Proceedings, 10th World Congress of Genetics Applied to Livestock Production

Studies of the Genetic Background of a Teat Defect in Pigs Born in Germany and Sweden

E. Jonas[1], H. Chalkias[1], C. Neuhoff[2], G. Lindgren[1] and D.J. de Koning[1].
[1]Swedish University of Agricultural Sciences, Uppsala,
[2]Institute of Animal Sciences, University of Bonn, Germany

ABSTRACT: To improve the understanding of the genetics underlying the inverted teat defect in pigs, we combined studies from Germany and Sweden. Based on a linkage study in Germany which revealed highly significant loci in an experimental population, confirmation of regions was attempted using commercial animals. A genome-wide association study on commercial pigs was also conducted in Sweden. Since both datasets of commercial pigs had a relatively low number of samples, we tested if microsatellite and data of SNP arrays have, in a combined analysis, enough power to validate loci with a higher reliability. We combined microsatellite data and haplotypes and verified associated alleles on chromosomes 1, 4, 6, 11, 14 and 18. Approaches like these present an opportunity to re-use old datasets and to increase the power of recent association studies when samples or further funding are not available.
Keywords: sow; inverted teat; inherited defect; meta analysis

Introduction

While many of the current studies aiming to identify the genetic background of complex traits are using high density SNP arrays for genome-wide association studies (GWAS), linkage studies based often on microsatellite markers are still being published. Datasets on both studies co-exist and even though in some populations both methodologies (linkage and association) were applied, most had only been genotyped with one or the other marker type (SNP array or microsatellite). The combination of different studies, especially their results via a meta-analysis has been used for different traits to increase the power and identify genetic regions which are valid across populations. It has been shown that pooling of data from studies of moderate power will be a useful tool to identify the genetic background of complex diseases but also to better distinguish between standards of study designs (Gu et al. (2001); Salanti et al. (2005)).

Typical examples for a limited number of studies with few samples are such of complex inherited diseases in livestock. Projects on the inverted teat defect in pigs have been conducted in different countries, among those one in Germany using animals from a commercial and an experimental population, genotyped using microsatellite markers, another in Sweden using commercial animals genotyped using the 60k SNP array (Jonas et al. (2007); Chalkias (2013)). Both studies in commercial animals (Jonas et al. (2007); Chalkias (2013)) were conducted using relatively few animals with similar genetic background, allowing only the identification of alleles with strong effects (if such effects exist). A combined analysis using data from those studies should assist to increase the power and result in a better identification of disease causing alleles.

Our study aimed to test if and how those studies can be pooled and if such a joint dataset will enable to verify previously reported loci and possibly enable to increase the total power for the identification of the genetic background of the inverted teat defect in pigs.

Materials and Methods

Animals and Phenotypes. Information from three different studies was used here: one experimental population and two commercial data sets. Datasets had been described previously (Jonas et al. (2007), Chalkias (2013)). The experimental population was based on a cross between Berlin miniature pigs and Duroc (Dumi), a study design aiming for the identification of QTL via linkage mapping. A total of 1070 animals (55 families) from the Dumi population as well as one commercial data set were sampled at the University Bonn, Germany. Animals of commercially used maternal lines (German Landrace and German Large White) were selected based on their phenotype using the discordant sib-pair design. Samples from affected and unaffected animals were collected at the slaughterhouse and samples from parents of discordant sib-pairs (one affected one unaffected) were provided by breeding companies and artificial insemination stations, resulting in a total of 130 families (Jonas et al. (2007)). Samples for the experiments conducted in Sweden were also collected using maternal lines (Yorkshire) and following the discordant sib-pair design; however no parental samples were collected. A total of 230 samples were collected in Sweden (115 animals with at least one inverted teat), leading to a total of 100 families and 15 unrelated pairs.

Experiments and analysis. Animals from the Dumi and commercial datasets of the University of Bonn were genotyped using microsatellite markers (N=72) across all chromosomes (Jonas et al. (2007)). The commercial families at the Swedish Agricultural University were genotyped using the Illumina porcine 60kb SNP array and SNP data passing the quality control criteria were further analysed (Chalkias (2013)).

Based on previous results from both studies, data on a few chromosomes were used for this preliminary study. A genome-wide association study using Plink (Purcell et al. (2007)) identified peaks on chromosome 4, 14 and

18 (P < 0.0001) in the Swedish commercial population (data not shown). Additionally markers on chromosomes 1, 6 and 11 were included in the analysis, leading to a total of 29 tested markers/regions. Allele frequencies of microsatellite markers were extracted and haplotypes were built (confidence intervals) for the SNP markers using haploview (Barrett et al. (2005)). Positions of microsatellites markers were identified using Ensembl (Sscrofa10.2) and merged to the SNP positions. The closest haplotypes to the microsatellite markers were selected and used to derive haplotypes in Phase (Stephens et al. (2001)). Examples are shown in Figure 1. Further allele frequencies of microsatellite markers and haplotypes (from commercial animals only) were compared and – if the frequencies were similar – alleles were coded accordingly, with the allele with the highest frequency giving a '1'. Data from commercial animals were then merged and an association analysis was performed for the separate and combined datasets using a family-based association test in FBAT (http://www.biostat.harvard.edu/*fbat/fbat.htm) (version 1.4). Details on the FBAT statistic are described previously (Jonas et al. 2007). For the analysis presented here, alleles and their frequencies, number of informative families, (direction of) Z-value as well as P-values were extracted from the results in FBAT.

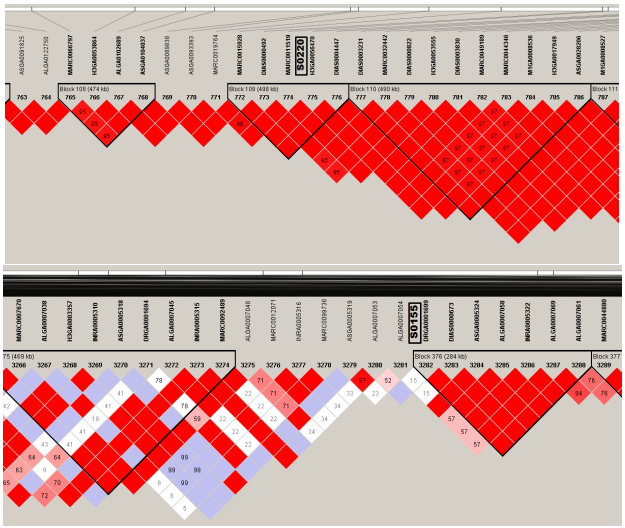

Figure 1: Screenshot of the haplotypes around markers S0220 (above) and S0155 (below) using Haploview.

Results and Discussion

Phenotypic data. Animals and data presented here were part of previous investigations into the genetic background of the inverted teat defect. Definitions of this defect were the same between the German and Swedish populations and examples of a normal and inverted teat are shown in Figure 2. Also other details, such as the development into a functional teat during lactation had been described (Chalkias (2013)) or hypothesized for both commercial populations. A total of 380 out of 904 F2 offspring from the experimental population had at least one inverted teat, affected animals had on average 5.2 inverted teats (Jonas et al. (2008)).

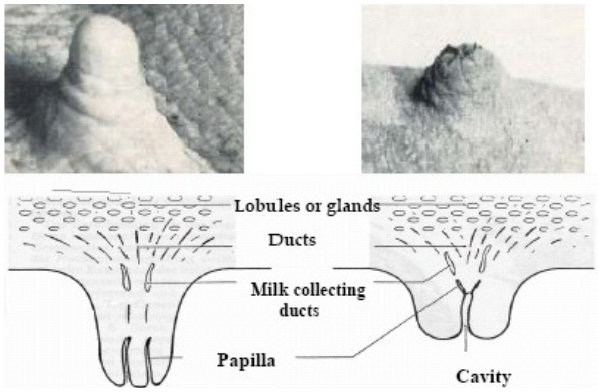

Figure 2: Picture and schematic drawing of a normal (left) and inverted (right) teat (Grosse Beilage et al. (1996), Steffens (1993), adapted by Yammuen-Art (2008)).

Animals of the commercial lines were already highly selected for the number of functional teats; still 11.3% of animals monitored at the slaughterhouse had at least one inverted teat. Detailed studies using animals of the Swedish Yorkshire population have shown that approximately 12% have non-functional teats (Chalkias et al. (2013)).

Analysis. Studies on the genetic background of the inverted teats using commercial animals did not lead to highly significant associations or linkage (Chalkias (2013), Jonas (2007)). However, QTL were previously identified using animals of the experimental Dumi population (Ün (2002), Oltmanns (2003)). An association analysis using FBAT did identify significant associations for a number of microsatellite markers in both populations genotyped at the University of Bonn (Jonas et al. (2008)).

With a peak at marker S0220 on chromosome 6 the most significant QTL was identified in Dumi, however not confirmed in the commercial animals (Jonas et al. (2008)). Two out of three alleles were significantly associated with the inverted teat defect in the Dumi population, one with positive and one with negative effect. A total of 46 and 38 families were informative. The two closest haplotype blocks (Figure 1) were phased using data from Yorkshire pigs and allele frequencies of haplotypes and microsatellite marker from the commercial animals were compared and coded in order to merge the datasets. The allele with the lowest frequency was significantly associated (P<0.05) in the German commercial animals with only 9 informative families and combined data with one additionally Swedish family (Table 1).

For some regions, a combined analysis did reveal an effect of the marker not previously identified in the experimental population. The additional families from the Yorkshire population did, at some loci, improve the results or identify an effect not previously confirmed in the commercial families from Germany (Table 1). However, if this analysis will be further tested additional parameters should be included for the combination of datasets, such as the

effect of the allele via the direction of the Z-value, which has currently not been used as coding criteria, but examined in the final results. It should also be tested if data from commercial animals can be merged with such from the experimental families.

Table 1. Results of the association analysis using FBAT

Marker[1]		P-value[2]		Fam[3]	Freq[4]
S0155^{ns}	0.013	0.157	0.005	11/2	0.03
SW1301^{sign}	0.249	0.005	0.023	72/29	0.22
SW1515^{sign}	0.029	0.166	0.010	85/43	0.42
SW1515^{sign}	0.033	0.414	0.023	19/6	0.03
SW1851^{sign}	0.010	0.439	0.013	18/15	0.08
SW373$^{n.t.}$	0.000	0.248	0.007	77/36	0.60
S0097^{sign}	0.000	0.612	0.001	79/32	0.61
S0097^{sign}	0.001	0.705	0.005	48/25	0.21
S0097^{sign}	0.012	0.317	0.007	7/1	0.01
S0035^{ns}	0.496	0.003	0.315	84/44	0.32
S0035^{ns}	0.003	0.020	0.151	59/28	0.20
S0059^{ns}	0.016	0.346	0.092	39/18	0.12
S0059^{ns}	0.058	0.052	0.439	29/13	0.09
S0059^{ns}	0.034	0.317	0.024	23/1	0.02
S0220^{sign}	0.011	0.317	0.007	9/1	0.00
S0300^{ns}	0.011	0.264	0.013	36/47	0.69
S0300^{ns}	0.011	0.317	0.008	36/1	0.04
SWR726$^{n.t.}$	0.020	0.456	0.030	31/39	0.49
S0009 (S0386)^{sign}	1.000	0.013	0.029	8/40	0.50
SW703^{sign}	0.111	0.050	0.688	20/48	0.47
SW703^{sign}	0.038	0.013	0.686	20/53	0.42
SW703^{sign}	0.564	0.046	0.059	2/4	0.01
SW857^{ns}	0.021	0.157	0.008	9/2	0.02
S0007^{sign}	0.303	0.035	0.024	24/38	0.21
S0007^{sign}	0.039	0.881	0.116	29/42	0.31
SW787^{ns}	0.011	0.042	0.001	66/35	0.30
SW787^{ns}	0.150	0.083	0.029	56/36	0.25
SWR414^{sign}	0.289	0.027	0.615	44/43	0.48
SWR414^{sign}	0.006	0.033	0.512	20/22	0.10
SWR414^{sign}	0.480	0.046	0.041	3/16	0.05

Shown are [1]Marker loci and indication of significance in the Dumi population with sign: at least one allele was significant, ns: no significant allele, n.t.: marker not tested; [2]P-values from the analysis of German and Swedish commercial families and the combined analysis; [3]Number of informative families in the German and Swedish commercial families (the combined analysis was the sum of both), [4]Allele frequency averaged over the two commercial and the combined dataset

For some of the microsatellite markers used for fine-mapping in the German commercial families, no haplotypes could be identified (using the confidence interval method). These additional markers were selected based on their heterozygosity, some were also not assigned to a clear position on the Ensembl SScrofa10.2 database (e.g. SWR726). Those markers are likely in less conserved regions in the porcine genome, the consequences this had for the linkage study performed previously remains unknown.

The alignment of microsatellite markers and haplotypes is very relevant and information additional to frequencies and significance levels might be useful. It might be interesting to analyse all marker regions and to compare the identified significant regions with those identified using candidate genes and expression analyses (Yammuen-Art (2008), Tetzlaff et al. (2009)).

Conclusion

We could show that our approach was successful in some of the targeted genetic regions. However, this combined approach is based on the assumption of a common genetic background in both commercial populations. The results presented here do therefore not provide final proof of the concept. We aim to test data subsets from animals genotyped for both marker types to validate the basic reliability of the method. The preliminary results presented here, suggest that there might be a possibility to improve results without additional genotyping. Results from such a combined analysis might be relevant for further region-specific (candidate gene) studies. The availability of databases and the porcine whole genome sequence makes such an approach now possible. A combination of datasets might be especially useful when samples are not available in a quantity or quality (degradation) required. A combined analysis will also reduce the need for additional animal experiments.

Acknowledgement

We would like to acknowledge the funding agencies of the research on the inverted teat defect (FBF, BMBF, Formas) and all colleagues who participated in the projects. EJ is currently funded by MistraBiotech.

Literature Cited

Svensson, C., and Hultgren, J. (2008). J. Dairy Sci. 91:1510-1518

Barrett, J.C., F1ury, B., Maller, J., and Daly, M.J. (2005). Bioinformatics.

Chalkias, H. (2013). Acta Universitatis agriculturae Sueciae 2013:79.

Chalkias, H., Rydhmer, L., and Lundeheim, N. (2013). Livest. Sci. 152: 127-34

Grosse Beilage. E., Steffens, S., and Schoon, H. A. et al. (1996). Tierärztliche Praxis 24: 31-35

Gu, C., Province, M.A., and Rao, D.C. (2001). Adv. Genet. 42:255-72

Jonas, E. (2007). Dissertation. Landwirtschaftliche Fakultät Universität Bonn

Jonas, E., Schreinemachers, H.J., and Kleinwächter, T., et al. (2008). Mamm. Gen. 19(2):127-38

Oltmanns, I. (2003) . Dissertation. Landwirtschaftliche Fakultät Universität Bonn

Purcell, S., Neale, B., and Todd-Brown, K., et al. (2007). American Journal of Human Genetics, 81

Salanti, G., Sanderson, S., and Higgins, J.P.T. (2005). Genet. Med. 7(1):13–20

Steffens (1993). SUS 3: 8-11

Stephens, M., Smith, N., and Donnelly, P. (2001). Am. J. Hum. Genet. 68: 978-989

Tetzlaff, S., Chomdej, S., Jonas, J., et al. (2009a): J. Anim. Breed. Genet. 126(3):237-41

Ün, C. (2002). Dissertation. Landwirtschaftliche Fakultät Universität Bonn

Yammuen-Art, S. (2008). Dissertation. Landwirtschaftliche Fakultät Universität Bonn