389e-05
BTB-02088288	12	55273312	0.31	0.24	3.55	2.157e-05
ARS-BFGL-NGS-56387	13	24355847	0.30	0.23	3.51	1.441e-05
ARS-BFGL-NGS-100044	24	49755342	0.06	0.03	27.93	6.389e-06
ARS-BFGL-NGS-23323	27	38457181	0.06	0.06	15.50	4.875e-05
dominant model <sup>†</sup>						
ARS-BFGL-NGS-14293	5	109229225	0.46	0.32	2.71	3.605e-05

<sup>#</sup>) F\_A / F\_U: Frequency of the minor allele in cases / control animals.

<sup>\*)</sup> Odds Ratios

<sup>†</sup>) For each of the three models the minor allele of the SNP under consideration was taken as reference allele. Numerical codes [0,1,2], [0,0,1] and [0,1,1] were used for zero, one and two copies of the minor allele in the additive, recessive and dominant model, respectively.

## Conclusion

BSP is a heterogeneous syndrome that varies in time of onset, symptoms, severity and duration. Moreover, it is not easy to distinguish BSP from BSS or even phenotypically strain hocks. BSP cases in this study were bulls kept in AI centers and were not in all cases diagnosed by veterinarians. Thus, it seems likely that imprecise phenotypes reduced the power in this study. Based on previous studies and the genetically heterogeneous nature of HSP in humans, a complex genetic background of BSP with several involved mutations and possibly incomplete penetrance due to e.g. environmental factors, seems likely. However, pedigree analysis and suggestive significance of several SNPs

confirm the widely assumed genetic background of BSP. Confirmation of the findings in this study by inclusion of more and accurately diagnosed BSP cases will be needed.

### **Acknowledgements**

We thank Daniele Vicario from Associazione Nazionale Allevatori Pezzata Rossa Italiana for reporting and providing BSP cases. Moreover we thank AI centers from Austria and Germany for support and reporting on BSP cases. This study was designed in cooperation with the board on Mendelian diseases and genetic conditions.

### **Literature Cited**

- Barazzoni A.M., Bompadre G., Grandis A., et al. (2003). *It. J. Anat. Embryol.* 108(3):167.
- Benjamini Y. and Hochberg Y. (1995). *J. R. Stat. Soc. B.*57: 289-300.
- Britney J.B., Martin S.W., Stone J.B. et al. (1984). *Prev. Vet. Med.* 3:45-52.
- Boukhris A., Schule R., Loureiro J.L., et al. (2013). *Am. J. Hum. Genet.* 93:118-123.
- Devlin, B. and K. Roeder, (1999). *Biometrics* 55 997–1004.
- Fink J.K. (2003). *Exp. Neurol. Suppl.* 1:S106-S10.
- Gentile A. and Testoni S. (2006). *Slov. Vet. Res.* 43(1):17-29.
- Greenough P.R. (2012). [www.merckmanuals.com](http://www.merckmanuals.com) (25.02.2014).
- Harding A.E. (1983). *Lancet* 1(8334):1151-1155.
- Voorman A., Lumley T., McKnight B., et al. (2011). *PLoS One* 5:e19416.
- Purcell S., Neale B., Todd-Brown K., et al. (2007). *Am. J. Hum. Genet.* 81: 559-575.
- R Development Core Team (2009). R Foundation for Statistical Computing, Vienna, Austria.
- Sponenberg D.P., Vanvleck L.D., McEntee K. (1985). *Vet. Med.* 80(8): 92-4.
- Tenszen A. (1998). *Can. Vet. J.* 39: 716-7.
- Tsaousidou M. K., Ouahchi K., Warner T.T. et al. (2008). *Am. J. Hum. Genet.* 82: 510-515, 2008
- Zimin A.V., Delcher A.L., Florea L. et al. (2009). *Genome Biol.* 10:R42-R42.