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# PIG GENETICS, INSIGHT IN MINIPIGS

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## ABSTRACT

The sequence of the pig has now been published. Associated with genomic tools such as high density genotyping, (re)sequencing, comparative genetic, it offers new opportunity to understand the genetic basis of complex traits. The challenge is now to get adequate phenotypes. For minipigs, focus is made on traits relevant for medicine, in order to use minipigs as a biomedical model. Because of the number of research fields, specific lines had to be developed and within the last decades, several minipig lines were made available. For example, to specifically study development and regression of cutaneous melanoma, the MeLiM line was built up to largely express this particular trait. It was then possible to study the genetic determinism of this trait and acquire knowledge relevant for human disease, and in a lesser extent also relevant for pig production. Currently, one of our aims is to develop a minipig line adequate for studies on human nutrition related diseases, such as obesity or atherosclerosis. This approach first requires the characterization of minipigs to understand the genetic determinism of their behavior but also some physiological parameters related to human nutrition.

**Key Words**: Genetic, minipig, selection

## INTRODUCTION

Swine breeding has a long history. The process of domestication, which begun circa 7000 b.c. includes selective breeding for specific characteristics, and may be considered a first step in genetic improvement. The process of domestication includes selective breeding for specific characteristics, and may be considered a first step in genetic improvement. Pig improvement has first been the result of empirical methods employed by individual farmers. Then selection became a technique scientifically based, progressing with the knowledge of the biology involved, and particularly with the development of genetics. Through centuries and geographical areas, the variety of breeding goals has conducted to a large number of pig breeds. Crossbreeding has also been used to create synthetic lines. Those breeds have been mainly developed for food subsistence. In the mid 20$^{th}$ century, pig has been viewed as an alternative to other animals for human oriented studies (Bustad and Mc Clellan, 1965). Most of the strains derived from a small number of reproducers taken from local populations of small size (e.g. Ossabaw, Lan Yu, Yucatan) and raised in a controlled environment, others were successively crossed with Asian populations and/or European populations for increasing diversity, introducing new coat colours or desirable characteristics. Some of the strains were then selected, generally for small size but also for other specific traits. Minipig strains were created under laboratory conditions in the USA, Russia, Japan, Taïwan, Germany, Czech Republic, Australia, sometimes derived from one another. In 1996, Thikonov enumerated 20 breeds of miniature pigs used for biomedical and biological studies. Some strains were

constituted for general research purposes (e.g. Hormel, Göttingen) and other for very specific studies (e.g. Yucatan strains, MeliM). Recently, Köhn (2011) described the constitution and major characteristics of nine micro- or minipig breeds including Clawn, Göttingen, Hanford, Troll, Sinclair, Westran and 3 Yucatan breeds. He also offered a schematic overview of minipig breeds used in biomedical research.

Advances in molecular biology as well as in statistics and computational facilities offer a new challenge for pig breeding and genetics in the next few years, which might increase the interest of minipig for biomedical research.

## CONSTITUTION OF SOME MINIPIG STRAINS

A description of the first small size pig strains developed for biomedical and pharmaceutical research purpose is given by Bustad and McClellan (1968). The very first project was initiated in 1949 at the Hormel Institute of the University of Minnesota, the strain between refered as Minnesota Miniatures or Hormel Miniatures. As detailed by Dettmers and Rempel (1968), it was started the crossbreeding of four American breeds; first 4 Guinea hogs females were mated to one wild boar from Catalina Island, then two and four years later, Piney Woods breeders were introduced and in 1957, the fourth breed stock was constituted of Ras-n-lansa (Guam pigs), introduced to reduce size. In total, only 4 males and 11 females were used from original breeds. The Hormel minipig strain was further on selected for small size (weight at 140 days of age) (Dettmers et al., 1965). In order to develop a white color strain, a Yorkshire boar was mated to Minnesota sows in 1963. As underlined by Simianer and Khön (2010), two founder breeds of the Minnesota Minipig are island-based strains, making use of the evolutionary phenomenon of so-called island dwarfism, reflecting the observation that isolated populations on small islands tend to develop dwarf variants in many mammalian species. Sinclair pigs derived directly from Hormel pigs.

Pigs from Florida swamps were bred by Pitman-Moore pharmaceutical company, which gave the name of the strain. Breeders were distributed to other laboratories to found new minipig strains (Hanford, VitaVet, Labco). The Hanford miniature swine was obtained by mating Pitman-Moore breeders with Palouse swine and a Louisiana swamp hog and later with introduction of Mexican pigs (Yucatan pigs) in their breeding stock.

The Berlin Miniature Pig breed (also known as Mini-Lewe) has been developed 20 years ago at the experimental Station of the Humboldt-University from a cross of Vietnamese Pot Belly Pigs, Saddle Back Pigs and German Landrace followed by consistent selection for small body weight (Hardge et al., 1999).

A detailed description of one the minipig breeds mostly used for pharmaceutical and biomedical research, the Göttingen minipig, was recently provided by Simianer and Köhn (2010). The development of the Göttingen minipig was initiated in the 1960's at the Friedland Experiment Station of the University of Göttingen, Germany. It was inspired by the existence of similar miniature breeds, like the Minnesota Minipig. The initial breeding stock was constituted of four male and two female Minnesota Minipigs and three male and eight female, and later four additional female potbelly Vietnamese pigs from German zoos. Göttingen Minipigs were selected for low body weight on the basis of the 154-day weight, then on weaning weight. In order to obtain a white color strain, German Landrace pigs were introduced by artificial insemination during the years 1965 to 1969. Separate lines of coloured and white minipigs were established. In 1969, the proportional representation of the three original breeds in the white line was 60% Vietnamese potbelly pigs, 33% Minnesota Minipigs and 7% German Landrace (reported by Simianer and Köhn, 2010). From 1992, efforts were

conducted in the Göttingen minipig population to improve population uniformity, health status, recorded breed characteristics, and to exclusively produce white animals. The current selection objective is mainly based on body weight and litter size. Additional traits are taken into account such as the number of teats, the hair coat, black hair or spots, abnormalities and different body shape parameters like saddle back, position of legs and jaws. As temperament is considered of major relevance for minipigs used as laboratory animals, further studies were conducted showing that behaviour scoring is of moderate heritability and that temperament can be included in selection objectives (Köhn et al., 2009).

Pure Yucatan strains were also developed from original 25 Yucatan pigs (also mentioned as hairless Mexican pigs) imported from Yucatan Peninsula of Mexico. Panepinto et al. (1978) constituted an original breeding stock at Colorado State University from these imported pigs. Another miniature pure breed regained some interest within the last decade: the Ossabaw breed (Sturek et al., 2007). Pigs originated from the Ossabaw Island (United States). Research on the obesity-prone Ossabaw miniature swine clearly indicates that these animals develop metabolism syndrom and cardiovascular disease when fed a high-calorie atherogenic diet, and might prove to be superior to Yucatan pig (Neeb et al., 2010).

Selection for other criterion than small size (or coat color) was applied in some minipig populations. Phillips et al. (1979) developed two divergent Yucatan lines selected for low or high glucose-tolerance. These lines were developed in order to provide a model for diabete studies. Tikhonov (1996) established a minipig line (Minisibs), from Vietnamese, Swedish Landrace and wild boar, with chromosomal polymorphism (2n=38, 37, 36). MeliM breed, Munich miniature (Troll) and one Sinclair line were dedicated to melanoma research.

## THE MELIM PIG BREED AND MELANOMA PREDISPOSITION

An example of a pig breed developed for a specific biomedical research, cutaneous melanomas, is the Melanoblastoma-bearing Libechov Minipig (MeLiM) breed (Horak et al., 1999). This breed was constituted at the Libechov Institute of Animal Physiology and Genetics in the Czech Republic because of the similarities between human and porcine skin (Zhang and Monteiro-Rivière, 1997). Initially, an inheritable form of cutaneous melanoma has been described in the Hormel miniature pig strain. A strain of Hormel miniature pigs, known as Sinclair pigs were founders of a herd that was bred to have a high incidence of spontaneous melanomas. A similar pig model of melanoma was established in Germany Munich Miniature Swine. The history of MeliM breed was described by Hruban et al. (2004). The experimental herd of laboratory pigs was founded by importation of 5 animals of the Hormel strain from the USA in 1967. These animals were crossbred with Landrace, Large White, Cornwall, Vietnamese pigs and Göttingen pigs. A few black piglets with melanoma which occurred in this genetically heterogeneous population and the MeLiM strain was then established using selective breeding for melanoma. In 2004, the contribution of each ancestral breed was: Hormel strain 34–53%, Vietnamese pig 10–18%, Landrace 3–7%, Cornwall 1–6%, Large White 0–6% and Göttingen breed 19–34%, depending on the family.

For further studies, MeliM breeders were imported from the Czech Republic to the CEA/INRA Laboratory of Radiobiology and Genome Study in Jouy-en-Josas (France). With such a strain, it was possible to further describe tumour development, but as melanoma incidence was demonstrated as heritable, it was possible to explore the genetic determinism of melanoma susceptibility and unravel genes involved in incidence of spontaneous cutaneous melanoma. Studies has been conducted both for gene localisation with linkage and association analyses and gene expression in melanoma. Consequently, for genetic analysis, affected boars

were crossed with healthy sows of the Duroc strain to create an F1 and a backcross generation (Bourneuf et al., 2011) These crossbred animals were first analysed with 3 microsatellite markers linked to *CDKN2A* gene, a candidate gene for human melanoma (Piepkorn, 2000). Allelic association, linkage analysis and haplotype analysis of these data led to exclusion of the *CDKN2A* gene as a candidate for melanoma susceptibility (Le Chalony et al., 2003). A second analysis with 137 microsatellites revealed 4 chromosomal regions potentially involved in melanoma susceptibility on chromosomes (SSC) 1, 2, 6 (near MC1R), 7 and 8. The 3 regions on SSC 1, 6 and 7 have counterparts on human chromosomes harboring melanoma candidate loci (Geffrotin et al., 2004). A third QTL analysis was performed on additional backcross individual with a higher number of markers (153). The results revealed QTL on SSC 1, 2, 13, 15 and 17. A focus on *MC1R* gene showed that one allele, which determines black coat colour in pigs, predisposes significantly to melanoma. A refined QTL analysis of the most significant QTL confirmed a linkage between SSC13 and melanoma susceptibility (Bourneuf et al., 2011). In order to investigate a potential association of *MITF* gene, an identified candidate gene localised on SSC13, with melanoma development. A single-marker association analysis with 23 SNPs conducted to exclude *MITF* gene as a causal gene. New developments to localize QTL focused on structural variation of genomes such as copy number variants (CNV) or insertion-deletions. A higher density genotyping, provided by the Illumina 64k pig Beadchip, was performed on the backcross pedigree to detect potential CNV events which might lead to new QTL localisations and candidate genes for melanoma susceptibility (Corominas et al., 2011). Gene expression in melanoma also allowed to identified putative candidate genes.

## PIG GENETIC TOOLS

The pig genome sequence has been released (Groenen et al., 2012). This is a major step for pig genetic studies in general, and will prove to be an important resource in use of pig model for biomedical research. As an example, Groenen et al. (2012) studied predicted porcine protein sequences and compared them with their human orthologues. They observed 112 positions where the porcine protein has the same amino acid that is implicated in a human disease and most of these changes in humans have been shown to increase risk in multifactorial traits, including obesity and diabetes. Also the pig genome sequence was mainly based on a Duroc animal, a Göttingen pig has also been sequenced (Vamathevan et al., 2013). From comparison between genome sequences, one of their conclusions was that the differences identified between minipig when compared to the Duroc pig, highlight the fundamental importance of having gene information from the exact breed used in biomedical research. The consequence is that further genetic knowledge should be seeked out in each particular minipig strain, either in a very general way by resequencing, or in a more focus manner on gene/region of interest. Additionally, they provided evidence, at least for drug development, that some target genes for cardiovascular drugs differed from human leading to potential functional divergence. Thus binding assays or dose-ranging investigations of drug target relationships may need to be carefully interpreted.

The constitution and use of the 60k single-nucleotide polymorphism (SNP) chip (Ramos et al., 2009) improved our possibility to localize region of interest (for an update of detected QTL on various traits, see pigqtldb http://www.animalgenome.org/cgi-bin/QTLdb/SS/index), but also to better understand pig evolution through domestication and selection. A recent example is given by Burgo-Paz et al. (2013) on American pig populations, including Ossabaw and Yucatan pigs. They observed that Ossabaw and Yucatan pigs were clearly differentiated,

despite an assumed shared Iberian ancestry. Yucatan was the closest breed to Spanish Iberian, whereas Ossabaw clustered among other American village pigs. An analysis of selection signatures revealed there could have been a common response among American populations involving a modification of their pattern of development and, perhaps, also by how they respond to external stimuli.

Another field of interest is the study of gut microbiota (the metagenome). Recent evidence suggests that the gut microbiota is an important contributing factor to obesity and related metabolic disorders. A study was conducted to characterise the intestinal microbiota in Göttingen minipigs and the Ossabaw minipigs (Pedersen et al. 2013). Obesity-related changes in the composition of the gut microbiota were found in lean versus obese Göttingen and Ossabaw minipigs, buth the changes differed from one strain to the other. In both pig models diet seems to be the defining factor that shapes the gut microbiota as observed by changes in different bacteria divisions between lean and obese minipigs. They showed that both Ossabaw and Göttingen minipigs may be useful animal models in studying gut microbiota and provide a better understanding of obesity and metabolic syndrome and the relationship to the gut microbiota. It provided new insight in exploring this relationship in other minipig strains.

## CONSTITUTION OF A NEW MINIPG BREED

As it has been explained, there are already numerous pig strains already used for biomedical research, some of them locally used, some of them largely used throughout the world for different research fields. For research involving physiological or genetic studies, the adequacy of the model, i.e. the pig strain, has to be carefully evaluated considering that the characteristics of the animals might less relevant in one strain than in another one. The constitution of new minipig breed at INRA was driven by the aim to produce a strain adequate for human nutrition studies oriented towards diabetes and obesity. The first step is to characterized different breeds (Vietnamese, Pitman-Moore and Yucatan) in order to estimate the (dis)advantages of each breed. Yucatan appeared to be a good basis, especially regarding behavior. Crossbreeding of different breeds at the beginning to benefit from the advantages of each foundation lines, to increase the variability of the traits and to decrease inbreeding, at least at the constitution of the line. The second step is to define a crossbreeding and/or selection strategy. Up to now, except selection conducted by Phillips et al. (1979) in Yucatan, no specific selection objective has been established in order to improve the adequacy of a pig line to human nutrition studies. Such a selection objective would have to take into account relevant metabolic parameters. This step has yet to be evaluated (which traits, which genetic parameters). Then the selection has to conducted minimizing the increase of inbreeding (using optimal contribution or a at least a family-based selection). Additional crossbreeding could be taken into consideration for introgressing interesting QTL or genes, using high density genotyping first for introgression then for controlling the initial genetic background.

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