Erythropoietin receptor gene polymorphism in Indian pig lines

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ABSTRACT

A restriction fragment length polymorphism (RFLP) based genotyping test was developed to assess a C/T polymorphism in the regulatory region of erythropoietin receptor (EPOR) gene of pigs. Homozygous CC animals were absent in all the genetic groups studied, whereas the locus appeared fixed with T alleles in indigenous animals. The association of allelic variants with traits like litter size at birth (LSB), litter size at weaning (LSW), litter weight at birth (LWB) and litter weight at weaning (LWW) were explored in Indian Large White Yorkshire (LWY), Duroc, Indigenous Ankamali and F1 crosses between LWY boars and Ankamali sows (CB). All the populations under study had very high frequency of T alleles, with desi group being fixed with T allele. Genotypes of EPOR gene were not associated with any of the litter traits. The allele frequencies remained under HWE except in desi group.

Key words: Erythropoietin receptor (EPOR), Litter traits, Pig

The domestic pig is a litter bearing animal which is an excellent source of red meat. Even though pig is a litter bearing animal, prenatal mortality is very high among them. About 20–30% of foetuses die before day 30 and another 10–20% dies during the period from day 40 to parturition (Klemcke et al. 2001). Lack of space within uterus for all the developing foeti was identified as an important reason for such loss of piglets. Further, it was suggested that intrauterine crowding resulted in impaired fetal erythropoiesis and that breed differences existed in the rate of blood cell development (Vallet et al. 2003). The concentration of circulating RBC’s is controlled by the hormone erythropoietin (EPO), which acts through the EPO receptor (EPOR). EPO enhances RBC count through inhibition of apoptosis or increased cell division. The coding sequence of EPOR gene was 1,843 bp, with a putative protein of 509 residues. A single nucleotide polymorphism (C/T) which created an extra GATA-1 site (T allele) in intron 4 of the swine EPOR gene was identified and a genotyping assay was described by them for the same, based on primer extension and mass spectrometry (Vallet et al. 2005). The foetal genotype was associated with a functional increase in the expression of the EPOR gene and improved foetal survival. The present article discusses the development of a PCR-RFLP based genotyping test for this polymorphism besides exploring its association with traits like litter size at birth (LSB), litter size at weaning (LSW), litter weight at birth (LWB), litter weight at weaning (LWW) pre-weaning mortality (PWM) and weight gained till weaning (WGW).

MATERIALS AND METHODS

The 5,425 bp sequence of EPOR gene submitted by Xi and Di (2008) was retrieved from Genbank (accession no. EU407778.1) and the primers described by Vallet et al. (2005) for amplifying a 116 bp product were located using the tool, ‘Primerblast’ (Ye et al. 2012). The product was predicted to lie between 2,308 and 2,423 bp. The polymorphic site described by Vallet et al. (2005) was located inside the extracted sequence. A new reverse primer was designed 5’-GGATGAGAGGCGTGGTCAA-3’ and used along with the forward primer, 5’-CTACCTGGGTCCCGTTCTG-3’ reported by Vallet et al. (2005), so as to amplify a 180 bp polymorphic fragment. Neb cutter (V2.0), a web based tool (Vincze et al. 2003) for restriction analysis was used to predict the expected restriction pattern. The set of fragments predicted were therefore 104 bp, 56 bp and 20 bp for C allele and 124 bp and 56 bp for T allele, upon digestion with the enzyme MnlI.

Data on LSB, LWB, LSW and LWW were recorded for the association study from 75 Large White Yorkshire (LWY) animals, 22 Ankamali sows, 45 crossbred (CB) and 18 Duroc sows. The CB animals were F1 crosses between Ankamali sows and LWY boars. Blood samples were drawn from ear vein of these animals and DNA was isolated using Phenol-Chloroform method. Polymerase chain reactions (PCR) were performed in a volume of 10 μl, so as to contain about 75 to 100 ng of template DNA, 5 pM each of forward and reverse primers, 200 μM of each dNTP’s, 2.5 mM of MgCl2, 0.75 U Taq polymerase and polymerase buffer to a
final concentration of 1X, diluted with autoclaved ultra-
filtered water. The preparations were amplified in a thermal
cycler. To avoid spurious amplification, 1.5% V/V
formamide (Sarkar et al. 1990) was also included in the
reaction mixture. The PCR products were co-incubated
with one unit of *Mni*I overnight in the same tube at 37°C, along
with its buffer in a volume of 15 μl. The digested amplicons
were resolved out in 6% non-denaturing polyacrylamide
gel along with 50 bp DNA ladder and were silver stained
according to the protocol developed by Qu et al. (2005).
Allele frequencies were calculated using direct count
method and were analysed using Chi square test for the
identification of differences between breed. The gene
frequencies were examined for deviations from Hardy-
Weinberg equilibrium using Chi squared test for testing the
equilibrium.

Association study was performed using the linear model
\[ Y_{ijklm} = \mu + G_i + B_j + A_k + S_l + S_{im} + e_{ijklmn} \]
in SAS (Version 9.1) where \( \mu \) is the general effect; \( G_i \) represented the genotypes of EPOR (CC or CT); \( B_j \) represented breed (j, 1–4); \( A_k \) represented groups based on age (k, 1–5); \( S_l \) represented season (l, 1–3); \( S_{im} \) represented sires (m, 1–44) and \( e_{ijklmn} \) stands for random error. The sows were
run on the basis of age as less than one year, 1 to 1 1/2
years, 11/2 to 2 years, 2 to 2 1/2 years and 2 1/2 to 3 years.

**RESULTS AND DISCUSSION**

The overall least square means and means observed in
different genetic groups under study are presented in Table
1. Considering breed wise means, litter size at weaning
\((P_{\leq 0.01})\) and weight gained till weaning \((P_{\leq 0.05})\) differed
significantly among genetic groups (breeds). The mean
LSW of LWY in the present study was marginally higher
than that observed by Singh et al. (2002) and Palve et al.
(2000) which were 7.84 and 7.48, respectively. The LSW
of Large White pigs was lower than that reported by Wolf
et al. (2008) in Czech population. The F_1 crosses between
LWY and desi of Andhra Pradesh performed better than
those from the present study (Prasanna et al. 2009).

The results point out that genotyping of polymorphic
region of EPOR gene is also possible using PCR-RFLP, as
against primer extension and mass spectrometry reported
by Vallet et al. (2005). The predicted as well as the observed
restriction pattern confirms the presence of a non
polymorphic restriction site within the PCR product. The
‘C’ allele of the gene presented an additional restriction
site for the enzyme *Mni*I (5’ CCTC(N)_7) whereas this site
would be absent in T allele in which the 5’ C is replaced by
the nucleotide T. The test provide a cheaper alternative to
primer extension and mass spectrometry used by the original
authors (Vallet et al. 2005) for the genotyping of this locus.
The restriction pattern observed is presented in Fig. 1. The
20 bp fragment was not observed on gel, except for this the
banning pattern observed was as predicted by Neb Cutter
(V 2.0).

Homozgous CC genotype was not observed in any of
the genetic groups under study. Ankamali pigs were
apparently fixed with T allele in this locus. The frequency
of C allele was highest in LWY sows. Between group allelic
and genotypic frequencies were analyzed using Chi square
test. Results indicated that the frequency of alleles and
genotypes in case of Ankamali differed significantly from
that of other genetic groups under study. The allele
frequencies and genotype frequencies observed in the study
are presented in Table 2. The figures in parenthesis represent
the number of observations.

**Table 1. Least squares means±SE of litter traits**

<table>
<thead>
<tr>
<th>Breed</th>
<th>LSB</th>
<th>LSW</th>
<th>LWB</th>
<th>LWW</th>
<th>PWM</th>
<th>WGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LWY (75)</td>
<td>10.53±1.03</td>
<td>8.05±0.85</td>
<td>10.71±1.28</td>
<td>68.58±7.41</td>
<td>2.56±0.80</td>
<td>46.71±5.54</td>
</tr>
<tr>
<td>Ankamali (22)</td>
<td>6.97±0.55</td>
<td>4.87±0.54</td>
<td>4.57±0.78</td>
<td>35.40±4.00</td>
<td>3.46±0.94</td>
<td>41.26±6.52</td>
</tr>
<tr>
<td>CB (45)</td>
<td>10.45±0.99</td>
<td>7.40±0.82</td>
<td>10.46±1.22</td>
<td>53.6±17.11</td>
<td>1.85±0.82</td>
<td>59.51±5.67</td>
</tr>
<tr>
<td>Duroc (18)</td>
<td>10.49±1.13</td>
<td>6.13±0.94</td>
<td>11.64±1.40</td>
<td>48.98±8.15</td>
<td>5.03±1.42</td>
<td>31.78±9.87</td>
</tr>
<tr>
<td>Overall (160)</td>
<td>9.41±1.12</td>
<td>6.46±1.05</td>
<td>8.66±1.43</td>
<td>50.38±9.21</td>
<td>3.27±0.18</td>
<td>42.07±1.36</td>
</tr>
</tbody>
</table>

Means with different superscript within a column differ significantly \(P_{\leq 0.01} ; *P_{\leq 0.05} ; \) Figures in parenthesis represent number of observations.

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**Fig. 1. Alleles from EPOR locus resolved on 6% polyacrylamide gel and visualized by silver stain. Lane 1, PCR product (180 bp); Lanes: 2,3,5, genotype CC–104 bp, 56 bp (20 bp fragment was not observed on gel); Lanes 3,6,7,8, genotype CT–124 bp, 104 bp, 56 bp.**
The allele frequencies were in equilibrium among Large White Yorkshire, Duroc and crossbred genetic groups. Chi square values were not calculated for Ankamali group as the observed frequency was zero for two of the genotypes.

Results from statistical analysis using general linear model revealed that genotype had no significant effect on any of the litter traits studied. Sire apparently had a significant effect on litter traits as observed by Raghavendran et al. (2013) and Akdag et al. (2009). Season of farrowing was not associated with any of the litter traits covered in the present study. In contrast, studies on LWy, Crossbred and Duroc pigs by Raghavendran et al. (2013) ruled out significant effect of sire on litter traits.

Age of sows appeared to be a significant (P<0.05) factor affecting pre, weaning mortality in piglets. Parity or age of sows consistently appeared to have a significant effect on litter traits as observed by Raghavendran et al. (2013) and Akdag et al. (2009). Season of farrowing was not associated with any of the litter traits covered in the present study. The report by Sai et al. (2009) that higher litter size was observed on rainy season was also in contrast with the findings of the present study. Wolf et al. (2008) also observed significant effect of herd-year-season on litter traits.

The Ankamali pigs used for the present study were obtained from the state of Kerala. The state is a narrow stretch of land, separated from Deccan plateau by the Western Ghats, which acts as a continuous natural boundary in the east, except for a few gaps. This should have allowed the animals to live in relative isolation resulting in the loss of C allele due to random drift or founder effect resulting in the fixation of T allele. Fixation of loci was reported earlier in indigenous pig breeds of China. The missing alleles, however, were found among European pig population of China, as reported by Bao et al. (2008) while working on Fucosyl transferase 1 gene. Therefore, the loss of allelic diversity in desi pigs of Kerala could not be viewed as an unusual finding.

The frequency of T allele reported in unselected pig populations of USA was as low as 0.028 (Vallet et al. 2005), whereas the values were much higher among all genetic groups covered in the present study (Table 2). The selective pressure exerted by environment in human populations of different habitats are well documented (Hancock et al. 2010). Adaptation to a particular environment could actually depend on the alleles of genes possessed by the individual. Considering the results of present study, the T allele apparently has some sort of selective advantage under the geographical and climatic conditions prevailing in Kerala, whereas C allele could be the favored one in USA.

The conditions for HWE was reported to hold good for most human populations and deviations usually resulted from changes in population structure, sampling and genotyping errors (Wigginton et al. 2005). Since, HWE is maintained in all genetic groups except desi, it could be assumed that pig populations by and large remain in HWE, possibly due to high fecundity. Since, the desi animals were monomorphic at this locus, HWE could not be tested.

Reports indicate that foetal, rather than maternal genotype of EPOR gene had significant effect on litter size at birth (Vallet et al. 2003; Vallet et al. 2005). The present study tested the hypothesis that EPOR genotype of dam had significant effect on litter traits. The study focused on maternal genotype rather than foetal genotype and had failed to identify any such association. Other litter traits like LSW, LWB and LWW were also tested for significant associations but nothing of that sort could be identified.

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REFERENCES


Table 2. Frequency of genotypes and alleles at EPOR locus

<table>
<thead>
<tr>
<th>Breed</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>TT</td>
</tr>
<tr>
<td>LWY (75)</td>
<td>0.27 (20)</td>
<td>0.73 (55)</td>
</tr>
<tr>
<td>Desi (22)</td>
<td>0.00** (0)</td>
<td>1.0** (22)</td>
</tr>
<tr>
<td>CB (45)</td>
<td>0.11 (5)</td>
<td>0.89 (40)</td>
</tr>
<tr>
<td>Duroc (18)</td>
<td>0.22 (4)</td>
<td>0.78 (14)</td>
</tr>
<tr>
<td>Overall (160)</td>
<td>0.18 (31)</td>
<td>0.82 (162)</td>
</tr>
</tbody>
</table>

** P<0.01; * P<0.05.


