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The MHC homozygous inbred Babraham pig as a resource for veterinary and translational medicine

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Abstract

The Babraham pig is a highly inbred breed first developed in the United Kingdom approximately 50 years ago. Previous reports indicate a very high degree of homozygosity across the genome, including the MHC region, but confirmation of homozygosity at the specific MHC loci was lacking. Using both direct sequencing and PCR-based sequence-specific typing, we confirm that Babraham pigs are essentially homozygous at their MHC loci and formalize their MHC haplotype as Hp-55.6. This enhances the utility of the Babraham pig as a useful biomedical model for studies in which controlling for genetic variation is important.

Brief Communication

Pigs are a fundamental food producing animal and important biomedical model. The consumption of ~~pig meat~~pork continues to rise in both the developed and developing world, particularly in Asia~~,~~ (USDA 2017), and preventing and controlling infectious disease remains a priority~~.~~ (Beckham et al. 2018; Gay 2013). Reduced disease burden enables increases in farming density and outputs, improves animal welfare and can reduce the chance of zoonotic disease transmission. Healthier animals can also significantly improve the health and livelihood of small scale and subsistence producers. In addition, the similarities in physiology and organ size mean that pigs are an excellent disease model with the potential to provide organs for human transplantation~~.~~ (Ekser et al. 2017; Lunney 2007). To enable future disease, vaccine, and translational research, a more detailed understanding of the genetic variation that underpins differential immune responses in pigs is essential.

Domesticated pigs have maintained a significant level of genetic diversity, both within and between breeds, despite strong selection for production traits and inbreeding (Yang et al. 2017). Inevitably, this diversity correlates with significant variation at the polymorphic immune loci. For the pig major histocompatibility complex (MHC, also referred to as swine leukocyte antigen (SLA)), there are currently 238 MHC class I alleles and 223 MHC class II alleles described for *Sus scrofa* in the Immuno-Polymorphism Database (IPD)-MHC database (http://www.ebi.ac.uk/ipd/mhc/group/SLA) (Maccari et al. 2017). The antibody lambda locus also appears to be highly polymorphic, even among commercial pigs with a similar genetic background (Guo et al. 2016), and the T cell loci appear variable in gene content, at least between breeds (Schwartz, J.C., T. Connelley, and J.A. Hammond, unpublished). This diversity is problematic for infectious disease research and quantitative trait mapping studies in which complex and uncontrolled genetic variation may confound results and reduce statistical power. Immunogenetic variation also presents significant problems for preclinical studies with the pig as a model and future efforts to enable xenotransplantation. ~~Inbred pig models are therefore~~For example, porcine endogenous retroviruses (PERVs), encoded in the pig genome, have impeded organ xenotransplantation as they pose a risk if passed to humans. These elements have recently been removed using genome editing in an outbred pig (Niu et al. 2017). The same work on a large inbred pig (that can produce suitably sized organs) would decrease the possibility of additional uncharacterized PERVs that are likely in outbred populations. Furthermore , having a defined MHC allows one to predict tolerance and associated clinical interventions. For instance, the power of the inbred Babraham pig model

has recently been demonstrated in a genome-wide analysis which identified minor histocompatibility antigens involved in corneal transplant rejection (Nicholls et al. 2016). This work was only possible using individuals with a controlled genetic background and a defined MHC region in order to facilitate controlled matching and mismatching of histocompatibility loci. Large inbred pig models are therefore fundamentally important scientific resources.

In the UK, and likely the whole world, the Babraham ~~pigs are now~~pig is the only extant example of a large inbred ~~pigs.~~pig breed. While there are several MHC inbred miniature pig breeds that have been developed, including the NIH and Yucatan miniature pigs (Choi et al. 2016; Sachs et al. 1976), these are less representative of commercial breeds. As a consequence ~~they have~~, the Babraham pig has great potential to play an important role in studying infectious diseases in pigs~~,~~ and as a preclinical model for human disease. Babraham pigs were derived from a Large White commercial background by Dr Richard Binns at the Babraham Institute (United Kingdom) during the 1970s (Signer et al. 1999). Multiple skin grafts were performed across potential parents and selective breeding was carried out between those individuals in which least cross-rejection was observed. This was continued for five generations, rejecting defective individuals and those with residual skin graft rejection, and which produced individuals that tolerated skin grafts. This tolerance indicated functional homozygosity at least for the MHC antigens and probably also for a high proportion of minor histocompatibility loci. After 20 generations, a restriction fragment length polymorphism study demonstrated a level of inbreeding homozygosity comparable to inbred strains of mice (Signer et al. 1999). ~~Recently,~~During the period from this 1999 study until 2016, there were approximately 15 generations and recovery from a bottleneck of 13 sows and 2 boars (animal records from The Pirbright Institute). At this point, SNP analysis of single nucleotide polymorphisms (SNP) using ~~a 60k SNP~~the Illumina PorcineSNP60 chip suggested approximately 85% homozygosity across the Babraham genome, based on 59,852 genotyped SNPs (Nicholls et al. 2016). Other animals with the same level of inbreeding and homozygosity are not available for large veterinary species.

The SNP chip analysis by Nicholls et al. (2016) indicated complete homozygosity across the MHC region of chromosome 7. However, measuring MHC variation using commercial SNP assays is not always accurate as much of the variation over polymorphic loci falls below the minimum minor allele frequency to be included in the assay. In addition, structurally variable

haplotypes often confound the mapping of second generation sequencing technologies that produce relatively short reads used to design the SNP chip. Indeed, using SNPchimp (Nicolazzi et al. 2015) to search the Illumina 60k SNP array revealed only two SNPs present in the vicinity of the classical MHC class I loci (i.e. within the interval of Sscrofa10.2, chr 7: 24,600,000 – 24,765,000). Confirming that the Babraham MHC is homozygous would add significant value to this animal line as ~~a~~ both a veterinary and biomedical model. We therefore ~~sought~~used two different typing methods to confirm MHC homozygosity and formalise the Babraham MHC haplotype.

The genes targeted for cDNA sequencing were the classical MHC class I genes *SLA-1*, *SLA-2*, and *SLA-3*, the non-classical class I genes *SLA-6*, *SLA-7*, and *SLA-8*, and the class II genes *SLA-DQA*, *SLA-DQB1*, and *SLA-DRB1*. All known SLA alleles within the IPD-MHC database were downloaded and used for oligonucleotide primer design (Table 1). ~~PCR amplicons were generated from cDNA~~Total RNA was extracted from peripheral blood mononuclear cells derived from six animals (as distantly related as possible) using TRIzol (Thermo Fisher Scientific) following manufacturer's instructions. Complementary DNA (cDNA) was generated using the Superscript III reverse transcriptase kit (Thermo Fisher Scientific) following manufacturer's instructions. PCR amplicons were generated from this cDNA, ligated into pGEM-T Easy vector (Promega), and transformed into NEB 5-alpha chemically-competent *Escherichia coli* (New England Biolabs). Approximately 584 individual clones were selected by positive colony PCR result and submitted to Source BioScience (United Kingdom) for sequencing. Sanger chain-termination sequencing was performed using either of the vector-specific T7 (forward) or SP6 (reverse) primers. The chromatograms from the individual sequencing reads were then compared to the known alleles within the IPD-MHC database.

To further confirm SLA homozygosity in the Babraham pigs, ~~SLA~~ genotyping of *SLA-1*, *SLA-2*, *SLA-3*, *DRB1*, *DQB1*, and *DQA* was performed on the genomic DNA from 22 animals (including the six animals used for cDNA analysis) using PCR-based assays with sequence-specific typing primers (PCR-SSP) as previously described (Ho et al. 2009b; Ho et al. 2010). The typing primer panel has since been modified to accommodate for the increasing number of SLA alleles and allele groups (details not shown).

Both SSP typing and sequencing methods confirmed homozygosity at the *SLA-1*, *SLA-2*, *SLA-DQA*, *SLA-DRB1*, and *SLA-DQB1* loci (Table 2). The sequenced region of *SLA-DQB1*, containing the majority of both beta domains, could not differentiate between alleles *SLA-DQB1*08:01* and *SLA-DQB1*08:02*, which differ from each other at two nucleotide positions outside of the sequenced region (i.e. at positions +52 and +606-). This gene was nevertheless identical over the sequenced region in all animals based on reads from eight clones per animal. Only three sequencing reads from two animals were recovered for *SLA-3*. One of these reads corresponded with *SLA-3*04:02* and the remaining two reads corresponded with *SLA-3*04:03*, indicating that at least one of the six animals is a heterozygote at this locus. These two alleles differ only in the alpha-3 domain, by both a 12-bp insertion in *SLA-3*04:03* and a single non-synonymous mutation nine bp upstream of the insertion. However, it is uncertain what, if any, influence these differences have on peptide-binding and receptor interactions, especially as this region is distal from the peptide-binding regions of the alpha-1 and alpha-2 domains. The paucity of *SLA-3* reads is likely due to the co-amplification of *SLA-3* cDNA with *SLA-1* and *SLA-2*, both of which are considered more highly expressed (Lunney et al. 2009; Tennant et al. 2007). The haplotype that corresponds to the genotype *SLA-1*14:02-SLA-3*04:03-SLA-2*11:04-DRB1*05:01-DQB1*08:01* has been previously designated by the ISAG/IUIS-VIC SLA Nomenclature Committee Hp-55.6. The class I haplotype Hp-55.0 was originally described in the ESK-4 cell line (Ho et al. 2009a), while the class II haplotype Hp-0.6 has been detected in several pig breeds including Yucatan (Smith et al. 2005), Austrian Pietrain (Essler et al. 2013), Chinese Bama miniature pigs (Gao et al. 2014), as well as the SK-RST cell line (Ho et al. 2009a).

Sequencing reads were additionally obtained for the non-classical MHC class I genes (*SLA-6*, *SLA-7*, and *SLA-8*) due to broad primer specificity. A total of six identical reads from three animals were identified for *SLA-6*, all of which contained the intron between the first two alpha domains, and thus originated from either unspliced mRNA or contaminating genomic DNA. Despite this, both exons were in frame and putatively functional. All of these reads also differed by at least 4 bp from the nine known *SLA-6* alleles in IPD-MHC, with five alleles being equally close (*SLA-6*01:01*, *SLA-6*03:01*, *SLA-6*04:01*, *SLA-6*05:01*, and *SLA-6*06:01*). Reads specific for *SLA-7* (n=1) and *SLA-8* (n=8, from 4 animals) were also detected, likely due to the degenerate nature of the *SLA-6* primers used for cDNA amplification. Only three alleles of *SLA-7* are currently described within the IPD-MHC

database, and the closest of these, *SLA-7*01:01*, differs by 2 bp to the single read sequenced from the Babraham samples. For *SLA-8*, all of the ~~sequences were~~sequencing reads were identical to each other and were also exact matches for known alleles *SLA-8*01:01*, *SLA-8*04:01*, and *SLA-8*05:01*. As the sequenced reads did not span the entire transcript, it could not be ascertained which, if any, of these alleles correspond to the Babraham *SLA-8*. Thus, the sequencing results suggested that the Babraham animals were identical to each other for at least the *SLA-6* and *SLA-8* non-classical MHC class I loci, while only a single read was obtained for *SLA-7*.

~~This study shows that the~~For inclusion into the IPD-MHC database, the Babraham-derived alleles presented here have been deposited into GenBank (accessions: MH107868 - MH107877). This study shows that the inbred Babraham pigs are functionally MHC homozygous. Taken together with the high level of inbreeding as measured by SNPs over the entire genome (Nicholls et al. 2016), this confirms the Babraham pig as a very valuable model for swine and human disease research, as well in wider biomedical applications. ~~The~~This value can only increase as our ability to edit mammalian genomes and produce gene-edited animals improves.

Table 1 – Oligonucleotide primers used for amplification of SLA genes

gene	orientation	sequence (5'-)	cDNA position	domain
SLA-1,-2, -3, -7, and *-8*	sense	GACACGCAGTTCGTGHGGTTC	153-163	α1
SLA-6	sense	AGGACCCGCGTCTGGAGAAG	150	α1
SLA-1,-2,-3,-6, -7, and *-8*	anti-sense	CTGGAAGGTCCCATCCCCTG	789-799	α3
SLA-1,-2,-3, -6, and *-7*	anti-sense	GCTGCACMTGGCAGGTGTAGC	851-861	α3
SLA-DQA	sense	GAGCGCCTGTGGAGGTGAAG	54	leader
SLA-DQA	sense	GACCATGTTGCCTCCTATGGC	85	α1
SLA-DQA	anti-sense	CAGATGAGGGTGTTGGGCTGAC	398	α2
SLA-DQA	anti-sense	GACAGAGTGCCCGTTCTTCAAC	462	α2
SLA-DQB1	sense	GAGACTCTCCACAGGATTTCGTG	98	β1
SLA-DQB1	anti-sense	ACTGTAGGTTGCACTCGCCG	395	β2
SLA-DRB1	sense	GGGACAYCSCACMGCATTTC	89	β1
SLA-DRB1	sense	GAGTGYCRTTTCTTCAVYGGGAC	127	β1
SLA-DRB1	anti-sense	CAGAGCAGACCAGGAGGTTGTG	421	β2
SLA-DRB1	anti-sense	GGTCCAGTCTCCATTAGGGATC	552	β2

Table 2 – Classical MHC class I and class II genotypes of inbred Babraham pigs

	SLA-1	*SLA-2*	*SLA-3*	*DRB1*	*DQA*	*DQB1*
Sequencing	**14:02*[1]	**11:04*[2]	**04:03*[3]*/*04:02*	**05:01*	**01:03*	**08:01* or **08:02*
SSP typing	**14:02*	**11:04*	**04:XX*	**05:XX*	**01:XX*	**08:XX*

[1] previously known as *SLA-1*es11*
[2] previously known as *SLA-2*es22*
[3] previously known as *SLA-3*04es32*

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