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Generation and characterization of RAG2 knockout pigs as animal model for severe combined immunodeficiency

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ABSTRACT

Pigs with severe combined immunodeficiency (SCID) are versatile animal models for human medical research because of their biological similarities to humans, suitable body size, and longevity for practical research. SCID pigs with defined mutation(s) can be an invaluable tool for research on porcine immunity. In this study, we produced RAG2-knockout pigs via somatic cell nuclear transfer and analyzed their phenotype. The V(D)J recombination processes were confirmed as being inactivated. They consistently lacked mature T and B cells but had substantial numbers of cells considered to be T- or B-cell progenitors as well as NK cells. They also lacked thymic medulla and lymphoid aggregations in the spleen, mesenteric lymph nodes, and ileal Peyer's patches. We showed more severe immunological defects in the RAG2 and IL2RG double-knockout pig through this study. Thus, SCID pigs could be promising animal models not only for translational medical research but also for immunological studies of pigs themselves.

1. Introduction

Immunodeficient animal models are versatile tools in biomedical research. They are naturally used as research models of immunity. In addition, they can be used as animal platforms for the generation of humanized animals that steadily maintain transplanted tissues and/or cells of humans and can be used for *in vivo* biological research of human. They are also useful in the preclinical trials of stem cell-based regenerative medicine to evaluate the effectiveness and safety of the transplantation of human stem cells, such as iPS cells. Severely immunodeficient mice models, such as NOG (Ito et al., 2002), NSG (Shultz et al., 2005), and BRG (Traggiai et al., 2004) mice have been intensively developed. The functional reconstitution of the human hematopoietic and immune systems has been achieved by transplanting human hematopoietic stem cells into these immunodeficient mice (Ito et al., 2002; Shultz et al., 2005; Traggiai et al., 2004), and research on human-specific infections has been performed using these models (Akkina, 2013; Bility et al., 2012; Bility et al., 2014). These models have also been used in human cancer and stem cell research (Cunningham et al., 2012; Shultz et al., 2014). However, mice models have considerable limitations for modeling human biological and clinical conditions. Their small size narrows the scope of surgical and clinical applications, and their short longevity precludes long-term evaluation of post-transplantation effects. Moreover, there are several genetic lines of difference in adaptive and innate immune systems (Mestas and Hughes, 2004) as well as inflammatory responses (Seok et al., 2013) between mice and humans.

Pigs are invaluable animal models in human medical research because of their similarities to humans in physiology, anatomy, nutrition, and genetics. In fact, several genetically modified pigs have been shown to recapitulate human disease with higher fidelity than mice models (Fan and Lai, 2013; Prather et al., 2013). Therefore, immunodeficient pigs can be excellent animal models for biomedical research, such as the development of humanized tissues and organs for transplantation and long-term evaluation of transplanted cancer or stem cells of human origin. Our group (Suzuki et al., 2012) and Watanabe et al. (2013) previously reported

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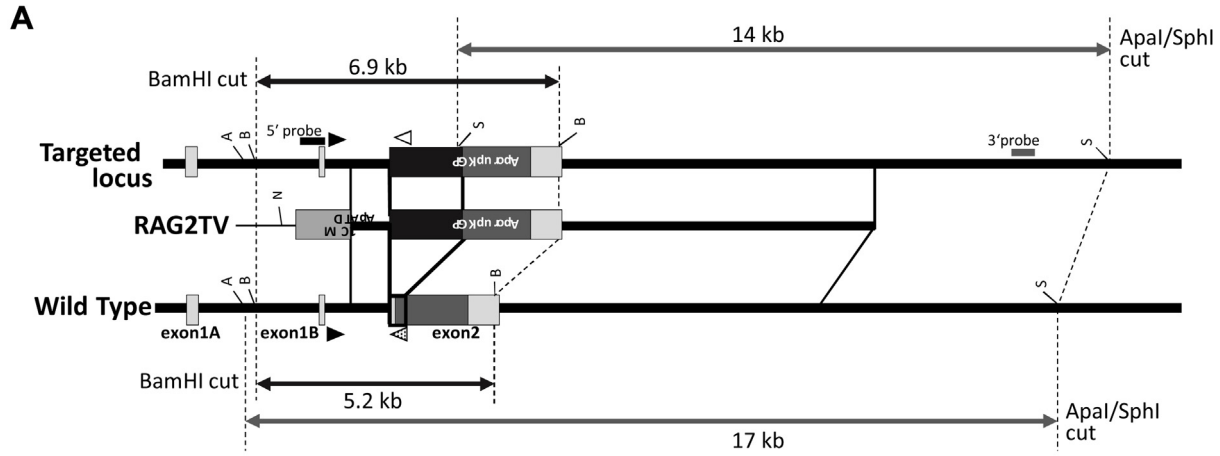

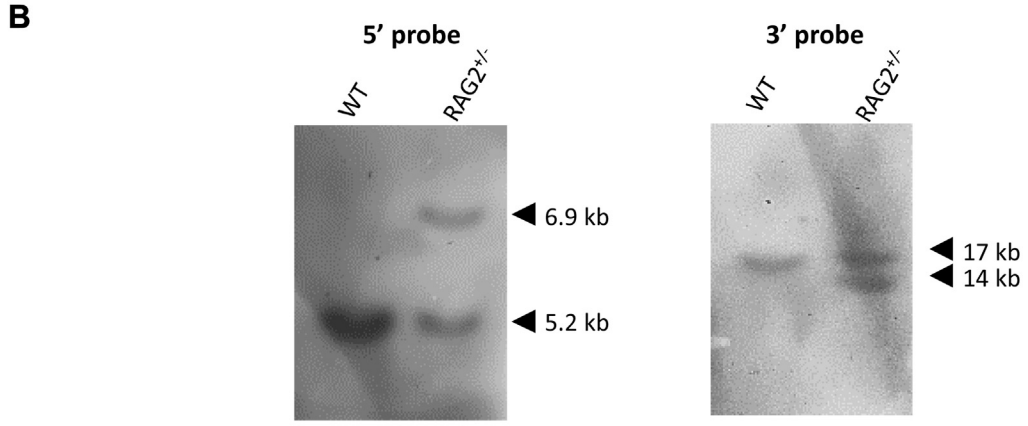

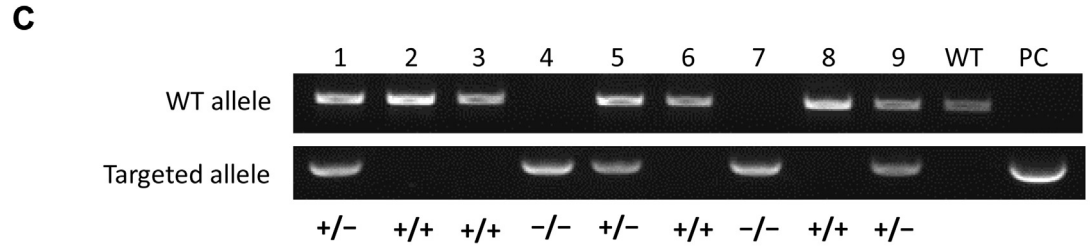

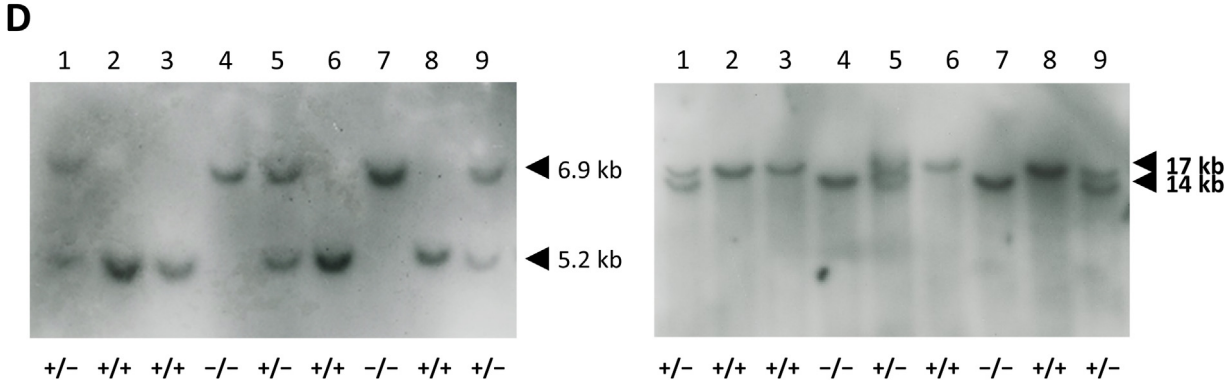

Fig. 1. Targeted disruption of porcine *RAG2* gene and genotyping of RAG2 targeted pigs.
(A) The endogenous *RAG2* locus (lower; exons are indicated by black boxes and coding regions are indicated by gray boxes), TV (middle) and the predicted configuration of a targeted allele (upper) are shown. Positions of three primers for diagnostic PCR are indicated by arrowheads (black, white, or dotted). Positions of Southern blotting probes are indicated by bars. The bidirectional arrows indicate DNA fragments to be detected by Southern blot analysis of *Bam*HI or *Apa*I/*Sph*I-digested genomic DNA. The upper and lower arrows, respectively, show fragments derived from a targeted and wild-type allele. Restriction sites are abbreviated as follows: A, *Apa* I; B, *Bam* HI; and S, *Sph* I.
(B) Southern blot analysis of *Bam* HI (5′ probe) or *Apa* I/*Sph* I (3′ probe)-digested genomic DNA from one PCR-positive cloned fetus ($RAG2^{+/-}$) and a wild-type control (WT).