Proc. Assoc. Advmt. Anim. Breed. Genet. ***17****: 131-134*

BIOCHEMICAL AND DNA POLYMORPHISMS AND THEIR ASSOCIATIONS WITH OSTEOCHONDRAL DISEASES AND PRODUCTION TRAITS IN PIGS

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SUMMARY

This study reports association of five blood types, three enzymes, two proteins, *Escherichia coli* F18 receptor (ECF18R) gene and Ryanodin receptor (RYR) gene with six production, four meat quality traits and two osteochondral (OC) diseases in Swiss pig populations. Data were on 3,918 animals for production traits and 303 animals for meat quality traits and OC. A mixed linear model with allele substitution effects was used for each trait by marker analysis (144 analyses). Significant marker-trait associations are presented. In general, heritability estimates for production and meat quality traits were higher than those for OC lesions. Blood types lack significant associations with many traits except H and S types. Enzymes (mainly, glucose phosphate isomerase) and protein polymorphisms show significant associations with daily weight gain, premium cuts and back fat as well as OC lesions. The RYR and ECF18R genes significantly affected all growth, production and lean meat content traits and OC lesions; RYR also affected pH values. This study reported many new marker-trait associations particularly between the incidence of OC lesions and polymorphisms at glucose phosphate isomerase, 6-phosphogluconate dehydrogenase, postalbumin 1A, RYR and ECF18R loci. These results would be useful in selection and for further functional –omics investigations.

INTRODUCTION

The conventional biochemical markers (such as enzymes and proteins), blood types, and DNA tests can be used in animal breeding, if they have strong associations with economically important traits and diseases. Such markers for complex traits would play an important role in emerging areas of integrated genetics, transcriptomics and proteomics in the form of systems genetics (Kadarmideen *et al*. 2006). Meat quality and quantity, growth and feed efficiency are important economic traits. Osteochondrosis (OC), characterized by abnormal ossification of cartilages at bone joints, is often the cause of leg weakness in pigs. These OC lesions have mixed inheritance with polygenic heritability of 0.12-0.38 (Kadarmideen *et al*. 2004) and a segregating major gene (Kadarmideen and Janss 2005). In the Swiss pig breeding program, some blood types, proteins and enzyme systems are assayed for routine parentage control and a few other DNA tests are conducted. It would be useful to identify if associations exist between these markers and economically important traits for breeding and further systems genetics investigations. The main aim of this study was to investigate and quantify the association of blood types and polymorphisms in enzymes, proteins, Escherichia coli (E. Coli) F18 receptor (ECF18R) gene and Ryanodin receptor (RYR) gene with several traits including OC.

MATERIALS AND METHODS

Biochemical and DNA marker genotypes. Materials were from SUISAG, a stock company for services in pig production (http://www.suisag.ch). The RYR gene is used in selecting pigs against porcine stress syndrome and *ECF18R* gene is used to select against susceptibility to diarrhoea. Blood

group typed were A, S, H, G and E. Enzyme systems typed were glucose phosphate isomerase (GPI), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase 2 (PGM), postalbumin 1A (Po1A) and alpha-1-B glycoprotein (A1BG). The information on these markers dated from 1996 to 2003. Genotyping methods for the markers tested in this study are described in Vogeli *et al.* (1996) and in Meijerink *et al*. (1997).

Phenotypes and pedigree. Traits considered in this study (along with their abbreviations) are given in Table 1. Further details on recording and quantitative genetics of all traits and OC lesions are described in Kadarmideen *et al*. (2004). Information on marker types was available for 3,918 animals recorded for onfarm traits and 303 animals recorded for station-tested traits and OC lesions. Pedigree was constructed suitable for sire models with relationship among sires (523 sires in onfarm dataset and 113 sires in station test data).

Statistical model and analyses. A mixed linear model estimated environmental/non-genetic, polygenic as well as allele substitution effects for each trait by each system as:

$$\mathbf{Y= X\beta + Wg+Zs+e}$$

where $\mathbf{y}$ was a vector of records, $\mathbf{X}$ was a design matrix relating fixed effects in β to $\mathbf{y}$. Fixed effects (and their levels) fitted in the model for onfarm dataset include farm (77) and year and month of performance test (58), breed effect (3; Large White or Landrace or Duroc) and sex (2; male and female). In the station test data set, fixed effects (and their levels) include stable period (36) and slaughter day in slaughter house (86) among others. Weight at end of test and age (in days) at slaughter were covariates. Design matrix, $\mathbf{Z}$, related records to random sire genetic effects in vector **s**. The vector **e** contained residual effects. The matrix **W** is a *r* x *c* design matrix with *r* = number of records and *c* = number of unique alleles at the locus: each element of **W** contains number of copies of a given allele (0 or 1 or 2) and **g** is an allele substitution effect (linear effect of substituting 0 or 1 or 2 copies of a given allele for the other allele at the same locus). In the actual analysis, the missing class effect was included and estimated for each locus and the effect of one allele was set to zero effect due to linear dependency in estimating allelic effects. Estimation of genetic and environmental parameters was performed by single-trait analysis. There were 12 trait by 12 marker (=144) mixed model analyses, for which ASReml software (Gilmour *et al.* 2002) was used. An *F-ratio* test statistic and the associated *p-values* for each one of 144 analyses were collated to determine significance.

RESULTS AND DISCUSSION

Mean, standard deviations (SD) and abbreviations for all traits used in the analysis are given in Table 1 along with their univariate estimate of heritability (h^2) and standard error (s.e.). Heritability estimates for production, FCR and IMF traits were high compared to that of pH and OC lesions. These descriptive statistics as well as heritability estimates were very similar to those obtained from earlier analysis on the same data set (Kadarmideen *et al*., 2004). In this study, heritability estimates for 3 additional traits recorded on farm are reported (DWG_f, BFT and PPC_f). Allele frequencies of 12 marker loci were estimated by simple allele counting at each locus. There were missing or unknown blood/serum/genotypes for some of these marker systems (up to 58%). For blood types, allele A was more frequent; for GPI and PGM, allele 'B' was more frequent than allele 'A'; for Po1A system, allele 'A' and 'B' were more frequent. For RYR gene, the stress susceptibility T allele remains at very low frequency (0.01); it can not be completely eliminated due to importation of boars who are T

carriers. For ECF18R gene, the frequency of resistant 'A' allele was 0.43 while susceptibility 'G' allele was 0.57, showing that there is still a room for selection against susceptible genotypes.

Table 1. Descriptive statistics and univariate estimates of heritability (with s.e.) for different traits analysed by allele substitution mixed models

Traits and units	Abbreviation	N	Mean	SD	h^2 (s.e.)
Daily Weight Gain (g) on station (30-103 kg)	DWG_s	303	882.81	85.31	0.21 (0.07)
Daily Weight Gain (g) on farm (0-97.5 kg)	DWG_f	3918	605.0	51.21	0.28 (0.07)
Feed Conversion Ratio	FCR	303	2.54	0.14	0.42 (0.07)
Back fat (mm)	BFT	3918	10.6	1.42	0.43 (0.08)
Proportion Premium cuts (%) on farm	PPC_f	3918	58.20	1.51	0.60 (0.08)
Proportion Premium cuts (%) on station	PPC_s	303	57.20	2.45	0.54 (0.28)
Intra Muscular Fat (%)	IMF	303	2.12	0.56	0.66 (0.18)
pH after 1 hr	pH1	303	6.27	0.16	0.12 (0.07)
pH after 30 hr	pH30	303	5.41	0.06	0.18 (0.09)
Reflectance MLD (1-100 unigalvo units)	H30	303	32.73	3.08	0.24 (0.12)
Distal epiphyseal cartilage of ulna (1-6)	DEU	303	2.31	0.83	0.06 (0.06)
Condylus Medialis Femoris (1-6)	CMF	303	1.33	0.46	0.15 (0.07)

Table 2. P-values resulting from testing the association between blood types, enzyme, protein, E. Coli and RYR polymorphisms with production traits and osteochondral diseases in pigs based on 3918 records on DWG_f, PPC_f and BFT) and 303 records (for pH1, pH30, DEU and CMF) using allele substitution mixed models

Loci (alleles)	No.	DWG_f	PPC_f	BFT	pH1	pH30	DEU	CMF
A (2)	3248	0.2866	0.0722*	0.2086	0.5833	0.3654	0.2360	0.5695
S (2)	1798	0.6126	0.5712	0.6065	0.0405**	0.1559	0.5268	0.0062***
H (4)	3193	0.0018***	0.0057***	0.0030***	0.0192**	0.2305	0.8894	0.0377**
G(4)	1689	0.7047	0.7041	0.9139	0.2433	0.0434**	0.3642	0.1839
E(4)	1632	0.1219	0.0442**	0.0104**	0.3519	0.7578	0.9227	0.2000
GPI(2)	2881	0.7945	0.1177	0.0899*	0.8188	0.8107	0.0038***	0.0235**
PGD(2)	2799	0.0456**	0.4677	0.2726	0.1329	0.6717	0.8241	0.0459**
PGM(2)	1757	0.3946	0.2020	0.2467	0.4458	0.4876	0.0210**	0.4314
Po1A(14)	1686	0.0229**	0.0094***	0.0020***	0.0822*	0.0690*	0.0113**	0.0358**
A1BG(2)	3758	0.0003***	0.1467	0.1467	0.0998*	0.0624*	0.0802*	0.0657**
RYR(2)	1302	0.0000***	0.0000***	0.0000***	0.0161**	0.0075**	0.0166**	0.0100**
ECF18R(2)	3507	0.0700*	0.0479**	0.0562*	0.3654	0.4414	0.7065	0.0199**

***Significant association between the trait and the marker at $P < 0.001$
**Significant association between the trait and the marker at $P < 0.05$
*Significant association between the trait and the marker at $P < 0.10$

P-values from association test results are in Table 2. Most of the markers were not significantly associated with station-tested traits such as IMF, FCR, DWG_s, PPC_s and H30 (possibly due to low number of records); hence p-values for these traits are not presented in Table 2. Blood groups, in general, lacks significant associations with many traits analysed, except H and S types. Enzyme and protein show significant associations, mostly with DWG_f, PPC_f and BFT. RYR gene significantly affected all onfarm traits, pH and OC. Enzyme GPI affects OC with allele B decreasing the incidence, in relation to allele A. This GPI allele B also decreases growth and lean meat and increases BFT. Proteins Po1A and A1BG affected not only onfarm traits but also OC.

Actual allele substitution effects at all loci analysed (results not shown) revealed that s.e. were high for station-tested traits than s.e. for onfarm traits (due to low sample size). Sstatistically significant allele substitutions effects were the following. A1BG allele S, compared to F allele, was associated with increased DWG_f, and BFT and decreased PPC_f and decreased OC lesions. A1BG-S allele seems to decrease pH values of muscle. The effects of RYR-C and T alleles are not largely different on growth, premium cuts and BFT. However,.the T allele decreases pH1 and pH30 values, typical findings of halothane gene; this is confirmed here by the opposite effect of RYR-C allele. The ECF18R-A allele increases growth and BFT but decreases premium cuts, compared to G allele. Association with meat quality and growth agree with biological expectation for RYR and EcoliF18 genotypes but the biological relationship with OC needs further investigation. It must be noted that loci S, H, PHI, PGD, A1BG, RYR and ECF18R are located on porcine chromosome 6 and hence the associations and substitution effects might be confounded due to strong linkage disequilibrium between most of these loci. It is known that RYR and ECF18R are genes with significant effects on meat quality and growth, respectively but very few studies have investigated this in the population genetics and mixed model context; further this is the first report for Swiss pig populations.

In conclusion, this study reported many new associations, specifically for the first time on the association between incidence of two osteochondral lesions and GPI, PGD, Po1A, RYR and ECF18R which has potential value for other livestock species and human. These association results may serve as a basis for further molecular genetic and -omic investigations.

ACKNOWLEDGEMENTS

Author thanks SuisAG company for provision of trait data and Dr. Peter Vogeli at ETH Zurich, Switzerland for geno- and phenotyping and for useful discussions.

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