

## Practical Application of DNA Markers for Genetic Improvement

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### Summary

PIC has been developing and using DNA markers in its improvement program for more than a decade. Markers have been developed for both input and output traits and for both quantitative traits and major genes. Most recently we have used genomics to assist in the selection against developmental abnormalities. This paper discusses the challenges involved in finding solutions for such multi-factorial problems using susceptibility to scrotal hernia as an example.

### Introduction

DNA markers are used routinely within the PIC genetic improvement program. Examples, include polymorphisms within ESR (litter size see Short et al., 1997), MC4R (growth and fatness, Kim et al., 2000), PRKAG3 (meat quality, Milan et al., 2000, Ciobanu et al., 2001) and FUT1 (resistance to *E. coli* F18, Meijerink, et al., 2000, Frydendahl et al., 2003).

In 1998 PIC began receiving reports from some customers who were observing an elevated incidence of scrotal hernias among cross-bred progeny of a newly launched parent boar product. A number of actions were taken in response to this problem.

Developmental abnormalities such as scrotal hernia are observed at low frequencies in pig populations. Genetic modeling studies have suggested that scrotal hernias are inherited through major gene effects with a polygenic background variation, (see Thaller et al., 1996). The PIC lines associated with the affected crossbred progeny had not previously shown a significantly elevated incidence when mated pure, suggesting, if genetics was a cause, that there would be genetic involvement of more than one line. Since known causes of developmental abnormalities include single and multi-gene genetic patterns as well as infections and chemical involvements as non-genetic causes, a multi-disciplinary team was assembled to ascertain possible causes and solutions.

The initial scrotal hernia team consisted of geneticists, veterinarians, nutritionists, and two academics, as well as individuals with practical farm experience. Since it was clear that there were farms experiencing significant problems with scrotal hernias, while other farms using identical genetics (including in some cases the same sires) were not having problems, the first task of the team was to attempt to understand what was causing the difference.

## Results

In order to review possible genetic associations, single sire matings were instituted on twelve cooperating commercial farms. A total of 478 individual boars were mated to 5,670 sows, resulting in the births of approximately 19,500 males. As well as using the data for genetic parameter estimation, we reviewed a range of other possible causes, including castration techniques; examination by researchers at Arizona State Universities for gross anatomical abnormalities (none were found); a parity review; examination of differences in birth weight; when the boars entered into studs and other seasonal effects; the health of the sow farms; the gestation length of the sows; and a review of the scientific literature. In spite of finding no consistent patterns, we pursued the theory that we were looking at a genotype by environment interaction with both the genotype and the environment being difficult to define. The remainder of this paper deals with the genetic approaches that we tried.

Family selection was the first and easiest action. Nine sire groups that appeared to have a higher incidence of scrotal hernias among their offspring, were culled. Additionally, any single incidence of a scrotal herniated boar in a litter resulted in culling of the entire litter. This is a very blunt selection tool, akin to throwing the baby out with the bathwater. A sharper selection tool was needed.

A routine purebred and crossbred data collection method was developed, and in 1999, PIC quantitative geneticists implemented a classical BLUP EBV system for scrotal hernia to support selection against the trait in pure lines.

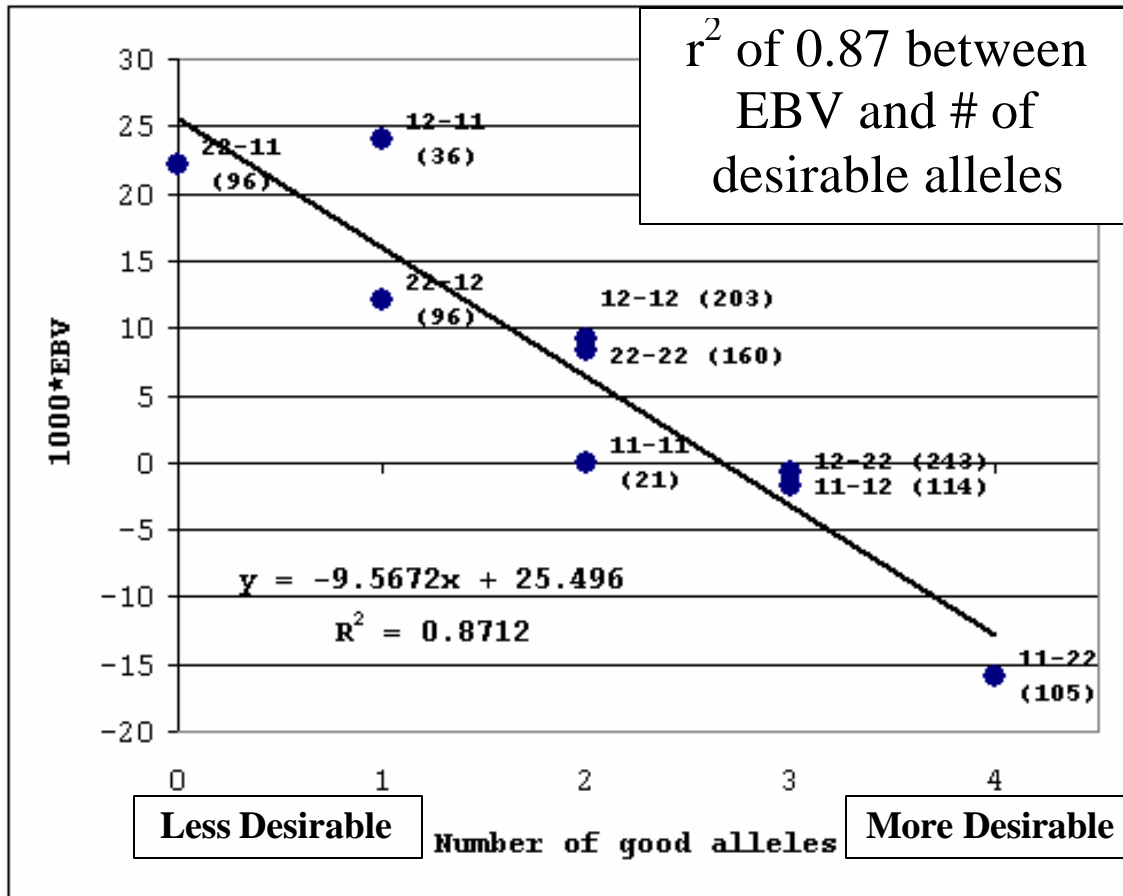
It was recognized from the outset that a developmental abnormality question with genetic involvement could be most effectively addressed using DNA markers. As a consequence, a comprehensive gene discovery program was instituted in 1999. A major challenge for this work was the creation of suitable databases (quality and quantity) of tissues and phenotypes of different lines and crossings. Once these were established, we used a candidate gene approach (over 40 markers from more than a dozen genes ultimately were investigated) as well as a genome scan approach (using AFLP<sup>TM</sup> and microsatellites).

We detected two markers that showed strong association with scrotal hernia in a subset of families in one of the lines. Evaluation of the transmission of these closely linked markers was consistent with the presence of a major locus for scrotal hernia, at least in this particular line. These markers also showed association with scrotal hernia in a number of unrelated samples from the same line, providing additional support for the occurrence of a major gene in the region of the markers for a proportion of samples in that line. However, in further analyses the markers showed incomplete linkage disequilibrium with the trait in one of the lines, and no association at all in the other lines.

The positional information was used to develop more markers in this region of the genome. Ultimately, we identified two markers in two different genomic regions that appeared to have a strong association with our BLUP EBV in one of the lines. This relationship is illustrated in Figure 1.



**Figure 1:** Relationship between PICmarq™ genotypes for two Scrotal Hernia markers and BLUP EBVs for Scrotal Hernia among 1,000 boars.



Explanation of symbols: Genotypes are given for two genes, each with two alleles, “22-11” means that the first gene has two copies of allele #2, while the second gene has two copies of allele #1. Numbers in ( ) are the number of boars of this genotype.

These markers have been incorporated into the genetic improvement program as part of the EBV calculation for susceptibility to scrotal hernias. This development together with the implementation of Global BLUP analysis (see below) provides a very robust estimate of susceptibility for hernias.

- ✓ Global BLUP is used to evaluate scrotal hernia EBVs based on the litters of over 60,000 living sows currently contributing to PIC’s PICtraq™ genetic database from PIC herds around the world.

## Conclusion

Two Developmental Abnormality markers have now joined a growing panel of other PICmarq™ DNA markers for traits such as meat quality, disease resistance and performance traits, routinely used to aid selection in our sire lines. These markers add greater accuracy to the quantitative BLUP evaluations.

The combined effects of the family culling and use of the scrotal hernia EBV have resulted in a significant reduction in the incidence of scrotal hernias in one of the affected lines (see Table 1).

Table 1: Trend in SH incidence for the sire line at the Genetic Nucleus

Period	Incidence
January – June 2000	1.87
July 2000 – June 2001	1.80
July 2001 – June 2002	0.58
July 2002 – June 2003	0.75

While we have not yet been able to completely elucidate the genetic model, nor to identify the environmental risk factors, a combination of quantitative and molecular approaches has solved the problem to the extent that we no longer see an elevated incidence of hernias among cross-bred progeny of the parent boar line.

These markers extend the range of traits where PIC currently uses DNA marker information (PICmarq™) in its genetic improvement program. Markers are now routinely used to select for coat color, growth and leanness, litter size, reproductive longevity, aspects of meat quality and disease resistance as well as in quality control and traceability.

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