INVITED REVIEW

Rescuing Lethal Phenotypes Induced by Disruption of Genes in Mice: a Review of Novel Strategies

Nándor LIPTÁK[1]*, Zoltán GÁL[1]*, Bálint BIRÓ, László HIRIPI[1], Orsolya Ivett HOFFMANN[1]

** These authors contributed equally to this work.*

[1]Animal Biotechnology Department, NARIC-Agricultural Biotechnology Institute, Gödöllő, Hungary

Received July 16, 2020
Accepted November 18, 2020
Epub Ahead of Print January 14, 2021

Summary

Approximately 35 % of the mouse genes are indispensable for life, thus, global knock-out (KO) of those genes may result in embryonic or early postnatal lethality due to developmental abnormalities. Several KO mouse lines are valuable human disease models, but viable homozygous mutant mice are frequently required to mirror most symptoms of a human disease. The site-specific gene editing systems, the transcription activator-like effector nucleases (TALENs), Zinc-finger nucleases (ZFNs) and the clustered regularly interspaced short palindrome repeat-associated Cas9 nuclease (CRISPR/Cas9) made the generation of KO mice more efficient than before, but the homozygous lethality is still an undesired side-effect in case of many genes. The literature search was conducted using PubMed and Web of Science databases until June 30th, 2020. The following terms were combined to find relevant studies: "lethality", "mice", "knock-out", "deficient", "embryonic", "perinatal", "rescue". Additional manual search was also performed to find the related human diseases in the Online Mendelian Inheritance in Man (OMIM) database and to check the citations of the selected studies for rescuing methods. In this review, the possible solutions for rescuing human disease-relevant homozygous KO mice lethal phenotypes were summarized.

Key words

Knock-out mice • CRISPR/Cas9 • Lethality • Knock-out rabbits

Corresponding author

N. Lipták, NARIC-Agricultural Biotechnology Institute, Animal Biotechnology Department, Szent-Györgyi Albert St. 4, H-2100 Gödöllő, Hungary. Fax: +36-28-526-151. E-mail: liptak.nandor@abc.naik.hu

Introduction

Generating KO animals gives the opportunity to observe a whole organism if a gene is disrupted and it provides an answer to the origin and course of the appearance of various diseases. The production of these animal models is efficient enough nowadays although, a long journey led to the techniques of developing models that are now easy to produce.

The first two methods to generate KO mice were gene trapping (Gossler *et al.* 1989) and gene targeting (Mansour *et al.* 1988). Both methods required embryonic stem cells (ESCs), produced chimeric mice and were neither cost nor time effective. Transposon systems were also practical tools to disrupt genes in mice (Dupuy *et al.* 2001), however, transposon-based approaches proved to be very effective in creating transgenic animals later (Garrels *et al.* 2011, Katter *et al.* 2013). Site-specific endonucleases, TALENs, ZFNs and CRISPR/Cas9 are the latest members of the gene-editing toolbox. TALENs and ZFNs require engineered proteins, while CRISPR/Cas9 is RNA-guided. CRISPR/Cas9 gene editing requires the Cas9 mRNA or protein and the single guide RNA (sgRNA), which consists of the trans-activating RNA and CRISPR RNA. All of the aforementioned endonucleases induce site-specific double-strand breaks (DSBs) in the genome, which are usually

repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is predominant during the G1 phase, while HDR is active in S and G2 phases (Zaboikin *et al.* 2017). Both HDR and NHEJ can evoke small indels and point mutations, but HDR may also generate large insertions in the targeted genes if homologous template DNA is available. CRISPR/Cas9 system became the most efficient and broad spread tool for creating KO laboratory animals, e.g. mice (Wang *et al.* 2013), rats (Yoshimi *et al.* 2014), rabbits (Yang *et al.* 2014), etc., and a less expensive alternative of the previously described TALEN and ZFN applications (Ceasar *et al.* 2016). The widely accepted method of disrupt genes in animals with site-specific endonucleases is the microinjection of those gene-editing constructs into once-cell stage embryos.

Systemic phenotyping data, which were provided by International Mouse Phenotyping Consortium revealed that approximately 35 % of the mouse genes were essential for viability (Brown and Moore 2012). Several reports, along with recent studies claimed that heterozygous mutant mice did not develop the symptoms of a human disease and the homozygous KO mice were not viable.

The novel strategies to overcome KO mouse embryonic and postnatal lethality are described in detail in the following sections and Table 1, along with the related human diseases.

Mosaic inactivation of the target gene for rescuing the KO lethal phenotype

The generation of chimeric mice with gene targeting using ESCs was successful to establish KO mice (Crosby *et al.* 1998, Lindahl *et al.* 1998), but this method was laborious and expensive.

Mutations in the serine protease inhibitor Kazal-type 5 (SPINK5) gene were associated with Netherton syndrome, an autosomal recessive disorder which caused dermatitis, severe dehydration due to the malfunctioning epidermal barrier (OMIM 256500) (Chavanas *et al.* 2000). *Spink5* KO *(Spink5*$^{-/-}$) mice were created by insertional mutagenesis (Yang *et al.* 2004) for studying Netherton syndrome, but *Spink5*$^{-/-}$ mice died within few hours after birth. Mosaic inactivation of the *Spink5* gene using TALEN resulted in viable *Spink5*$^{-/-}$ KO mice, an appropriate animal model for human Netherton syndrome (Kasparek *et al.* 2016). Mosaicism could occur normally if the gene-editing endonucleases activated after the one-cell embryonic stage. One-cell stage embryos were microinjected with different concentrations of TALEN mRNA. Mild skin phenotype was observed in 8 % and 17 % of the pups from the higher concentrations of TALEN mRNA-groups (Kasparek *et al.* 2016).

Mutations of cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene were identified as potential basis of autoimmune lymphoproliferative syndrome 5 (ALPS 5, OMIM 616100 (Schubert *et al.* 2014)). Loss of *Ctla-4* caused premature lethality in case of gene-targeted *Ctla-4*$^{-/-}$ KO mice (Waterhouse *et al.* 1995). This issue remained unsolved until the invention of two-cell microinjection (Wang *et al.* 2017). This method was further developed to create KO mosaic mice with lethal mutations by one-step microinjection of the CRISPR/Cas9 reagents into one blastomere of two-cell stage embryos. Among others, a premature lethal phenotype, which was caused by *Ctla-4*$^{-/-}$ mutation was rescued and *Ctla-4*$^{-/-}$ KO mice survived for more than five months (Wu *et al.* 2019).

Disruption of other genes involved in the affected pathway

In the first two reports, embryonic lethal phenotype caused by the deletion of *Mouse double minute 2 homolog* (*Mdm2*) was rescued by the disruption of *p53* (Jones *et al.* 1995, Luna *et al.* 1995). *Mdm2*$^{-/-}$*p53*$^{-/-}$ double KO mice became a valuable model for studying human tumorigenesis (OMIM 614401, (Xiao *et al.* 1995)).

SPINK5 gene encodes lympho-epithelial Kazal-type related inhibitor (LEKTI), an inhibitor of kallikrein-related peptidases 5, 7 (KLK5, 7) and other serine proteases in the epidermis (Chavanas *et al.* 2000). In newborn *Spink5*$^{-/-}$ mice, elevated activation of the pro-kallikrein-cascade in the epidermis and stratum corneum was observed earlier (Sales *et al.* 2010). Taking advantage of this pathway, *Klk5*$^{+/-}$ mouse line was generated, crossed with *Spink5*$^{+/-}$ mice to create *Klk5*$^{-/-}$*Spink5*$^{-/-}$ double KO mice for modeling Netherton syndrome. The loss of *Klk5* rescued the neonatal lethal phenotype, which was evoked by *Spink5* deficiency but the life span of *Klk5*$^{-/-}$*Spink5*$^{-/-}$ mice was not as long as wild type (wt) littermates (Furio *et al.* 2015). *Klk5-7* were disrupted by gene-targeting and TALEN, respectively, then *Klk5*$^{-/-}$*Klk7*$^{-/-}$ double KO mice mated with *Spink5*$^{+/-}$ mice. Triple KO mice developed as normally as wt mice and fatal dehydration or severe defects of the epidermal barrier were not detected (Kasparek *et al.* 2017). In a very recent study, the deletion of *Klk5* and *Camp (Cathelicidin antimicrobial peptide*) were also sufficient to alleviate the severe symptoms evoked by the disruption of *Spink5* in mice (Zingkou *et al.* 2020).

Table 1. The novel strategies to overcome KO mouse embryonic and postnatal lethality.

Human genetic disorders, OMIM entries	Affected human genes	KO mice with lethal phenotype	Methods for phenotype rescuing
ALPS 5, 616100	CTLA-4	Premature lethality (Waterhouse *et al.* 1995)	Two-cell microinjection, highly effective, CRISPR/Cas9 (Wu *et al.* 2019).
Accelerated tumor formation, 614401	MDM2	Embryonic lethality (Jones *et al.* 1995, Luna *et al.* 1995)	Disruption of *p53* (Jones *et al.* 1995, Luna *et al.* 1995)
MODY-2, 125851	GCK	Embryonic lethality (Bali *et al.* 1995)	Tissue-specific KO mice, modest success (Postic *et al.* 1999, Terauchi *et al.* 1995, Zhang *et al.* 2004) Homozygous mutant rabbits CRISPR/Cas9 (Song *et al.* 2020)
Lipodystrophy, 151660 HGPS, 176670 EDMD, 181350	LMNA	Premature lethality (Mounkes *et al.* 2003, Sullivan *et al.* 1999)	KO rabbits, CRISPR/Cas9, modest success (Sui *et al.* 2019)
Netherton syndrome, 256500	SPINK5	Neonatal lethality (Yang *et al.* 2004)	Mosaic mice, TALEN (Kasparek *et al.* 2016); Disruption of *Klk 5* (Furio *et al.* 2015); *Klk5* and *Klk7* (Kasparek *et al.* 2017); *Klk5* and *Camp* (Zingkou *et al.* 2020)
Craniosynostosis, Chitayat syndrome, 611888	ERF	Embryonic lethality (Yamamoto *et al.* 1998)	Tetraploid complementation (Yamamoto *et al.* 1998), lentiviral gene transfer (Okada *et al.* 2007)
SCAR26, 617633	XRCC1	Embryonic lethality (Tebbs *et al.* 1999)	Transgene complementation (Tebbs *et al.* 2003)
Chronic pancreatitis, 167800, 608189	SPINK1	Perinatal lethality (Ohmuraya *et al.* 2005)	*Spink3*$^{-/-}$; XX^{SPINK1} knock-in mice (Sakata *et al.* 2016)
CDG-Ia, 212065	PMM2	Embryonic lethality (Thiel *et al.* 2006)	Mannose drinking, modest success (Schneider *et al.* 2011), (Chan *et al.* 2016)
CDG-Ib, 602579	MPI	(DeRossi *et al.* 2006)	Hypomorphic mice (Sharma *et al.* 2014)
CDG-It, 614921	PGM1	(Balakrishnan *et al.* 2019)	No solutions yet, galactose drinking was inefficient (Balakrishnan *et al.* 2019)
Premature ovarian failure, 147380	INHA	(Matzuk *et al.* 1992)	Bigenic mice, GeneSwitch method (Pierson *et al.* 2000)

Abbrevations: ALPS 5: autoimmune lymphoproliferative syndrome 5, CTLA-4: cytotoxic T-lymphocyte antigen-4, MDM2: mouse double minute 2 homolog, MODY-2: maturity-onset diabetes of the young 2, GCK: glu cokinase, *Klk: Kallikrein-related peptidase*, HPGS: Hutchinson-Gilford progeria syndrome, EDMD: Emery-Dreifuss muscular dystrophy, LMNA: nuclear lamin A, SPINK: serine protease inhibitor Kazal-type, *Camp: Cathelicidin antimicrobial peptide*, ERF: ETS proto-oncogene 2 (ETS2) repressor factor, SCAR26: spinocerebellar ataxia-26, XRCC1: x-ray cross-complementing 1, CDG: congenital disorder of glycosylation, PMM2: phosphomannomutase 2, MPI: phosphomannose isomerase, PGM1: phosphoglucomutase 1, INHA: inhibin alpha.

Inducible KO models

Conditional KO technologies were developed for temporal gene expression in mice; e.g. the inducible KO (iKO) method, in which the target gene could be switch on and off with doxycycline treatment (Zeng *et al.* 2008); the mifepristone-inducible GeneSwitch approach (Wang *et al.* 1994), etc.

Polymorphism in INHIBIN alpha (INHA) promoter was associated with premature ovarian failure (OMIM: 147380 (Harris *et al.* 2005)). *Inha* deficient mice (*Inha*$^{-/-}$) died 12-17 weeks after birth due to gonadal tumors (Froguel *et al.* 1992). GeneSwitch approach was successfully applied to rescue the lethal phenotype which was evoked by the disruption of *Inha* in mice. Bigenic mice were produced by crossing of transgenic mice with liver-specific mifepristone-induced chimeric nuclear receptor (GLVP) and transgenic target mice containing a GVLP-responsive promoter upstream of polio-virus internal ribosome entry site-linked sequences coding of inhibin A (Pierson *et al.* 2000).

Overcome perinatal lethality by integration of the target gene into the X-chromosome

SPINK1 mutations may lead to various types of chronic pancreatitis (OMIM 167800, 608189) (Witt *et al.* 2000)). *Spink3*, the mouse homologue of human SPINK1 was disrupted by gene targeting, but *Spink3* KO (*Spink3*$^{-/-}$) mice died within two weeks after birth due to the improper inactivation of intrapancreatic trypsinogen and *Spink3*$^{+/-}$ mice did not develop this disorder (Ohmuraya *et al.* 2005). For rescuing the lethal phenotype, human SPINK1 minigene was integrated into the X-chromosome. Mosaic expression of the SPINK1 mRNA was achieved by the random inactivation of the X-chromosome (Sakata *et al.* 2016). *Spink3*$^{+/-}$ and XX^{SPINK1} knock-in mice were mated to generate *Spink3*$^{-/-}$; XX^{SPINK1} mice. *Spink3*$^{-/-}$; XX^{SPINK1} mice also showed the symptoms of chronic pancreatitis such as loss of acinar cells, intralobular fibrosis but reached sexual maturity and therefore proved to be a valuable model for the human disorder (Sakata *et al.* 2016).

Tetraploid complementation assay

Placental defects induced by gene deletions are frequently responsible for embryonic lethality in mice (for more details, see review (Rossant and Cross, 2001)). ETS proto-oncogene 2 (ETS2) repressor factor (ERF) mutations evoke craniosynostosis (Twigg *et al.* 2013) and Chitayat syndrome (OMIM 611888 (Chitayat *et al.* 1993)). *Ets proto-oncogene 2* (*Ets2*) was disrupted by gene targeting, but *Ets2*$^{-/-}$ embryos were arrested at day 8 (Yamamoto *et al.* 1998). Tetraploid complementation assay could be a valuable method where a tetraploid embryo (morula or blastocyst stage) is aggregated with diploid ESCs. The aggregation results a normally developed fetus, which is exclusively derived from the ESCs, while the extra-embryonic tissues are completely derived from the tetraploid cells. *Ets2*-deficient embryos were efficiently rescued by tetraploid complementation (Yamamoto *et al.* 1998).

Lethal phenotype rescuing by transgene-complementation

X-ray cross-complementing 1 (XRCC1) protein is an indispensable part of the DNA single-strand break repair system. (Whitehouse *et al.* 2001). Spinocerebellar ataxia was evoked by mutations in the XRCC1 in human patients (Hoch *et al.* 2017). *Xrcc1*$^{+/-}$ mice were developed by gene targeting earlier to study the functions of that gene, but *Xrcc1*$^{-/-}$ mouse embryos aborted between day 6 and 8, thus, the function of that protein in adult mice was not possible to assess (Tebbs *et al.* 1999).

Xrcc1 minigene was integrated into the mouse genome to rescue the phenotype. Although the *Xrcc1* mRNA level was only 10 % in transgenic mice compared with wt littermates, this reduced *Xrcc1* mRNA level was enough the overcome embryonic lethality (Tebbs *et al.* 2003). Lentiviral gene transfer was also effective in rescuing the lethal phenotype evoked by *Ets2*, *Mitogen-activated protein kinase* (*Mapk*) 14 and *Mapk1* deficiency in mice (Okada *et al.* 2007).

Tissue-specific deletion of the target gene

Mutations in the glucokinase gene (GCK) were associated with maturity-onset diabetes of the young 2 (MODY-2, OMIM 125851, (Froguel *et al.* 1992)). Heterozygous *Glucokinase* (*Gck*$^{+/-}$) mutant mice were generated by gene targeting as a MODY-2 animal model, but their plasma glucose and insulin levels were similar compared to *Gck*$^{+/+}$ mice. Unfortunately, the complete lack of the enzyme resulted in lethality at embryonic day 9.5 (Bali *et al.* 1995). Numerous mouse lines were created with liver or pancreatic β-cell-specific deletion of

the *Gck* gene to prevent embryonic lethality. Pancreatic-specific $Gck^{-/-}$ mice died seven days after birth due to glycosuria and severe dehydration, while $Gck^{+/-}$ mice showed only mild diabetes (Postic *et al.* 1999, Terauchi *et al.* 1995). Liver-specific $Gck^{-/-}$ mice were created using Cre/Lox technology. The lack of hepatic *Gck* rescued the lethal phenotype, which was observed in global and pancreatic $Gck^{-/-}$ mice. Hepatic $Gck^{-/-}$ mice showed mild hyperglycemia and impaired insulin secretion. (Postic *et al.* 1999) These data were confirmed by another research group later (Zhang *et al.* 2004).

Biochemical approaches

Several attempts were made to create adequate KO mouse models for different types of congenital disorder of glycosylation (CDG), e.g. CDG-Ia, OMIM 212065 (Dupre *et al.* 2001); CDG-It, OMIM 614921 (Ondruskova *et al.* 2014), CDG-Ib, OMIM 602579 (Niehues *et al.* 1998), etc. *Phosphomannose isomerase* KO ($Mpi^{-/-}$) mice died during embryonic development due to mannose 6-phosphate accumulation (DeRossi *et al.* 2006). *Phosphomannomutase 2*-deficient ($Pmm2^{-/-}$) mouse line, a promising model for human CDG-Ia could not be established due to embryonic lethality (Thiel *et al.* 2006). *Phosphoglucomutase* 2 KO ($Pgm2^{-/-}$) newborn mice were not detected after ten $Pgm2^{+/-}$x $Pgm2^{+/-}$ crossings, and the $Pgm2^{+/-}$ mice had different glycosylation pattern compared to human patients with CDG-It (Balakrishnan *et al.* 2019). Prenatal mannose supplementation was utilized to overcome embryonic lethality of $Pmm2^{-/-}$ mice (Schneider *et al.* 2011). $Pmm2^{R137H/F118l}$ compound heterozygous mouse line was created as an analog of the human CDG-Ia-associated $Pmm2^{R141H/F122L}$ genotype. 9 mg/ml mannose were added to the drinking water of female $Pmm2^{+/F118L}$ before mating with $Pmm2^{R137H/+}$ males and during pregnancy. This mannose-drinking protocol proved to be an efficient method to rescue lethality of $Pmm2^{R137H/F118L}$ (Schneider *et al.* 2011) and $Pmm2^{F115L/F115L}$ embryos (Chan *et al.* 2016), but not in case of $Pmm2^{R137H/F118L}$ mice, a model of the human CDG-Ia-related $Pmm2^{R141H/F119L}$ genotype (Chan *et al.* 2016). Strikingly, mannose treatment worsened embryonic lethality in the *Phosphomannose isomerase* KO ($Mpi^{-/-}$) (DeRossi *et al.* 2006) and *Mpi* hypomorphic mouse line, models of human CDG-Ib (Sharma *et al.* 2014).

Unfortunately, drinking galactose (9 mg/ml) to pregnant $Pgm2^{+/-}$ mice, model of human CDG-It was not effective to induce survival of $Pgm2^{-/-}$ mice beyond embryonic development (Balakrishnan *et al.* 2019).

Disruption of the target gene in rabbits

If a KO mouse line is not able to develop the symptoms of a human disease, using other laboratory animals could be a practical option.

GCK mutant rabbits were generated using the CRISPR/Cas9 system for modeling MODY-2 (Froguel *et al.* 1992). Both $GCK^{+/-}$ and $GCK^{-/-}$ rabbits with frameshift mutations (GCK-FS) died before sexual maturity. Heterozygous GCK mutant rabbits with non-frameshift mutation (GCK-NFS) were viable, fertile, hence homozygous GCK-NFS rabbit line could be established. Homozygous GCK-NFS rabbits had similar symptoms as human MODY-2 patients (elevated fasting serum glucose, decreased serum insulin, glycosuria), thus may serve as a valuable model for human MODY-2 (Song *et al.* 2020).

Nuclear lamin A gene (LMNA) mutations were related to numerous human diseases, e.g. lipodystrophy (OMIM 151660, (Shackleton *et al.* 2000)), Hutchinson-Gilford progeria syndrome (HGPS, OMIM 176670, (Cao and Hegele, 2003)), Emery-Dreifuss muscular dystrophy (EDMD, OMIM 181350, (Bonne *et al.* 1999)). $Lmna^{-/-}$ KO mice showed similar symptoms as human EDMD and HPGS patients but died at 8 and 4 weeks of age, respectively. $Lmna^{+/-}$ mutant mice developed as normal as wt mice, but human-EDMD or HPGS-like symptoms were not observed (Mounkes *et al.* 2003, Sullivan *et al.* 1999). $LMNA^{-/-}$ KO rabbits created by CRISPR/Cas9 system as a model for human LMNA mutations-related disorders (Sui *et al.* 2019). $LMNA^{-/-}$ KO rabbits had dilated cardiomyopathy, lipodystrophy and premature aging, reflecting human EDMD, lipodystrophy and HPGS. However, $LMNA^{-/-}$ rabbits had shorter life span compared with $Lmna^{-/-}$ mice (Sui *et al.* 2019), indicating a limited application of the $LMNA^{-/-}$ rabbit line for studying human diseases.

Conclusions

Embryonic and postnatal lethality limited the translational value of many KO mouse lines in the past three decades. The most promising method to rescue a lethal phenotype is the creation of mosaic mice by TALEN or CRISPR/Cas9 system. This one-step technique can be performed on either one-cell or two-cell

stage embryos, but two-cell microinjection is more efficient. The disruption of other genes is a convincing method as well but it requires the development or purchasing of further KO mouse lines and also needs several crossings to establish double or triple KO mice. Mannose supplementation was utilized in several KO mouse lines, which were developed for studying various types of human CDG. The success of this approach was restricted to *Pmm2*$^{R137H/F118L}$ and *Pmm2*$^{F115L/F115L}$ genotypes, modeling human CDG-Ia. KO rabbits may provide an alternative of KO mice to overcome embryonic or postnatal lethality in the future, but the establishment and characterization of a KO rabbit line could be expensive and time consuming.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the National Research, Development and Innovation Office (NKFIH) grant no. 124708. The project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

References

BALAKRISHNAN B, VERHEIJEN J, LUPO A, RAYMOND K, TURGEON C, YANG Y, CARTER KL, WHITEHEAD KJ, KOZICZ T, MORAVA E, LAI K: A novel phosphoglucomutase-deficient mouse model reveals aberrant glycosylation and early embryonic lethality. J Inherit Metab Dis 42: 998-1007, 2019. https://doi.org/10.1002/jimd.12110

BALI D, SVETLANOV A, LEE HW, FUSCO-DEMANE D, LEISER M, LI B, BARZILAI N, SURANA M, HOU H, FLEISCHER N, DEPINHO R, ROSSETTI L, EFRAT S: Animal model for maturity-onset diabetes of the young generated by disruption of the mouse glucokinase gene. J Biol Chem 270: 21464-21467, 1995. https://doi.org/10.1074/jbc.270.37.21464

BONNE G, Di BARLETTA MR, VARNOUS S, BECANE HM, HAMMOUDA EH, MERLINI L, MUNTONI F, GREENBERG CR, GARY F, URTIZBEREA JA, DUBOC D, FARDEAU M, TONIOLO D, SCHWARTZ K: Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. Nat Genet 21: 285-288, 1999. https://doi.org/10.1038/6799

BROWN SD, MOORE MW: The International Mouse Phenotyping Consortium: past and future perspectives on mouse phenotyping. Mamm Genome 23: 632-640, 2012. https://doi.org/10.1007/s00335-012-9427-x

CAO H, HEGELE RA: LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). J Hum Genet 48: 271-274, 2003. https://doi.org/10.1007/s10038-003-0025-3

CEASAR SA, RAJAN V, PRYKHOZHIJ SV, BERMAN JN, IGNACIMUTHU S: Insert, remove or replace: A highly advanced genome editing system using CRISPR/Cas9. Biochim Biophys Acta 1863: 2333-2344, 2016. https://doi.org/10.1016/j.bbamcr.2016.06.009

CHAN, B, CLASQUIN, M, SMOLEN, GA, HISTEN, G, POWE, J, CHEN, Y, LIN, Z, LU, C, LIU, Y, CANG, Y, YAN, Z, XIA, Y, THOMPSON, R, SINGLETON, C, DORSCH, M, SILVERMAN, L, SU, SM, FREEZE, HH, JIN S: A mouse model of a human congenital disorder of glycosylation caused by loss of PMM2. Hum Mol Genet 25: 2182-2193, 2016. https://doi.org/10.1093/hmg/ddw085

CHAVANAS S, BODEMER C, ROCHAT A, HAMEL-TEILLAC D, ALI M, IRVINE AD, BONAFE JL, WILKINSON J, TAIEB A, BARRANDON Y, HARPER JI, De PROST Y, HOVNANIAN A: Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet 25: 141-142, 2000. https://doi.org/10.1038/75977

CHITAYAT D, HAJ-CHAHINE S, STALKER HJ, AZOUZ EM, COTE A, HALAL F: Hyperphalangism, facial anomalies, hallux valgus, and bronchomalacia: a new syndrome? Am J Med Genet 45: 1-4, 1993. https://doi.org/10.1002/ajmg.1320450103

CROSBY JR, SEIFERT RA, SORIANO P, BOWEN-POPE DF: Chimaeric analysis reveals role of Pdgf receptors in all muscle lineages. Nat Genet 18: 385-388, 1998. https://doi.org/10.1038/ng0498-385

DEROSSI C, BODE L, EKLUND EA, ZHANG F, DAVIS JA, WESTPHAL V, WANG L, BOROWSKY AD, FREEZE HH: Ablation of mouse phosphomannose isomerase (Mpi) causes mannose 6-phosphate accumulation, toxicity, and embryonic lethality. J Biol Chem 281: 5916-5927, 2006. https://doi.org/10.1074/jbc.M511982200

DUPRE T, CUER M, BARROT S, BARNIER A, CORMIER-DAIRE V, MUNNICH A, DURAND G, SETA N: Congenital disorder of glycosylation Ia with deficient phosphomannomutase activity but normal plasma glycoprotein pattern. Clin Chem 47: 132-134, 2001. https://doi.org/10.1093/clinchem/47.1.132

DUPUY AJ, FRITZ S, LARGAESPADA DA: Transposition and gene disruption in the male germline of the mouse. Genesis 30: 82-88, 2001. https://doi.org/10.1002/gene.1037

FROGUEL P, VAXILLAIRE M, SUN F, VELHO G, ZOUALI H, BUTEL MO, LESAGE S, VIONNET N, CLÉMENT K, FOUGEROUSSE F, TANIZAWA Y, WEISSENBACH J, BECKMANN JS, LATHROP GM, PASSA P, PERMUTT MA, COHEN D: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. Nature 356: 162-164, 1992. https://doi.org/10.1038/356162a0

FURIO L, PAMPALAKIS G, MICHAEL IP, NAGY A, SOTIROPOULOU G, HOVNANIAN A: KLK5 inactivation reverses cutaneous hallmarks of Netherton syndrome. PLoS Genet 11: e1005389, 2015. https://doi.org/10.1371/journal.pgen.1005389

GARRELS W, MATES, L, HOLLER S, DALDA A, TAYLOR U, PETERSEN B, NIEMANN H, IZSVAK Z, IVICS Z, KUES WA: Germline transgenic pigs by Sleeping Beauty transposition in porcine zygotes and targeted integration in the pig genome. PLoS One 6: e23573, 2011. https://doi.org/10.1371/journal.pone.0023573

GOSSLER A, JOYNER AL, ROSSANT J, SKARNES WC: Mouse embryonic stem cells and reporter constructs to detect developmentally regulated genes. Science 244: 463-465, 1989. https://doi.org/10.1126/science.2497519

HARRIS SE, CHAND AL, WINSHIP IM, GERSAK K, NISHI Y, YANASE T, NAWATA H, SHELLING AN: INHA promoter polymorphisms are associated with premature ovarian failure. Mol Hum Reprod 11: 779-784, 2005. https://doi.org/10.1093/molehr/gah219

HOCH NC, HANZLIKOVA H, RULTEN SL, TETREAULT M, KOMULAINEN E, JU LM, HORNYAK P, ZENG ZH, GITTENS W, REY SA, STARAS K, MANCINI GMS, MCKINNON PJ, WANG ZQ, WAGNER JD, YOON G, CALDECOTT KW, CONSORTIUM CRC: XRCC1 mutation is associated with PARP1 hyperactivation and cerebellar ataxia. Nature 541: 87-91, 2017. https://doi.org/10.1038/nature20790

JONES SN, ROE AE, DONEHOWER LA, BRADLEY A: Rescue of embryonic lethality in Mdm2-deficient mice by absence of P53. Nature 378: 206-208, 1995. https://doi.org/10.1038/378206a0

KASPAREK P, ILENINOVA Z, HANECKOVA R, KANCHEV I, JENICKOVA I, SEDLACEK R: A viable mouse model for Netherton syndrome based on mosaic inactivation of the Spink5 gene. Biol Chem 397: 1287-1292, 2016. https://doi.org/10.1515/hsz-2016-0194

KASPAREK P, ILENINOVA Z, ZBODAKOVA O, KANCHEV I, BENADA O, CHALUPSKY K, BRATTSAND M, BECK IM, SEDLACEK R: KLK5 and KLK7 ablation fully rescues lethality of Netherton syndrome-like phenotype. PLoS Genet 13: e1006566, 2017. https://doi.org/10.1371/journal.pgen.1006566

KATTER K, GEURTS AM, HOFFMANN O, MATES L, LANDA V, HIRIPI L, MORENO C, LAZAR J, BASHIR S, ZIDEK V, POPOVA E, JERCHOW B, BECKER K, DEVARAJ A, WALTER I, GRZYBOWKSI M, CORBETT M, FILHO AR, HODGES MR, BADER M, IVICS Z, JACOB HJ, PRAVENEC M, BOSZE Z, RULICKE T, IZSVAK Z: Transposon-mediated transgenesis, transgenic rescue, and tissue-specific gene expression in rodents and rabbits. FASEB J 27: 930-941, 2013. https://doi.org/10.1096/fj.12-205526

LINDAHL P, HELLSTROM M, KALEN M, KARLSSON L, PEKNY M, PEKNA M, SORIANO P, BETSHOLTZ C: Paracrine PDGF-B/PDGF-Rbeta signaling controls mesangial cell development in kidney glomeruli. Development 125: 3313-3322, 1998.

LUNA RMD, WAGNER DS, LOZANO G: Rescue of early embryonic lethality in Mdm2-deficient mice by deletion of P53. Nature 378: 203-206, 1995. https://doi.org/10.1038/378203a0

MANSOUR SL, THOMAS KR, CAPECCHI MR: Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. Nature 336: 348-352, 1988. https://doi.org/10.1038/336348a0

MATZUK MM, FINEGOLD MJ, SU JG, HSUEH AJ, BRADLEY A: Alpha-inhibin is a tumour-suppressor gene with gonadal specificity in mice. Nature 360: 313-319, 1992. https://doi.org/10.1038/360313a0

MOUNKES LC, KOZLOV S, HERNANDEZ L, SULLIVAN T, STEWART CL: A progeroid syndrome in mice is caused by defects in A-type lamins. Nature 423: 298-301, 2003. https://doi.org/10.1038/nature01631

NIEHUES R, HASILIK M, ALTON G, KORNER C, SCHIEBE-SUKUMAR M, KOCH HG, ZIMMER KP, WU RR, HARMS E, REITER K, VON FIGURA K, FREEZE HH, HARMS HK, MARQUARDT T: Carbohydrate-deficient glycoprotein syndrome type Ib - Phosphomannose isomerase deficiency and mannose therapy. J Clin Inv 101: 1414-1420, 1998. https://doi.org/10.1172/JCI2350

OHMURAYA M, HIROTA M, ARAKI, M, MIZUSHIMA N, MATSUI M, MIZUMOTO T, HARUNA K, KUME S, TAKEYA M, OGAWA M, ARAKI K, YAMAMURA K: Autophagic cell death of pancreatic acinar cells in serine protease inhibitor Kazal type 3-deficient mice. Gastroenterology 129: 696-705, 2005. https://doi.org/10.1053/j.gastro.2005.05.057

OKADA Y, UESHIN Y, ISOTANI A, SAITO-FUJITA T, NAKASHIMA H, KIMURA K, MIZOGUCHI A, OH-HORA M, MORI Y, OGATA M, OSHIMA RG, OKABE M, IKAWA M: Complementation of placental defects and embryonic lethality by trophoblast-specific lentiviral gene transfer. Nat Biotechnol 25: 233-237, 2007. https://doi.org/10.1038/nbt1280

ONDRUSKOVA N, HONZIK T, VONDRACKOVA A, TESAROVA M, ZEMAN J, HANSIKOVA H: Glycogen storage disease-like phenotype with central nervous system involvement in a PGM1-CDG patient. Neuro Endocrinol Lett 35: 137-141, 2014.

PIERSON TM, WANG Y, DEMAYO FJ, MATZUK MM, TSAI SY, OMALLEY BW: Regulable expression of inhibin A in wild-type and inhibin alpha null mice. Mol Endocrinol 14: 1075-1085, 2000. https://doi.org/10.1210/me.14.7.1075

POSTIC C, SHIOTA M, NISWENDER KD, JETTON TL, CHEN YJ, MOATES JM, SHELTON KD, LINDNER J, CHERRINGTON AD, MAGNUSON MA: Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. J Biol Chem 274: 305-315, 1999. https://doi.org/10.1074/jbc.274.1.305

ROSSANT J, CROSS JC: Placental development: lessons from mouse mutants. Nat Rev Genet 2: 538-548, 2001. https://doi.org/10.1038/35080570

SAKATA K, ARAKI K, NAKANO H, NISHINA T, KOMAZAWA-SAKON S, MURAI S, LEE GE, HASHIMOTO D, SUZUKI C, UCHIYAMA Y, NOTOHARA K, GUKOVSKAYA AS, GUKOVSKY I, YAMAMURA K, BABA H, OHMURAYA M: Novel method to rescue a lethal phenotype through integration of target gene onto the X-chromosome. Sci Rep 6: 37200, 2016. https://doi.org/10.1038/srep37200

SALES KU, MASEDUNSKAS A, BEY AL, RASMUSSEN AL, WEIGERT R, LIST K, SZABO R, OVERBEEK PA, BUGGE TH: Matriptase initiates activation of epidermal pro-kallikrein and disease onset in a mouse model of Netherton syndrome. Nat Genet 42: 676-683, 2010. https://doi.org/10.1038/ng.629

SCHNEIDER A, THIEL C, RINDERMANN J, DEROSSI C, POPOVICI D, HOFFMANN GF, GRONE HJ, KORNER C: Successful prenatal mannose treatment for congenital disorder of glycosylation-Ia in mice. Nat Med 18: 71-73, 2011. https://doi.org/10.1038/nm.2548

SCHUBERT D, BODE C, KENEFECK R, HOU TZ, WING JB, KENNEDY A, BULASHEVSKA A, PETERSEN BS, SCHAFFER AA, GRUNING BA, UNGER S, FREDE N, BAUMANN U, WITTE, T, SCHMIDT RE, DUECKERS G, NIEHUES T, SENEVIRATNE S, KANARIOU M, SPECKMANN C, ET AL.: Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 20: 1410-1416, 2014. https://doi.org/10.1038/nm.3746

SHACKLETON S, LLOYD DJ, JACKSON SNJ, EVANS R, NIERMEIJER MF, SINGH BM, SCHMIDT H, BRABANT G, KUMAR S, DURRINGTON PN, GREGORY S, O'RAHILLY S, TREMBATH RC: LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. Nat Genet 24: 153-156, 2000. https://doi.org/10.1038/72807

SHARMA V, NAYAK J, DEROSSI C, CHARBONO A, ICHIKAWA M, NG BG, GRAJALES-ESQUIVEL E, SRIVASTAVA A, WANG L, HE P, SCOTT DA, RUSSELL J, CONTRERAS E, GUESS CM, KRAJEWSKI S, DEL RIO-TSONIS K, FREEZE HH: Mannose supplements induce embryonic lethality and blindness in phosphomannose isomerase hypomorphic mice. FASEB J 28: 1854-1869, 2014. https://doi.org/10.1096/fj.13-245514

SONG Y, SUI T, ZHANG Y, WANG Y, CHEN M, DENG J, CHAI Z, LAI L, LI Z: Genetic deletion of a short fragment of glucokinase in rabbit by CRISPR/Cas9 leading to hyperglycemia and other typical features seen in MODY-2. Cell Mol Life Sci 77: 3265-3277, 2020. https://doi.org/10.1007/s00018-019-03354-4

SUI T, LIU D, LIU T, DENG J, CHEN M, XU Y, SONG Y, OUYANG H, LAI L, LI Z: LMNA-mutated rabbits: A model of premature aging syndrome with muscular dystrophy and dilated cardiomyopathy. Aging Dis 10: 102-115, 2019. https://doi.org/10.14336/AD.2018.0209

SULLIVAN T, ESCALANTE-ALCALDE D, BHATT H, ANVER M, BHAT N, NAGASHIMA K, STEWART CL, BURKE B: Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J Cell Biol 147: 913-920, 1999. https://doi.org/10.1083/jcb.147.5.913

TEBBS RS, FLANNERY ML, MENESES JJ, HARTMANN A, TUCKER JD, THOMPSON LH, CLEAVER JE, PEDERSEN RA: Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. Dev Biol 208: 513-529, 1999. https://doi.org/10.1006/dbio.1999.9232

TEBBS RS, THOMPSON LH, CLEAVER JE: Rescue of Xrcc1 knockout mouse embryo lethality by transgene-complementation. DNA Repair 2: 1405-1417, 2003. https://doi.org/10.1016/j.dnarep.2003.08.007

TERAUCHI Y, SAKURA H, YASUDA K, IWAMOTO K, TAKAHASHI N, ITO K, KASAI H, SUZUKI H, UEDA O, KAMADA N, JISHAGE K, KOMEDA K, NODA M, KANAZAWA Y, TANIGUCHI S, MIWA I, AKANUMA Y, KODAMA T, YAZAKI Y, KADOWAKI T: Pancreatic beta-cell-specific targeted disruption of glucokinase gene. Diabetes mellitus due to defective insulin secretion to glucose. J Biol Chem 270: 30253-30256, 1995. https://doi.org/10.1074/jbc.270.51.30253

THIEL C, LUBKE T, MATTHIJS G, VON FIGURA K, KORNER C: Targeted disruption of the mouse phosphomannomutase 2 gene causes early embryonic lethality. Mol Cell Biol 26: 5615-5620, 2006. https://doi.org/10.1128/MCB.02391-05

TWIGG SRF, VORGIA E, MCGOWAN SJ, PERAKI I, FENWICK AL, SHARMA VP, ALLEGRA M, ZARAGKOULIAS A, AKHA ES, KNIGHT SJL, LORD H, LESTER T, IZATT L, LAMPE AK, MOHAMMED SN, STEWART FJ, VERLOES A, WILSON LC, HEALY C, SHARPE PT, HAMMOND P, HUGHES J, TAYLOR S, JOHNSON D, WALL SA, MAVROTHALASSITIS G, WILKIE AOM: Reduced dosage of ERF causes complex craniosynostosis in humans and mice and links ERK1/2 signaling to regulation of osteogenesis. Nat Genet 45: 308-313, 2013. https://doi.org/10.1038/ng.2539

WANG H, YANG H, SHIVALILA CS, DAWLATY MM, CHENG AW, ZHANG F, JAENISCH R: One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153: 910-918, 2013. https://doi.org/10.1016/j.cell.2013.04.025

WANG L, LI MY, QU C, MIAO WY, YIN Q, LIAO J, CAO HT, HUANG M, WANG K, ZUO E, PENG G, ZHANG SX, CHEN G, LI Q, TANG K, YU Q, LI Z, WONG CC, XU G, JING N, YU X, LI J: CRISPR-Cas9-mediated genome editing in one blastomere of two-cell embryos reveals a novel Tet3 function in regulating neocortical development. Cell Res 27: 815-829, 2017. https://doi.org/10.1038/cr.2017.58

WANG YL, O'MALLEY BW JR, TSAI SY, O'MALLEY BW: A regulatory system for use in gene-transfer. Proc Natl Acad Sci U S A 91: 8180-8184, 1994. https://doi.org/10.1073/pnas.91.17.8180

WATERHOUSE P, PENNINGER JM, TIMMS E, WAKEHAM A, SHAHINIAN A, LEE KP, THOMPSON CB, GRIESSER H, MAK TW: Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 270: 985-988, 1995. https://doi.org/10.1126/science.270.5238.985

WHITEHOUSE CJ, TAYLOR RM, THISTLETHWAITE A, ZHANG H, KARIMI-BUSHERI F, LASKO DD, WEINFELD M, CALDECOTT KW: XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. Cell 104: 107-117, 2001. https://doi.org/10.1016/S0092-8674(01)00195-7

WITT H, LUCK W, HENNIES HC, CLASSEN M, KAGE A, LASS U, LANDT O, BECKER M: Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet 25: 213-216, 2000. https://doi.org/10.1038/76088

WU Y, ZHANG J, PENG B, TIAN D, ZHANG D, LI Y, FENG X, LIU J, LI J, ZHANG T, LIU X, LU J, CHEN B, WANG S: Generating viable mice with heritable embryonically lethal mutations using the CRISPR-Cas9 system in two-cell embryos. Nat Commun 10: 2883, 2019. https://doi.org/10.1038/s41467-019-10748-2

XIAO ZX, CHEN JD, LEVINE AJ, MODJTAHEDI N, XING J, SELLERS WR, LIVINGSTON DM: Interaction between the retinoblastoma protein and the oncoprotein Mdm2. Nature 375: 694-698, 1995. https://doi.org/10.1038/375694a0

YAMAMOTO H, FLANNERY ML, KUPRIYANOV S, PEARCE J, McKERCHER SR, HENKEL GW, MAKI RA, WERB Z, OSHIMA RG: Defective trophoblast function in mice with a targeted mutation of Ets2. Genes Dev 12: 1315-1326, 1998. https://doi.org/10.1101/gad.12.9.1315

YANG DS, XU J, ZHU TQ, FAN JL, LAI LX, ZHANG JF, CHEN YE: Effective gene targeting in rabbits using RNA-guided Cas9 nucleases. J Mol Cell Biol 6: 97-99, 2014. https://doi.org/10.1093/jmcb/mjt047

YANG T, LIANG D, KOCH PJ, HOHL D, KHERADMAND F, OVERBEEK PA: Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in Spink5-/- mice. Genes Dev 18: 2354-2358, 2004. https://doi.org/10.1101/gad.1232104

YOSHIMI K, KANEKO T, VOIGT B, MASHIMO T: Allele-specific genome editing and correction of disease-associated phenotypes in rats using the CRISPR-Cas platform. Nat Commun 5: 4240, 2014. https://doi.org/10.1038/ncomms5240

ZABOIKIN M, ZABOIKINA T, FRETER C, SRINIVASAKUMAR N: Non-homologous end joining and homology directed DNA repair frequency of double-stranded breaks introduced by genome editing reagents. PLoS One 12: e0169931, 2017. https://doi.org/10.1371/journal.pone.0169931

ZENG H, HORIE K, MADISEN L, PAVLOVA MN, GRAGEROVA G, ROHDE AD, SCHIMPF BA, LIANG Y, OJALA E, KRAMER F, ROTH P, SLOBODSKAYA O, DOLKA I, SOUTHON EA, TESSAROLLO L, BORNFELDT KE, GRAGEROV A, PAVLAKIS GN, GAITANARIS GA: An inducible and reversible mouse genetic rescue system. PLoS Genet 4: e1000069, 2008. https://doi.org/10.1371/journal.pgen.1000069

ZHANG YL, TAN XH, XIAO MF, LI H, MAO YQ, YANG X, TAN HR: Establishment of liver specific glucokinase gene knockout mice: a new animal model for screening anti-diabetic drugs. Acta Pharmacol Sin 25: 1659-1665, 2004.

ZINGKOU E, PAMPALAKIS G, SOTIROPOULOU G: Cathelicidin represents a new target for manipulation of skin inflammation in Netherton syndrome. Biochim Biophys Acta Mol Basis Dis 1866: 165831, 2020. https://doi.org/10.1016/j.bbadis.2020.165831