Rapid One-Step Generation of Genetically Modified Göttingen Minipigs for Human Disease Modelling

For decades Göttingen Minipigs has been used in biomedical research, with their many anatomical, physiological and pathophysiological similarities to humans, and as such play an important role as large animal models in translational studies. In recent years, the number of genetically altered Göttingen Minipigs has increased, as advanced genetic techniques simplify the generation of animals with precisely tailored modifications. These modifications are designed to replicate genetic alterations responsible for human disease. As such, genetically altered Göttingen Minipigs are valuable large animal disease models, but in addition also considered promising donors for xenotransplantation.

Currently used technologies for generation of genetically modified minipigs require a long development time (2-3 years) (refs 1-2), even when applying CRISPR approaches, due to the almost 4 months long gestation period and 7 to 8 months maturation period of the minipigs. To overcome this limitation, we have established a rapid and convenient method for the generation of genetically modified animals GENISYST® (Gene-Disease Integrative Systems Transgenesis) for in vivo gene gain-of-function and loss-of-function studies. This is the first report so far that demonstrates successful generation of genetically modified Göttingen Minipigs in a fraction of time and cost of any other available methods.

Application of GENISYST® for gain-of-function studies

For confirmation of GENISYST® applicability in gain-of-function studies, we used a green fluorescent protein (GFP) to mark tissues, which received gene delivery. We achieved high levels of GFP expression across a broad spectrum of tissues. In the liver of Göttingen Minipigs, expression of the GFP was achieved within eight weeks of GENISYST® administration (Figure 1). This was not only evident on a gene level, but also supported by immunofluorescent staining showing appearance of GFP protein in animals with GENISYST® (Figure 2). Altogether, this result shows the feasibility of our novel approach in rapid gain-of-function experiments in the Göttingen Minipigs model.

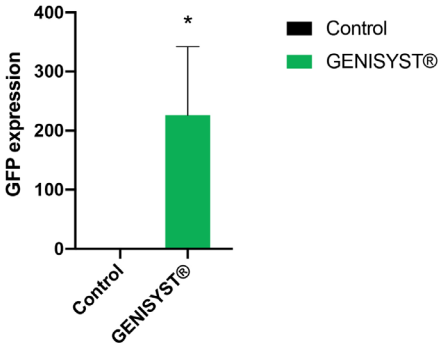

Figure 1: GFP gene expression in the minipig liver.

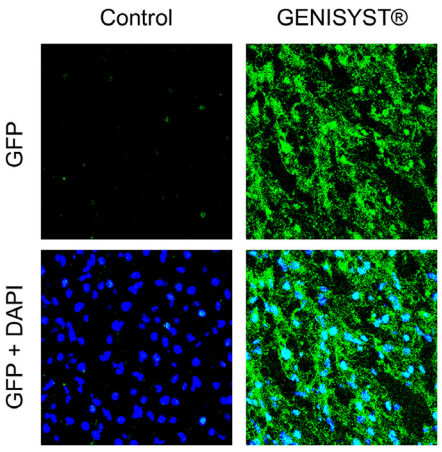

Figure 2: GFP protein expression in the minipig liver.

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Application of GENISYST® for loss-of-function studies

For confirmation of GENISYST® applicability in loss-of-function studies, we used a well-characterized disease model of PCSK-9 gene (Proprotein convertase subtilisin/kexin type 9) inactivation (ref 3). PCSK-9 is an enzyme of medical importance because of its activity in lipoprotein homeostasis (ref 4). Agents that block the action of PCSK-9, lower plasma concentrations of cholesterol reducing the risk of liver and cardiovascular disease, including inflammatory Non-Alcoholic Steatohepatitis (NASH) (ref 5). Within six weeks of GENISYST® administration to Göttingen Minipigs (ref 6), we achieved consistent reduction of PCSK-9 levels in the liver (Figure 3).

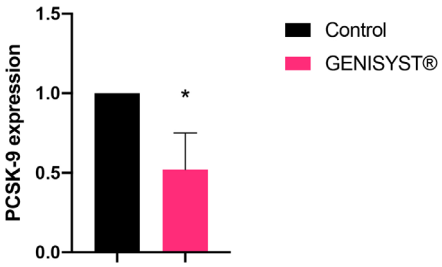

Figure 3: PCSK-9 gene expression in the minipig liver.

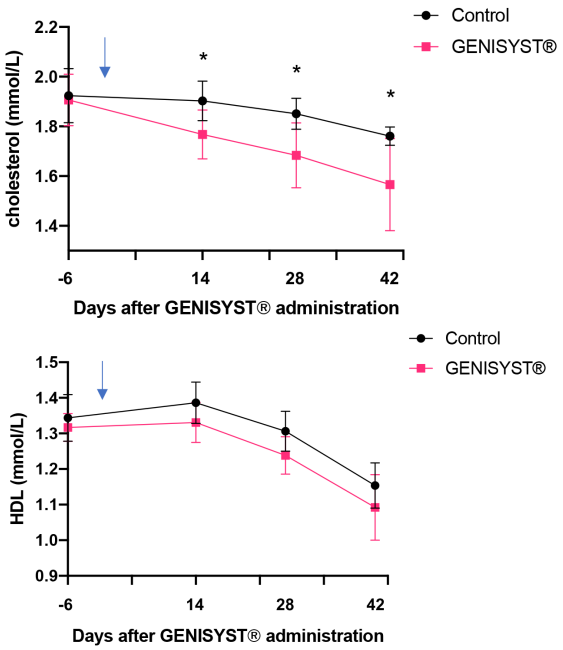

Figure 4: Lipid levels in the minipig blood.

This led to the physiological changes in the affected animals, recapitulating previously reported hallmarks of PCSK-9 inactivation: significant reduction in blood cholesterol levels (Figure 4) without concomitant changes in high-density lipoproteins (HDL, ref 3). This data confirms the applicability of GENISYST® in gene loss-of-function disease modelling in Göttingen Minipigs.

Conclusions

Our data indicates feasibility for rapid generation of genetically modified Göttingen Minipigs as models of human disease. GENISYST® facilitates accelerated generation of gain- or loss-of-function models, can be applied to model disease in a broad spectrum of tissues, and leads to a reduction in animals use. These features allow for significant cost and time savings over traditional methods.

References

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