Polydactyl Inheritance in the Pig

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Abstract

Two pigs were identified having “extra feet” (preaxial polydactyly) within a purebred population of Yorkshire pigs. Polydactyly is an inherited disorder in many species that may be controlled by either recessive or dominant genes. Experimental matings were conducted using pigs that had produced affected offspring with the result of 12 polydactyl offspring out of 95 piglets. One polydactyl-producing boar was also mated to 4 Duroc sows and 8 distantly related Yorkshire sows to produce 129 unaffected offspring. Together, these results suggest a recessive mode of inheritance, possibly with incomplete penetrance. Candidate genes, LMBRI, EN2, HOX-A10-13, GLI3, WNT2, WNT16, and SHH, were identified based on association with similar phenotypes in other species. Homologues for these genes are all found on SSC18. Sequencing and linkage studies showed no evidence for association with HOX-A10-13, WNT2, and WNT16. Results for the regions including GLI3, LMBRI, and SHH, however, were inconclusive. A whole genome scan was conducted on DNA samples from 10 affected pigs and 12 close relatives using the Illumina PorcineSNP60 BeadChip and compared with 69 more distantly related animals in the same population. No evidence was found for a major gene causing polydactyly. However, a 25-Mb stretch of homozygosity on SSC8 was identified as fairly unique to the family segregating for this trait. Therefore, this chromosome segment may play a role in development of polydactyly in concert with other genes.

Key words: candidate genes, polydactyl, SNP chip

The first report of a polydactyl pig was in 1931 (Curson 1931), with additional reports in 1938 (Hughes 1938), 1959 (Gaeddke 1959), and 1963 (Ptak 1963). Additionally, the National Swine Registry (the governing body of the Yorkshire breed of pigs) states in their requirements for registration that a Yorkshire pig with an extra dewclaw is not allowed to be registered (National Swine Registry 2007).

Other vertebrates have also been known to express different polydactyl phenotypes that are observed either simply by themselves or as one phenotype of a syndrome. Genes found responsible for polydactylism in other species include: Hedgehog and WNT gene families (Yang et al. 1998; Sheth et al. 2007; Zeller and Zuniga 2007), Sonic Hedgehog (SHH) (Hill 2007), a cis-acting regulator of SHH within LMBRI (Sagai et al. 2004; Huang et al. 2006; Wang et al. 2007), engrailed-2 (EN2) (Lawrence et al. 1999), TWIST1 (Firulli et al. 2007), GLI family zinc finger 3 (GLI3) (Fujoka et al. 2005), and the HOX genes (Tarchini et al. 2006; Sheth et al. 2007; Zeller and Zuniga 2007). Homologues for all of these candidate genes except TWIST1 reside on porcine chromosome 18.

After initial identification of 2 purebred Yorkshire pigs expressing a polydactyl phenotype, additional matings were conducted to positively identify if the phenotype was in fact genetic in nature and not due solely to environmental influences. Additional pigs with a range of polydactyl phenotypes were produced, and genetic markers were utilized to investigate the mode of inheritance and the genes causing the observed phenotype in our population of pigs.

Materials and Methods

Phenotypic Analysis

The polydactyl phenotypes observed ranged from extra dewclaws where only extra phalanges were present to extra feet that included extra carpal bones, metacarpals, and phalanges (Figure 1). In one case, the extra feet replaced the medial dewclaws (Figure 2). In cases of polydactyly in other species, a range of phenotypes have resulted from mutations in a single gene (e.g., Radhakrishna et al. 1999). Because pigs exhibiting all of these phenotypes occurred in the same family, pigs with any of the polydactyl traits were scored as affected, regardless of severity.

Population

This study originated in a closed breeding population of Yorkshire pigs located at the Iowa State University (ISU) swine breeding farm (Madrid, IA). From this population, a total of 3 boars (2 producing affected offspring), 9 dams
(5 producing affected offspring), and 127 offspring (12 affected) in 13 litters were included in this study. Two additional Yorkshire boars from Swine Genetics International (SGI) (Cambridge, IA) that had reports of producing pigs with extra dewclaws in other herds were used, producing 15 offspring (2 affected) in 2 litters. Additionally, 1 other boar and 12 other dams were mated with some of the above animals to assess the recessive or dominant nature of this trait, resulting in 143 offspring. All animals were raised under approved animal care regulations.

Two polydactyl pigs were initially identified. Pig 137-06 was a male pig (Figure 1A,B) having an “extra foot” on the medial side of both of his front feet. He was one of 8 piglets in a litter resulting from a mating between the 18-1 (sire) and 52-11 (dam) animals. Pig 156-08 was a female pig (Figure 1C) possessing an “extra foot” on the medial side of only one front foot (left) and was from a litter of 9 piglets that resulted from the mating of the 95-04 (sire) and 102-11 (dam) animals. All future matings involved at least one animal derived from the above 4 parents. Pictures of live pigs were taken at the farm, and X-rays were taken on 137-06 after he was euthanized. Pedigrees are provided in Figures 3 and 4.

Matings

The 95-04 boar was subsequently mated to the 52-11 and 102-11 dams, his cousin (137-09, a full sibling to the 137-06 barrow), and several of his daughters (156-05 twice, 156-06 twice, 156-07, 192-05, 192-07, and 207-03). The boar 156-03 was mated with his full sister, 192-07.

Additional matings were carried out to further test the inheritance of this phenotype (Figure 4). The sire 95-04 was mated to 4 Duroc (unrelated) females and 8 additional Yorkshire (unrelated) females. The 156-05 female was mated to a Duroc boar that had sired multiple litters but no affected piglets.

SGI possessed frozen semen on 2 boars, subsequently referred to as SGI_1 and SGI_2, that had previous reports of offspring with extra dewclaws. SGI_1 was mated to 137-09, and SGI_2 was mated to 156-06.

DNA

For adult or mature animals, blood samples were collected and used for DNA isolation. For planned matings, tail tissue samples were obtained at birth from each pig, placed into a labeled 1.7-ml tube, and stored at −80 °C. For each animal, a 25-mg tail sample was used for DNA isolation using the DNeasy kit from Qiagen (Valencia, CA) following the manufacturer’s protocol.

Candidate Gene Approach to SNP Genotyping

Single nucleotide polymorphisms (SNPs) were identified and genotyped in candidate genes as well as other genes

Figure 1. Original pigs affected with preaxial polydactyly in a breeding population of Yorkshire swine. (A) Yorkshire male (ID 137-06) expressing a preaxial polydactyl phenotype on both front feet. (B) Radiograph showing both front feet of the Yorkshire male (137-06) with preaxial polydactyly. (C) Yorkshire female (ID 156-08) expressing a preaxial polydactyl phenotype on one (left) front foot.
located throughout SSC18. The candidate genes analyzed on SSC18 included LMBR1, SHH, EN2, HOXA10-13, GLI3, WNT2, and WNT16, in which either SNPs were genotyped or sequencing was used if SNPs were not otherwise identified. In addition, SNPs from the following genes were used for linkage analysis of the chromosome: Leptin, GPR37, SPAM1, HYAL4, WASL, LMOD2, ASB15, SLCO1A1, AASS, FAM3C, WNT16, ING3, WNT2, PACAPR (Kollers et al. 2006), MPP6, and IGFBP1 (Mote and Rothschild 2006) spanning 83 of 91 cM of SSC18.

**Linkage Analysis**

An extension of the Elston–Stewart algorithm was used in a model-based linkage analysis to map the genomic location most likely to contain the locus causing the polydactyl phenotype. The implementation of the Elston–Stewart algorithm used here is described in Elston and Stewart (1971) and Fernandez et al. (2001, 2002), although use of linkage mapping has been previously described (Ott 1974). Likelihood ratios were calculated for each marker interval with complete penetrance assuming that the polydactyl mutation is at the center of this interval (L1) or that the polydactyl mutation is at another chromosomal location (L2). The log base 10 of this likelihood ratio (L1/L2) resulted in the logarithm of odds (LODs) score where a LOD score greater than 3 was classified as significant. Likelihood (L) can be expressed as

\[ L = \log \left( \frac{\text{Pr}(y|G) \times \text{Pr}(G)}{\text{Pr}(y|\bar{G}) \times \text{Pr}(\bar{G})} \right) \]

where \( y \) is a vector of polydactyl phenotypes and \( G \) is a vector of genotypes at the markers flanking the interval in question. Values equal to or below −3.0 were considered evidence against linkage. Scores above 3.0 were considered strong evidence for linkage.

**Large-Scale SNP Genotyping**

DNA samples from 10 affected animals (137-06, 156-08, X156-01, 177-07, 184-01, 191-13, 207-07, 251-01, 251-08, and 251-15) and 12 close relatives (sires: 18-1, 95-04 and his dam 18-5 [sister of 18-1]; dams: 52-11, 102-11; both dams and full siblings: 156-05, 156-06; full siblings: 137-07, X156-02, 251-03, 251-13, 251-16) were genotyped for 64 232 SNPs using the Illumina (San Diego, CA) 60K porcine SNP chip (Ramos et al. 2009). The same SNP chip was used to genotype 730 other animals from the same closed breeding population. GeneSeek, Inc. (Lincoln, NE) completed the genotyping.

**Figure 2.** Variation of the preaxial polydactyl phenotype seen in Yorkshire pigs. Both legs had what appeared to be an “extra foot” but one (left) is missing a dewclaw.

**Figure 3.** Pedigree of Yorkshire animals where polydactyl animals existed. Circles represent females. Squares represent males. Shaded figures represent polydactyl animals. Large circles and squares are parents, whereas smaller circles and squares represent the number of offspring from the mating. Mummies are not included in counts, though pigs born dead are.
Statistical Analysis of SNP Chip Data

Data from the 730 control animals combined with the 10 affected animals were used to remove from further analysis all SNPs that were not segregating in the population. Analyses were conducted comparing the 10 affected piglets with the 12 related pigs, then by comparing the 10 affected pigs with 69 of the most genetically similar of the 730 pigs from the general population.

First, regions that were inherited identical-by-descent (IBD) were predicted in the affected animals by searching for the longest stretches of homozygosity based on build 9 of the porcine genome after excluding all fixed markers. Next, the polydactyl pigs were compared with the 12 relatives using the software programs PLINK v1.05 (Purcell et al. 2007) with the DFAM analysis option for family-based association analyses and multifactor dimensionality reduction v1.1.0 (MDR; Moore et al. 2006) to look for single SNP effects and epistatic interactions between SNPs, respectively.

For all homozygous stretches that were thought to be significant based on the above analyses, the significance was assessed by comparing the affected animals with the 69 animals from the general population using PLINK v1.05 (Purcell et al. 2007). In each stretch, 1 SNP from each end and 1 in the middle were selected based on higher minor allele frequencies in the whole population to capture maximal variation. A haplotype association test was used to compare each set of 3 SNPs between the affected animals and unrelated control animals. All regions with any significance were examined for potential causative genes.

Results

Matings and Possible Mode of Inheritance

A complete analysis of all matings (within the polydactyl family, matings of boar 95-04 to distantly related or unrelated sows, and matings to commercial boars) and the resulting total number of litters farrowed, total pigs born, and the number of affected animals born can be seen in Table 1. Due to many complications, including fertility problems, neither of the 2 matings of affected pigs to affected pigs produced any offspring. No further attempts were made. Six matings producing 9 separate litters (3 repeat matings) occurred between pigs within our population that had at some time produced polydactyl offspring (Table 1).

This included 2 boars and 5 sows. Together, these matings produced 65 live offspring and 28 stillborn offspring that could be evaluated for phenotype. Of these 93 offspring, 1 had 2 extra feet, 1 had an extra foot but was missing a dewclaw, 4 had a single extra foot, 2 had 2 extra dewclaws each, and 4 had a single extra dewclaw for a total of 12 polydactyl offspring. These matings also appear to have produced a significantly higher proportion of dead offspring (at least 30 offspring/9 litters) and mummies (at least 4 mummies/9 litters) than expected in this herd; the remainder of this population of Yorkshire pigs showed an average of 5% of piglets born dead and 2% mummified.

Additional matings outside of our affected population were used to assess whether the trait could be dominant. The sire 95-04 was mated to 4 Duroc (unrelated) females producing 50 piglets and 8 additional Yorkshire (unrelated) females that produced 79 piglets with none being affected (Table 1). The mating of an unrelated Duroc boar to 156-05 (Yorkshire putative carrier) produced 14 unaffected piglets, even though she had previously produced 5 affected piglets.

Pedigree analysis of the initial 2 animals exhibiting the polydactyl phenotype showed that a common ancestor was found on both sides of each animal’s pedigree within 8–9 generations (data not shown). To compare the genetic basis of polydactyl in the ISU population with commercial swine, matings were made between boars from SGI (Cambridge, IA)
and our dams. Pedigree analysis of both SGI boars showed that they also had the same common relative in their pedigree as the ISU animals. SGI_1 was mated to the female 137-09 who had already produced an affected pig when mated to the 95-04 boar. The resulting litter produced 9 live piglets at birth with 2 individuals that possessed an extra dewclaw. One of the affected piglets had other birth defects such that he was unable to stand and had to be euthanized. The boar SGI_2 was mated to the female 156-06; the litter included 6 piglets born with no affected piglets.

SNP Segregation Analysis

After examining the WNT16 alleles the affected offspring inherited from each parent, it became clear that polydactyl offspring could inherit either allele from 95-04 (Table 2).

Linkage Analyses

Table 3 shows the genes analyzed for linkage analyses. No marker interval showed a significant association with the polydactyl phenotype in question with LOD scores between 0.15 and −69.85 (Table 3). Based on the linkage analysis results, the causative mutation can be rejected as being located between Leptin and WNT2 (LOD scores range from −3.08 to −69.85 throughout this stretch). Though other regions of SSC18, like the stretch from MPP6 to IGFBP1 (LOD score = 0.15), did not show strong evidence of association with the polydactyl phenotype, they should not be excluded from further analysis. This also left the region around SHH unable to be excluded (LOD score = −1.01).

60K SNP Chip Analysis

Comparisons of SNPs among affected and nonaffected pigs from the family did not identify SNPs that achieved significance using the max (T) empirical p-value (EMP2) option of PLINK but showed several SNPs on chromosome 7 with low empirical p-value (EMP1) values. MDR did not identify any significant epistatic interactions.

Based on the search for long IBD stretches in all affected animals, one especially long stretch (251 SNPs, 25 Mb) was identified on chromosome 8 (positions 36104826−61205897) that was homozygous among all 10 affected

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sire</th>
<th>Sire’s SNP genotype</th>
<th>Dam</th>
<th>Dam’s SNP genotype</th>
<th>Affected offspring’s genotypes</th>
<th>Unaffected offspring’s genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNT16</td>
<td>95-04</td>
<td>A/B</td>
<td>156-05</td>
<td>A/A</td>
<td>A/A (1), A/B (3)</td>
<td>A/A (10), A/B (9)</td>
</tr>
<tr>
<td>WNT16</td>
<td>95-04</td>
<td>A/B</td>
<td>156-06</td>
<td>B/B</td>
<td>A/B (1)</td>
<td>A/B (2), B/B (6)</td>
</tr>
<tr>
<td>WNT16-SNP2</td>
<td>95-04</td>
<td>A/B</td>
<td>156-05</td>
<td>B/B</td>
<td>A/B (3), B/B (1)</td>
<td>A/B (10), B/B (7)</td>
</tr>
<tr>
<td>WNT16-SNP2</td>
<td>95-04</td>
<td>A/B</td>
<td>156-06</td>
<td>A/A</td>
<td>A/B (1)</td>
<td>A/A (6), A/B (2)</td>
</tr>
</tbody>
</table>
animals. However, this region was also homozygous among 11 of the 12 unaffected pigs. Only 9% of the general population (N = 730 animals genotyped) shared the genotype of the affected animals throughout this stretch. The next longest stretch of homozygosity in the affected animals was 34 SNPs long on chromosome 15 (1.0 Mb from 24078571 to 25041363 bp).

When PLINK was used to compare the affected animals with the group of 69 pigs outside this family within regions shown by other methods to be potentially significant, haplotype analyses gave the following P values for the most significant haplotype in each region: SSC7 119765662–119940243 bp $P = 5.46 \times 10^{-14}$; SSC8 36104826–61205897 bp $P = 1.17 \times 10^{-8}$; and SSC18 553289–2383572 bp $P = 5.78 \times 10^{-9}$.

**Discussion**

Polydactyl pigs were rare in the general swine population but were commonly detected when animals were mated specifically to produce polydactyl offspring. In total, there were 14 affected pigs (half were born dead) of 110 pigs that were born in this project using pigs (carriers) originating from the founder animals of this population (Figure 3). However, when a boar that had produced polydactyl offspring was mated to distantly related and unrelated sows, no affected offspring were seen among 129 piglets of 12 sows. Together, these data suggest a recessive mode of inheritance for the trait.

At the same time, the number of affected offspring was less than expected for a simple recessive mode of inheritance. Based on Mendelian expectations, we should have seen approximately 28 polydactyl piglets (chi-squared = 6.6, $P = 0.01$). Therefore, this is unlikely to be a simple recessive mode of inheritance. Furthermore, an interesting note was that of the 155 pigs born in this project from parents (carriers or offspring of carriers), there were at least 40 pigs (26%) born dead and at least 13 mummified fetuses (8%), much more than normally expected (5% stillborn, 2% mummy) in this population. These data suggest some type of possible lethal expression also but was not different statistically from a recessive mode of inheritance for lethality.

Personal reports from other breeders that had sows with extra dewclaws, as well as the report from Hughes (1938), also suggest that there is not full penetrance with this trait as the affected by affected matings produced both affected and unaffected pigs.

Another possible explanation of the inheritance pattern is that the phenotype is controlled by 2 recessive loci, which could explain the homozygous stretch on chromosome 8 being present in all affected animals and their family members. This region was less common among the general population (9% homozygotes). If this locus is interacting with another significant locus, it could have produced the observed ratio of phenotypes. Additionally, a causative mutation within this region may exist that was not genotyped but could be heterozygous in some of the unaffected relatives, whereas being homozygous in the affected animals.

The production of affected offspring by mating ISU sows to SGI boars clearly suggested that this phenotype, in some form, also existed outside the breeding population at ISU with a similar genetic basis. This result indicates that the significant regions found in this population could be further studied in commercial populations to potentially identify a causative mutation.

### Table 3 LOD scores for intervals on chromosome 18

<table>
<thead>
<tr>
<th>Starting gene</th>
<th>Ending gene</th>
<th>LOD score for interval$^a$</th>
<th>Physical location of start gene (Mb)$^b$</th>
<th>Physical location of end gene (Mb)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMBR1</td>
<td>LEPTIN</td>
<td>−1.01</td>
<td>0.96</td>
<td>18.35$^c$</td>
</tr>
<tr>
<td>LEPTIN</td>
<td>GPR37</td>
<td>−3.08</td>
<td>18.35</td>
<td>21.14</td>
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<tr>
<td>GPR37</td>
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<td>21.75</td>
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<td>SPAM1</td>
<td>HYAL4</td>
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<td>21.91</td>
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<tr>
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<td>WASL</td>
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<td>21.91</td>
<td>21.96</td>
</tr>
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<td>21.99</td>
</tr>
<tr>
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<td>22.34</td>
</tr>
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<tr>
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<tr>
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<td>WNT2</td>
<td>PACAPR</td>
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<tr>
<td>PACAPR</td>
<td>MPP6</td>
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<td>46.58$^d$</td>
</tr>
<tr>
<td>MPP6</td>
<td>IGFBP1</td>
<td>0.15</td>
<td>46.58</td>
<td>48.65$^e$</td>
</tr>
</tbody>
</table>

$^a$ Based on recessive trait with full penetrance.

$^b$ Midpoint of each gene in megabases taken from ENSEMBL Pig Build 9.

$^c$ SHH is located in this interval at 1.74 Mb and EN2 at 2.01 Mb.

$^d$ HOXA10-13 is located in this interval at 44.18 Mb.

$^e$ GLI3 is located after this interval at 51.45 Mb.
Combined, these results suggest that the polydactyly phenotype in this population is not due to a single gene dominant mode of inheritance and is suggested to be recessive in nature, but without full penetrance. Based on the assumption of recessive inheritance, the candidate genes WNT16 and WNT2 can be rejected as containing causative mutations based on the inheritance patterns observed. The lack of mutations in EN2, HOX-A10-13, and GLI3 also suggests that they are not directly involved in causing porcine polydactyly in this population. The interval linkage analysis also rules out a causative mutation between Lepin and WNT2 and between PACAPR and MPP6 on chromosome 18.

**Funding**

Iowa Agriculture and Home Economics Experiment Station; State of Iowa and Hatch funding; US Department of Agriculture-Cooperative State Research, Education, and Extension Service National Needs Fellowship to B.M. and (2007-38420-17767 to D.G.).

**Acknowledgments**

Data collection and assistance provided by individuals from the farm crew at the Lauren Christian Swine Research Center, PIC USA, Dr James Koltes, Mr Dusty Loy, Ms Penny Fang, Mr Dan Mouw, and Dr Suniel Onderu and other members of the Rothschild Laboratory are greatly appreciated. Contribution of semen from SGI, Cambridge, IA is appreciated. We would like to thank the 2 reviewers and especially editorial board member, Dr E. Bailey, for their constructive feedback on this article.

**References**


Received January 22, 2010; Revised February 19, 2010; Accepted February 26, 2010

Corresponding Editor: Ernest Bailey