ASSOCIATION OF GENETIC VARIATIONS IN THE REGULATORY REGION OF
PIT1 WITH DIFFERENCES IN THE RATE OF EGG LAYING IN WHITE LEGHORN
CHICKENS

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INTRODUCTION
Pit1 (or GHF-1) is a major regulatory protein of pituitary cell differentiation as well as of the
expression of growth hormone, prolactin and thyroid-stimulating hormone (Bodner et al., 1988;
Voss and Rosenfeld, 1992). Genetic variations in this gene may therefore affect a variety of
phenotypes controlled by the pituitary axis. We have previously cloned the genomic sequence
of the promotor region and of intron 1 of the pit1 gene. A search for genetic variants in 785 bp
of the promotor/exon1 region and 1345 bp of the intron 1/exon2 region by Kansaku et al.
(2000) in various strains of chickens revealed several single-strand conformational polymorphisms (SSCP). In this communication we examined the association of two SSCP with
production traits in a non-selected White Leghorn population.

MATERIAL AND METHODS
SSCP analysis was conducted as described previously (Kansaku et al., 2000). The White
Leghorn strain analyzed was strain 7 (Gowe et al., 1993). It was developed at Agriculture
Canada (Ottawa) in 1950 and propagated as a closed breeding population without selection. In
each generation 100 sires were mated to two dams each, in order to maintain a large effective
population size. Inbreeding was estimated to be 2.6 % (Kuhnlein et al., 1990). Association
analyses were conducted using the NCSS program of Hintze (1997). Hardy-Weinberg
equilibrium was assessed using chi-square analysis as described by Weir (1990).

RESULTS AND DISCUSSION
A total of 330 hens were analyzed for the SSCP located in the segment A (regulatory region)
and segment D (intron 1). The alleles at the two loci were designated as Pit1-A and Pit1-a and
Pit1-D and Pit1-d, respectively. No double heterozygotes were observed and the alleles could
unambiguously be assigned to three haplotypes. The fourth combination of alleles was missing
as expected on the basis of maximal parsimony. The location of the SSCP and the unrooted
tree of the three haplotypes are indicated in figure 1 and the observed frequency of the
genotypes in table 1. Only three of the six possible genotypes occurred at frequencies above 5
% and were thus amendable for trait association studies.
Among the production traits analyzed only the rate of egg laying in two of three time periods
differed significantly among the genotypes (table 2). For other traits (body weight, egg weight
and egg specific gravity, each measured in three different time periods) differences were not
significant (data not shown).
The rates of egg laying were not normally distributed and no suitable transformation could be found. However, both parametric (GLM) and nonparametric (Kruskal-Wallis analysis by rank) analysis gave a similar result. The median daily rate of egg-laying between onset of egg-laying and 457 days of age among heterozygotes H2/H3 was 7.5% lower than among the homozygotes H3/H3 and 9% lower than among heterozygotes H1/H2, indicating that pit1 is a major gene affecting egg production.

Since genotypes H1/H2 and H2/H3 differed in their effect on egg laying, haplotypes H1 and H3 were not equivalent. Similarly, since genotype H2/H3 and H3/H3 differed, haplotypes H2 and H3 were also not equivalent. H3 differs from H1 and H2 by the SSCP in the promotor region (segment A) which may therefore be linked to the causative mutation or be the responsible mutation itself. Indeed, analysis of individual SSCP loci showed that only the SSCP in segment A was significantly associated with differences in the rate of egg laying.

The rate of egg laying is a fitness trait. Hence even in a randomly mated breeding population it is expected to lead to deviations from Hardy-Weinberg equilibrium. This is indeed observed. A goodness-of-fit test for disequilibrium among the genotypes defined by the three haplotypes yielded a chi-square of 331 (df = 3). A comparison of the heterozygotes representing the three pairs of haplotypes is shown in Table 3. The disequilibrium constants, defined as $D_{uv} = p_u p_v - 1/2 P_{uv}$, where $p_u$ and $p_v$ are the estimated haplotype frequencies and $P_{uv}$ the observed heterozygote frequency, were significant in all three cases.

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**Table 1. Observed frequency of genotypes in strain 7**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>1</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>H2</td>
<td>2</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td></td>
<td>264</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Median rate of egg laying for the most frequent genotypic classes

<table>
<thead>
<tr>
<th>Period</th>
<th>Median rate of egglaying (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H1/H2 (N = 40)</td>
<td>H2/H3 (N = 24)</td>
</tr>
<tr>
<td>AFE-275d</td>
<td>86.1\textsuperscript{A}</td>
<td>79.2\textsuperscript{B}</td>
</tr>
<tr>
<td>276d-356d</td>
<td>72.5</td>
<td>65.1</td>
</tr>
<tr>
<td>357d-457d</td>
<td>61.3\textsuperscript{A}</td>
<td>43.8\textsuperscript{B}</td>
</tr>
<tr>
<td>AFE-457d</td>
<td>72.7\textsuperscript{A}</td>
<td>63.7\textsuperscript{B}</td>
</tr>
</tbody>
</table>

\textsuperscript{A} Different subscripts indicate significant differences (Kruskal-Wallis Z-test)  
\textsuperscript{C} AFE is the age at first egg (on-set of egglaying)

The sign of the disequilibrium constant indicates that heterozygotes are more frequent (negative sign) or less frequent (positive sign) than the expected frequencies estimated from the haplotype frequencies. There was a deficiency of heterozygotes H2/H3 and an excess of heterozygotes H1/H2 which is concordant with the effects of these genotypes on the rate of egg laying.

Table 3. Disequilibrium between pairs of alleles

<table>
<thead>
<tr>
<th>Heterozygote</th>
<th>Disequilibrium constant</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1/H2</td>
<td>-0.054</td>
<td>307</td>
</tr>
<tr>
<td>H1/H3</td>
<td>0.053</td>
<td>71</td>
</tr>
<tr>
<td>H2/H3</td>
<td>0.049</td>
<td>96</td>
</tr>
</tbody>
</table>

In summary, the SSCP located in the promotor region was associated with differences in the rate of egg laying, while other production traits were not affected. The genotype distribution indicated genetic disequilibrium. Since inbreeding in the breeding population was relatively small and a series of other markers did not deviate from Hardy-Weinberg equilibrium, the deviation observed here may be a consequence of reduced reproductive fitness due to differences in the number of eggs laid. Whether other fitness traits such as fertility and hatchability are also affected remains to be determined.

REFERENCES

Weir, R.S. (1990) “Genetic Data Analysis” pp. 84-87 (Sinauer Assoc. Inc.).