

Discovery and Use of a Natural Mutation that Results in Severe Combined Immuno Deficiency in Pigs

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Summary and Implications

Piglets from the low residual feed intake (RFI) line at ISU were found to be affected with a lethal autosomal recessive mutation that causes Severe Combined Immunodeficiency (SCID). Bone marrow allotransplantation rescued the immune deficiency in four of nine attempted transfers; the other five exhibited signs of severe graft versus host disease and were euthanized. A genome wide association study identified a 5.6 Mb region that contained the causative mutation. Affected haplotypes were traced back to the founders of the RFI population, who were sourced from the purebred Yorkshire population. The SCID pigs will be useful as a biomedical model, as pigs are anatomically and genetically more similar to humans than SCID mice, which are now widely used. Development of a genetic test for the causative mutation will be valuable to the swine industry, allowing breeders to identify carriers.

Introduction

SCID is a primary immune deficiency that occurs naturally in humans, horses, and dogs. It is inherited as both an autosomal recessive and X-linked disease resulting in a disabled adaptive immune system, which is necessary for survival post-weaning. The disease has been artificially created using transgenics in mice and recently also in pigs for use as a biomedical model. We recently discovered the first natural cases of SCID in four piglets that died soon after arriving at Kansas State University for a study involving experimental infection with the PRRS virus. Two of the piglets died during the first seven days at KSU, which are used as an acclimation period before inoculation. Necropsy of these piglets showed severely underdeveloped thymus and lymph nodes, and the two piglets that were alive

at inoculation had not produced antibodies to the injected PRRS virus.

Remating the six parents of these four piglets resulted in litters that each produced SCID affected piglets. The disease segregated in an autosomal recessive manner. At birth, piglets appear healthy without any weight difference between affected and normal littermates. Immunoglobulins were undetectable in pre-suckling umbilical cord blood of affected piglets. Suckling provides affected piglets with maternal antibodies, which allow SCID piglets to remain healthy for several weeks after farrowing. Maternal antibody levels diminish after weaning, leaving SCID piglets susceptible to many pathogens. Normal and carrier piglets are able to mount an effective immune response to these pathogens, while their affected littermates succumb to these infections.

The purpose of this study was to identify the genomic region that harbors the causative mutation of SCID in pigs and to develop a genetic test to identify affected and carrier pigs.

Materials and Methods

Immunophenotyping was performed at Kansas State University using flow cytometry and ELISA. Flow cytometry showed that SCID piglets had low/no B- and T-cells, but NK-cells were present. ELISA was used to measure antibody levels in umbilical cord blood that was drawn before piglets suckled.

The Illumina Porcine SNP60 Beadchip was used to genotype 20 affected piglets, 6 known carrier parents, 50 unaffected littermates, and 97 ancestors. The dfam option of Plink was used to perform a genome wide association study to identify the region that contains the causative mutation. The SNP in a 1 Mb region surrounding a strong candidate gene in the identified region were phased using PHASE (2.1.1).

In order to rescue affected piglets such that they can be raised to breeding age, nine affected piglets from four litters were given completely swine leukocyte antigen (SLA)-matched bone marrow transfers.

Results and Discussion

The genome-wide association study identified a 5.6 Mb region associated with the SCID phenotype. A credible candidate gene, based on interspecies comparative information, was identified in the 5.6 Mb region. Twenty-three SNP in a 1 Mb region surrounding the candidate gene were used for analysis by PHASE. This analysis showed two haplotypes that segregated with affected status, either in a homozygous or compound heterozygous state. The identified haplotypes were traced through the pedigree of

the RFI line back to the founder generation. The founders were sourced from a purebred Yorkshire population.

Single SNP genotyping enabled us to identify carrier and affected pigs in the ISU RFI population. Genotyping was carried out using allele specific primers developed for SNPs that segregate with the disease phenotype located in the 1 Mb window around the candidate gene.

Bone marrow transfers were performed on nine SCID piglets from four litters. Donors were completely SLA-matched to affected recipients. Four piglets were successfully transplanted, however five showed signs of graft versus host disease after transfer and were euthanized. The four surviving transplanted piglets were documented to have a reconstituted adaptive immune system.

In conclusion, we have identified the first naturally occurring SCID pigs. As the mutation may be present in commercial populations, a genetic test would be valuable to identify carriers and avoid mating carriers to reduce mortality in the nursery. The SCID pigs are currently also being developed as a model for biomedical research.

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