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Generation of *LEPR* Knockout Rabbits with CRISPR/CAS9 System

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Abstract—The *LEPR* gene encodes a leptin hormone receptor, and its mutations are associated with morbid obesity, dysregulation of lipid metabolism, and fertility defects in humans. Spontaneous *Lepr* mutations have been described in rodents, and *Lepr* knockout animals have been generated, in particular, using the CRISPR/Cas9 system. Lipid metabolism in rodents significantly differs from that in humans or rabbits, and rabbits are therefore considered as the most relevant model of morbid obesity and lipid metabolism dysregulation in humans. *LEPR* knockout rabbits have not been reported so far. In this work a *LEPR* knockout rabbit was generated by introducing a deletion of the region around *LEPR* exon 10 using the CRISPR/Cas9 system. The body weight of the knockout rabbit was significantly higher than the average body weight of the wild type rabbits. CRISPR/Cas9-mediated generation of *LEPR* knockout rabbits will allow the development of a model of morbid obesity and endocrine defects due to leptin receptor mutations in humans.

Keywords: CRISPR/Cas9, *LEPR*, leptin, genetically modified rabbit

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The peptide hormone leptin (16 kDa) is produced in white adipose tissue; is classed as adipokine; and regulates eating behavior, energy metabolism, and many other neuroendocrine functions [1]. Leptin exerts its effects by binding to a transmembrane receptor, which is encoded by the *LEPR* gene and belongs to the gp130 cytokine receptor family [2]. Main leptin effects are mediated by activation of the JAK-STAT signaling pathway and signaling proteins, such as SOCS3, PTP1b, MAP kinases, and the insulin receptor substrate (IRS) [2]. A long isoform of the leptin receptor consists of three domains: extracellular, transmembrane, and intracellular. A soluble leptin receptor isoform has been described as cleaved extracellular domain, which circulates in the blood and acts as a leptin-binding protein [3]. The interaction of leptin with its receptor is necessary for the regulation of appetite, energy expenditure, maintenance of glucose homeostasis, and fertility. More than 50 clinical cases of obesity due to *LEPR* mutations have been described in humans [4]. Genetically modified rodents with a *Lepr* knockout have been constructed, but their phenotype fails to exactly reproduce the symptoms observed in humans [5]. Leptin is known to play a role in the induction and regulation of chronic inflammatory processes, degenerative diseases, autoimmune disorders, type 2 diabetes mellitus, and cancer [6]. The reproductive function is also regulated by leptin, and fertility is compromised in female mice with a tissue-specific *Lepr* knockout in the reproductive system [7]. However, the full spectrum of effects associated with activation or inhibition of leptin receptors is still incompletely understood.

Mice of diabetes (db) and obese (ob) strains with a phenotype characterized by metabolic defects, hyperglycemia, obesity, and signs of diabetes were described in the 1960s–1970s [8]. More recent studies have shown that leptin gene mutations are responsible for the ob/ob phenotype [9] and *Lepr* mutations, for the db/db phenotype [10]. A 106-bp insertion between exons 18 and 19 has been found to result in production of a nonfunctional truncated protein in the mice [10]. Mice with a Cre-inducible *Lepr* knockout due to a deletion of exon 1 or exon 17 have been obtained using the classic gene knockout technology [11, 12]. Mice

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with *Lepr* knock-out in various tissues (neurons, liver, pancreas, adipose tissue, endothelial cells, etc.) have been constructed on the basis of these strains [12–16]. The leptin effect on *Lepr* in the hypothalamus, but not in other tissues, has been shown to regulate appetite in experimental animals [12]. A spontaneous *Lepr* mutation has been found to cause obesity in rats (the Zucker, or *fatty* (fa/fa), strain) [17]. With the development of genome editing techniques, *Lepr* knockout rats have been generated by introducing indels in exon 4 of the gene with the use of the CRISPR/Cas9 system [18]. However, the phenotype of rodents carrying *Lepr* mutations fails to exactly reproduce the signs and symptoms observed in humans [5]. This could be due to the fact that, in mice, besides several other features, unlike humans or rabbits, plasma cholesterol is bound to a high-density lipoproteins, thus making mice resistant to the atherosclerosis. Thus, genetically modified rabbits, rather than mice, are promising as models of lipid metabolism disorders [19]. Rabbits with *LEPR* knock-out have not been described so far. The objective of this work was therefore to generate *LEPR* knockout rabbits with the use of the CRISPR/Cas9 technology.

The rabbit *LEPR* gene is located in chromosome 13 and contains 28 exons, including alternative ones. In total, 14 isoforms of *LEPR* transcripts are known to code for five protein isoforms. A guide RNA for nuclease SpCas9 was designed using the ChopChop tool (https://chopchop.cbu.uib.no/). The nucleotide sequence of one of the longest *LEPR* transcripts (XM_051856399, transcript variant 8, which codes for protein isoform X1 and consists of 21 exons) was used as a query. Two guide RNAs with the highest ranking scores were thus selected: sgLepr-1 (GCTGACACGATCATTCACAACGG) and sgLepr-2 (ATAATTCCGCATAGCGATGATGG). Regions targeted by the guide RNAs occur in *LEPR* exon 10 and are found in all transcript isoforms.

Next, a rabbit genomic region including *LEPR* exon 10 with guide RNA targets was amplified to detect possible single nucleotide polymorphisms (SNPs). Genomic DNA was isolated from tissues of a Soviet Chinchilla rabbit with a GeneElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, USA, G1N350). A 642-bp fragment was amplified in a reaction mixture (25 μL), which contained a 1× PrimeSTAR® HS premix (Takara Bio, Japan, R040), 160 nM forward (F, 5'-gtctttatctgatatcctgcttt-3') and reverse (R, 5'-gttagctcactatacccacaga-3') primers, and 20 ng of genomic DNA. PCR conditions were as follows: 94°C for 3 min; 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 45 s; 72°C for 3 min; and 4°C permanently. The PCR product was purified from the reaction mixture with a DNA Clean & Concentrator-25 kit (Zymo Research, United States, D4034) and sequenced using the F primer by the Sanger method. Two SNPs were detected in the target of the sgLepr-1 guide RNA: C/G in a protospacer position 2 and G/A in the protospacer adjacent motif (PAM) (data not shown). A single SNP (A/T in a protospacer position 20) was detected in the sgLepr-2 guide RNA (Fig. 1a). Based on the findings, the sgLepr-2 guide RNA was chosen for generating *LEPR* knockout rabbits. We assumed that the presence of a single SNP in the genome implies that at least one allele would be cleaved efficiently by nuclease SpCas9.

The sgLepr-2 guide RNA was then produced for zygote microinjection. First, on the backbone of the pX330-U6-Chimeric_BB-CBh-hSpCas9 (hereafter referred to as pX300; Addgene #42230) pX330-sgLepr-2 plasmid was constructed by annealing the single stranded synthetic oligonucleotides 5'-caccgataattccgcatagcgatga-3' and 5'-aaactcatcgctatgcggaattatc-3' (Evrogen, Russia) and cloning the resulting double-stranded product into the BbsI site of pX330. To obtain the DNA template for in vitro transcription (IVT), a 122-bp fragment was amplified on the template of pX330-sgLepr-2 with primers 5'-tgtaatacgactcactatagggataattccgcatagcgatga-3' and 5'-aaaagcaccgactcggtgcc-3'. The reaction mixture (50 μL) contained a 1× PrimeSTAR® HS premix, 200 nM primers, and 1 ng of pX330-sgLepr-2. PCR conditions were as follows: 95°C for 2 min; five cycles of 95°C for 15 s and 72°C for 20 s; five cycles of 95°C for 15 s, 66°C for 10 s, and 72°C for 20 s; 25 cycles of 95°C for 15 s, 63°C for 10 s, and 72°C for 20 s; 72°C for 10 min; and 4°C permanently. The PCR product was purified using a MinElute® Reaction Cleanup kit (Qiagen, Germany, 28204). Its concentration was measured with a Nanophometer ND-120 spectrophotometer (Implen, Germany). The PCR-amplified IVT template concentration was 29 ng/μL.

Next, IVT was carried out using a HiScribe® T7 High Yield RNA Synthesis kit (New England Biolabs, United States, 02040S) according to a protocol for short RNA transcripts of <300 nt. The reaction mixture (20 μL) contained 15.2 ng/μL PCR-amplified template. The reaction was incubated at 37°C for 5 h, then reaction was supplemented with 1 μL of 2 units/μL TURBO DNase (Ambion, Lithuania, AM2238) to degrade the template DNA, and further incubated at 37°C for 20 min. The guide RNA synthesized in the IVT reaction was purified from the reaction mixture with a miRNeasy Mini kit (Qiagen, 217004) and stored in aliquots at –80°C.

Activity of the sgLepr-2 guide RNA was assessed via an in vitro test for substrate cleavage by a ribonucleoprotein (RNP) complex. Recombinant SpCas9 was obtained as in [20]. SpCas9 and the sgLepr-2 guide RNA were diluted to 3 μM with a 1× Orange buffer (Thermo Scientific, BO5) and nuclease-free water (Ambion, AM9937), respectively. The in vitro cleavage reaction mixture (30 μL) contained 150 nM SpCas9, 150 nM sgLepr-2 or a control guide RNA that lacks targets in the rabbit genome (sgScr, protospacer 5'-gcactaccagagctaactca-3'), and 3.5 ng/μL DNA sub-

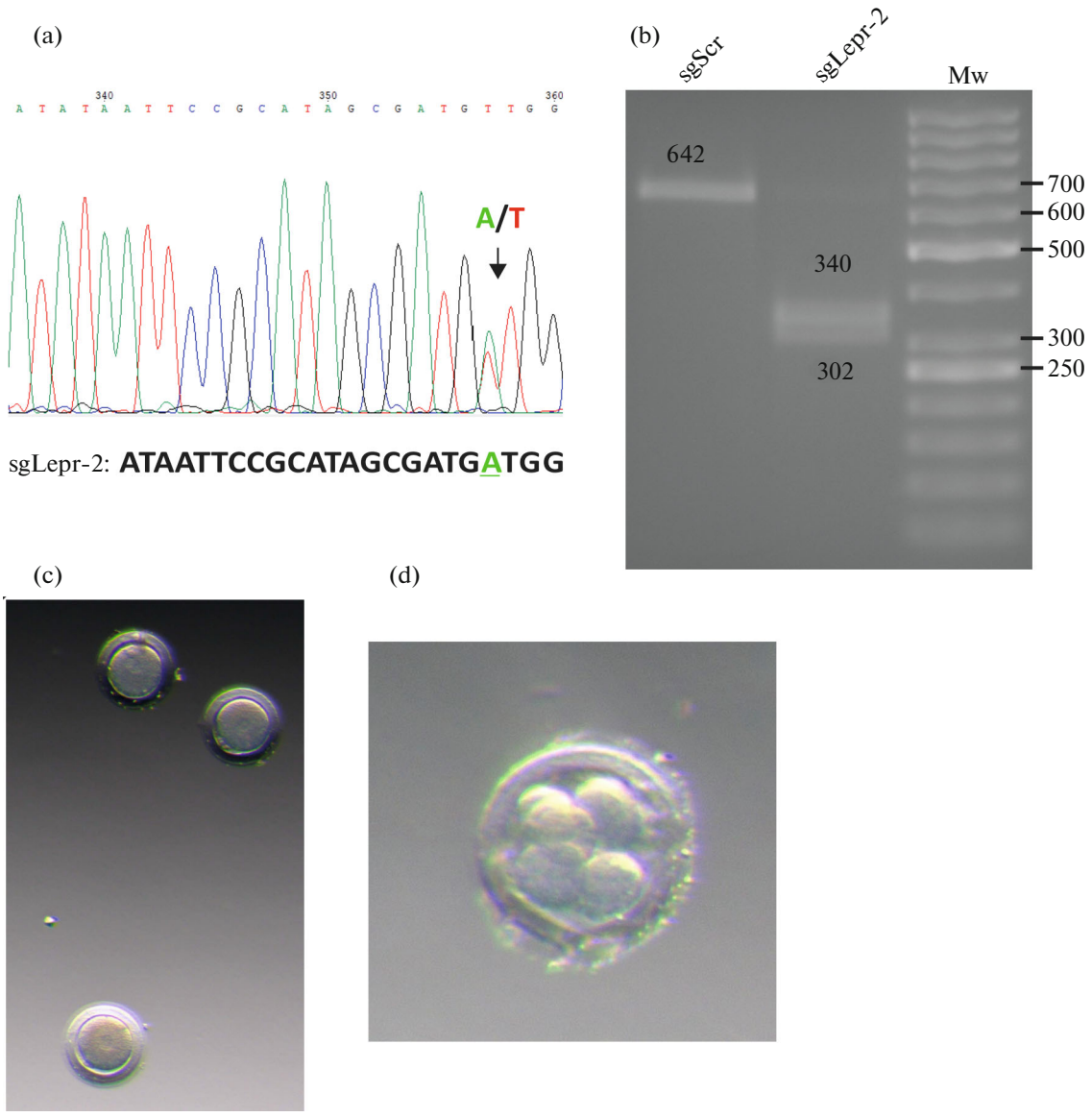

Fig. 1. Development of a technique to knockout the *LEPR* gene in rabbits with the CRISPR/Cas9 system. (a) Sanger sequencing of the sgLepr-2 target in the rabbit genome. SNP A/T in a position 20 of the protospacer is shown with an arrow. The nucleotide sequence of the sgLepr-2 target, including the protospacer and PAM, is shown below the chromatogram. (b) Test for in vitro cleavage of a DNA substrate with SpCas9 RNP. The 642-bp PCR product was incubated with SpCas9 RNP, which was produced using sgLepr-2 or control sgRNA having no target in the rabbit genome (sgScr). The DNA substrate was cleaved to yield products of the expected sizes (340 and 302 bp) only when sgLepr-2 was used. Mw, O'GeneRuler 50bp DNA Ladder (Thermo Scientific, USA, SM1133). The sizes of several DNA marker bands are shown on the right. (c, d) Rabbit embryos imaged (c) before microinjection and (d) 120 h after microinjection of the genome editing reagents. The embryos cultured in a growth medium in Petri dishes were photographed using a Nikon SMZ-800 microscope (Japan).

strate (the 642-bp product amplified from genomic DNA of a Soviet Chinchilla rabbit as described above) in the 1× Orange buffer. First, SpCas9 and guide RNA were combined and incubated at room temperature for 10 min to assemble RNP. Then the DNA substrate (3 μL) was added, and the reaction mixture was incubated at 37°C for 30 min. To degrade guide RNA, the mixture was supplemented with 1 μL of 10 μg/μL RNAse and incubated at 37°C for 10 min. To degrade SpCas9 and release the DNA substrate from its complex with RNP, the mixture was supplemented with 1 μL of 20 μg/μL proteinase K and incubated at 56°C for 10 min. The mixture was then analysed by electrophoresis in 2% TAE-agarose gel. The results of the in vitro test for substrate cleavage by RNP are shown in Fig. 1b. The sgLepr-2 guide RNA, but not the control scrambled guide RNA (sgScr), ensured the cleavage of the 642-bp PCR product into two fragments of expected sizes, 302 and 340 bp (Fig. 1b). The result showed that the sgLepr-2 guide RNA is suitable for

introducing double-strand breaks into the rabbit *LEPR* gene as determined by the guide RNA sequence.

To obtain rabbit zygotes, Soviet Chinchilla breed donor females were mated naturally and sacrificed 16–18 h after mating. The uterus, oviducts, and ovaries were collected. Zygotes were isolated by flushing the oviducts with a washing solution (Hanks' solution (PanEko, Russia, R020p) supplemented with gentamycin (Dal'khimfarm, Russia, an 800× solution) and 2% fetal bovine serum (FBS) (Cytiva, United States)); the solution temperature was 25–29°C. The washing solution was injected into a dissected oviduct from the uterine horn side with a syringe, collected in a Petri dish, and filtered through a Miniflush® embryo collection filter (Minitube, Germany). Zygotes were sought under a microscope (magnification 150–200×) and transferred with a plastic capillary into DMEM/F12 (PanEko, Russia) supplemented with 10% FBS and 100 units/mL penicillin and 100 μg/mL streptomycin under a layer of mineral oil (Origio, Denmark). Components of the CRISPR/Cas9 system were injected into the zygotes (as described below) to estimate the efficiency of *LEPR* gene editing with the sgLepr-2 guide RNA, and the zygotes were cultured for 120 h. Rabbit zygotes before microinjection and 120 h after microinjection are shown in Figs. 1c and 1d, respectively. After microinjection, the zygotes were cultured for 120 h, and the embryos were then tested for *LEPR* gene editing. The embryos were lysed as in [21], and the 642-bp genomic fragment was amplified with the F and R primers using a GenPak PCR Core kit (Laboratoriya Izogen, Russia, U 1010-08). 20 ul amplification reactions were carried out as follows: 94°C for 2 min; 40 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 45 s; and 72°C for 2 min. A heteroduplex analysis was then performed to identify the edited embryos. The PCR products were incubated at 95°C for 5 min and chilled to room temperature at a rate of 2°C/s from 95 to 85°C and 0.1°C/s from 85 to 25°C. The reaction mixture (5 μL) was resolved in 8% TBE-polyacrylamide gel. Editing was inferred from the presence of additional bands as compared with samples amplified from genomic DNA of noninjected zygotes (wt). The results of a heteroduplex analysis of six injected embryos (1–6) are shown in Fig. 2a. The DNA fragment was not amplified to a sufficient amount in the case of sample 5. All other samples showed additional bands, which were absent in wild-type samples and suggested the formation of heteroduplexes. To verify the editing of the *LEPR* locus, Sanger sequencing of the PCR products was carried out with the F primer and the sequencing results were analyzed using the ICE Analysis online tool (Synthego, United States, https://ice.synthego.com/). CRISPR/Cas9-mediated editing was confirmed for embryos 2 and 3 (Figs. 2b, 2c). According to the ICE analysis, the indel rates were 89 and 92%, respectively, meaning that the editing of both alleles of the target locus was efficient as early as at the single- or two-cell embryo stage. Sample 1 proved to be wild type. Samples 4 and 6 showed A/T polymorphism (data not shown) as characteristic of the genomic target of sgLepr-2 (Fig. 1a), and the polymorphism was most likely responsible for the formation of additional bands in gel in the heteroduplex analysis (Fig. 2a). It is of interest to note that the A/A genotype was observed in both of the edited samples (Fig. 2b), while the genotypes A/A, A/T, and T/T were detected in the three unedited embryos (data not shown). Although the results of analysis are not statistically significant, nevertheless they indicate that, as expected, only the *LEPR* allele A can be edited. The result indicates additionally that the genomic targets of guide RNAs are important to sequence in experimental animals. To summarize, reagents for efficient CRISPR/Cas9-mediated editing of the rabbit *LEPR* gene were obtained in our work.

Next, to produce *LEPR* knockout rabbits, the components of the genome editing system were prepared as a mixture of the SpCas9 mRNA (25 ng/μL, TriLink Biotechnologies, United States, L-7206) and the sgLepr-2 guide RNA (7.5 ng/μL) in a TE buffer (10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA) and microinjected into the cytoplasm of isolated zygotes in the Ooklin medium (PanEko) under a Zeiss Axiovert 200M microscope (magnification 400×), using a Narishige micromanipulator (Japan) and an Eppendorf FemtoJet injector (Eppendorf, France). Microinjection needles were manufactured using a Sutter Co P-97 puller (United States); a holding pipette was made using a Narishige PC-10 puller (Japan) and a Narishige MF-900 microforge (Japan). Zygotes that survived microinjection were transferred into DMEM/F12 supplemented with 10% FBS (see above) and cultured at 38.5°C, 100% humidity, and 7% CO_2 for 24 h prior to transplantation into recipients.

Healthy adult White Giant breed females were used as recipients in embryo transplantation. Ovulation was induced with the gonadotropin-releasing hormone analog Surfagon (Mosagrogen, Russia) at 1 μg per rabbit 15–24 h before transplantation. Laparotomy was performed to access the oviducts, and injected embryos were transferred into the ampullar region of the oviduct. Anesthesia was achieved using 0.5% Novocain (Solopharm, Russia) and Propofol Kabi (Fresenius Kabi, Germany) as recommended by the manufacturer. Spermwash (Gynotec, Canada) or Ooklin (PanEko) was used as a transplantation medium.

Newborn rabbits were recovered via cesarean section 30 days after embryo transplantation. Rabbits were anesthetized as above. Laparotomy was carried out to access the uterus, the embryo-containing horn was dissected, and fetuses were extracted. The uterus was then extirpated, bleeding controlled, and the wound sutured in layers. The newborn rabbits were transferred to a foster mother prepared beforehand.

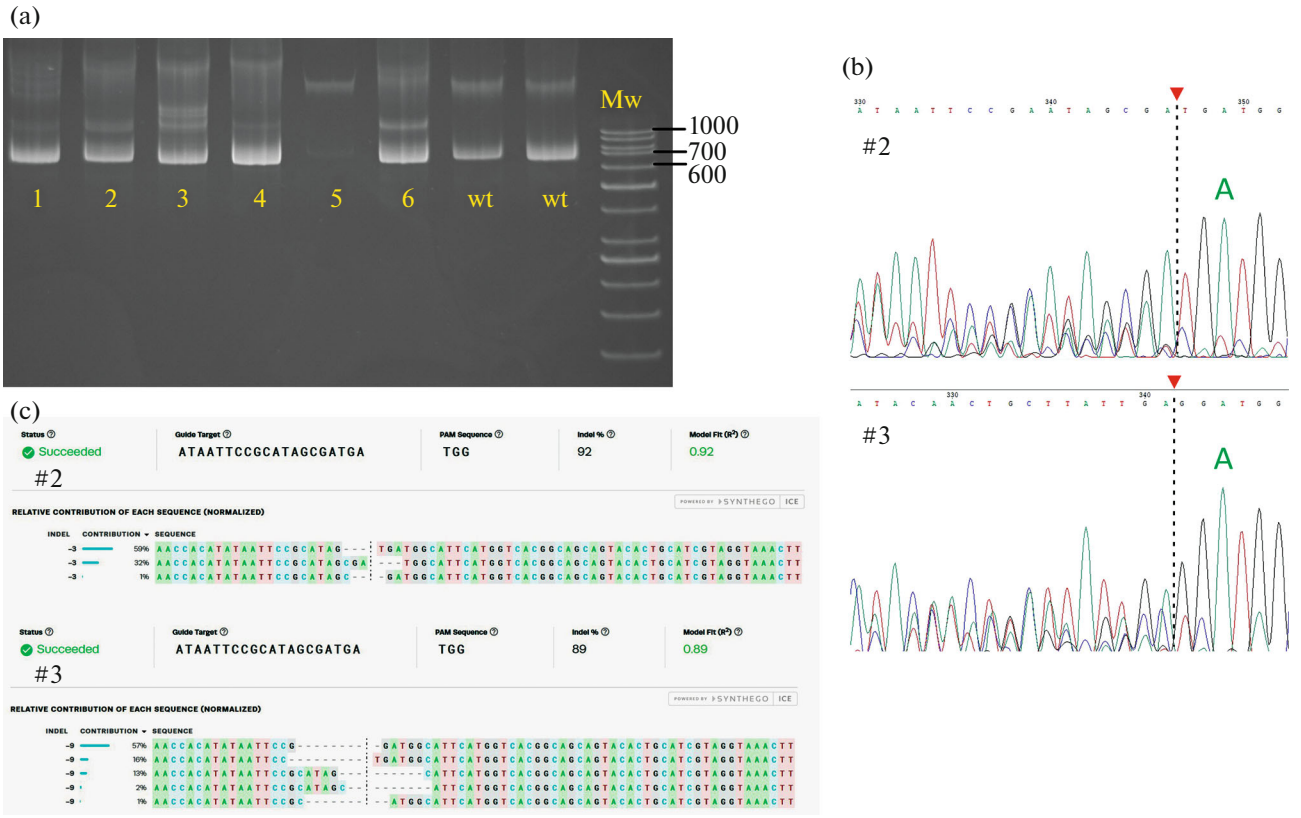

Fig. 2. Efficient editing of the *LEPR* gene in rabbit embryos. (a) Heteroduplex analysis of the PCR products amplified on the template of genomic DNA of six rabbit embryos (1–6). The embryos were microinjected with a mixture of the SpCas9 mRNA and sgLepr-2. The PCR mixtures (5 μL) were resolved by PAGE in 8% gel. Mw, O'GeneRuler 50bp DNA Ladder. The sizes of several DNA marker bands are shown on the right. (b) Sanger sequencing of samples 2 and 3. A vertical dotted line and a red triangle show the SpCas9 cleavage site. The SNP genotype (A) is indicated at the top of the chromatogram. (c) ICE analysis of the Sanger chromatograms of samples 2 and 3. The nucleotide sequences show the identified indels. The indel rates in samples 2 and 3 were 92 and 89%, respectively.

In total, 36 embryos were obtained and microinjected at the stage of two pronuclei in two experiments. Cleavage was observed in 32 embryos 24 h after microinjection. Of these, 22 embryos were transplanted into recipient 1 (11 embryos per oviduct) and 10 embryos (5 embryos per oviduct), into recipient 2. Recipient 1 died from infectious complications after transplantation. Recipient 2 developed pregnancy, and a single live newborn rabbit (a female) was recovered via cesarean section.

To genotype the newborn rabbit, genomic DNA was isolated from ear tissue as in [22] and the 642-bp fragment containing the target for sgLepr-2 guide RNA was amplified as described above. Genomic DNA of a wild-type rabbit was used as a negative control. PCR products were resolved by PAGE in 8% gel. In addition to the 642-bp fragment, which corresponded to the wild-type genome (Fig. 3, lane KO), a fragment of approximately 350 bp was amplified and was detected only in the knockout sample (KO). The PCR products were purified from agarose gel with a QIAEX II kit (Qiagen, #20021) and sequenced by Sanger sequencing with the F primer. The nucleotide sequence of the 642-bp DNA fragment was found to correspond in fact to the wild-type genome (data not shown). The short 356-bp PCR fragment amplified in the KO sample was found to result from a 295-bp deletion and a 9-bp insertion of the random sequence TTTAAAGTA (Fig. 3c). It is of interest to note that the allele T of the SNP occurring in the sgLepr-2 target was detected in the wild-type PCR product, thus explaining the lack of editing in this allele.

Thus, a genetically modified rabbit that carried a deletion of the region of *LEPR* exon 10 was obtained with the use of the CRISPR/Cas9 system. Schemes of the wild-type *LEPR* locus (wt) and the locus with the deletion of the region of exons 10 and 11 (KO) are shown in Fig. 3b. A nucleotide sequence analysis of the short PCR product showed that the deletion included 58 bp of exon 10, full-length 144-bp intron 10, and a 93-bp part of exon 11. A hybrid exon consisting of the 5' region of exon 10 and the 3' region of exon 11 formed in the genome as a result of the deletion (Fig. 3b). A splicing of the hybrid exon most probably will ultilize the acceptor site of exon 10 and the donor site of exon 11 of the *LEPR* gene. A nucleotide

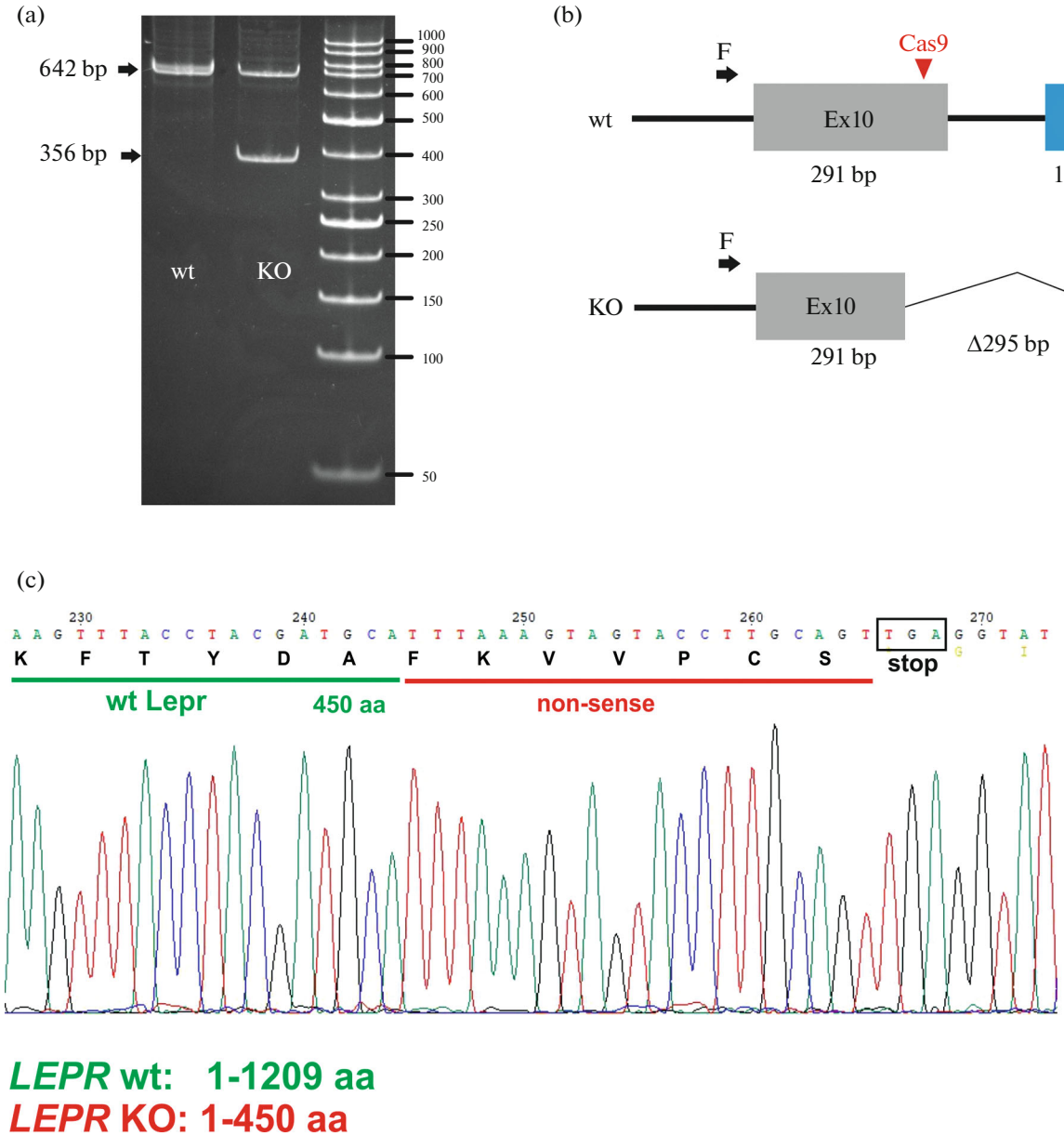

Fig. 3. Molecular genetic analysis of the *LEPR* knockout rabbit. (a) PCR products amplified on the template of wild-type (wt) or *LEPR* knockout (KO) rabbit genomic DNA were resolved by PAGE in 8% gel. The 642-bp fragment was amplified from wild-type genomic DNA, while the 356-bp fragment was amplified only in the KO sample. Sizes of the DNA molecular weight marker O'GeneRuler 50 bp DNA Ladder (ThermoFisher Scientific, United States) are shown on the right. The respective PCR products are indicated with arrows. (b) A scheme of the wild-type (wt) *LEPR* genomic locus shows the region of exons 10 and 11, which are 291 and 118 bp, respectively. Annealing sites for the F and R oligonucleotide primers are shown as black arrows. The KO panel shows the scheme of the edited *LEPR* locus. The cleavage site of SpCas9 nuclease guided by the sgLepr-2 guide RNA is shown with a red triangle. (c) Sanger sequencing of the 356-bp PCR product. Translation of the open reading frame of the knockout allele of the XM_051856399 transcript is shown under the nucleotide sequence. The wild-type Lepr protein (underlined green) is synthesized from the 1st to the 450th amino acid residue, which is followed by the nonsense peptide FKVVPCS (underlined red) resulting from the insertion of random 9 bp (TTTAAAGTA). Translation stops at the premature translation termination codon TGA (framed).

sequence analysis of the *LEPR* transcript (XM_051856399) showed that a premature stop codon was generated by the deletion (Fig. 3c). Translation of the resulting transcript is expected to start from the ATG translation initiation codon in *LEPR* exon 2 and to yield a product consisting of the 450 N-terminal amino acid residues of the Lepr protein that are followed by the senseless peptide FKVVPCS encoded in frame with the Lepr part. Then translation would terminate at the TGA translation termination codon

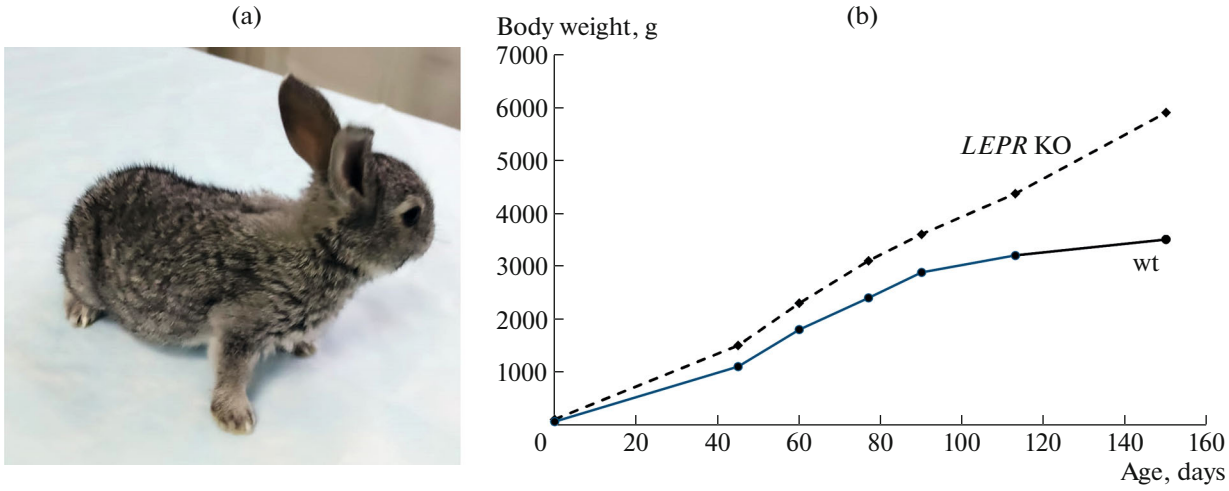

Fig. 4. Body weight gain of the *LEPR* knockout rabbit. (a) The genome-edited rabbit at two weeks of age. (b) A body weight gain curve of the *LEPR* knockout rabbit (*LEPR* KO) in comparison with an average body weight gain curve of wild-type Soviet Chinchilla rabbits (wt).

(Fig. 3c). Thus, our molecular genetic analysis indicates that the deletion introduced using the CRISPR/Cas9 system leads to synthesis of a truncated nonfunctional protein, thereby causing a *LEPR* knockout.

Usually, F0 animals are mosaic and carry several modified alleles in the genome. Only two PCR products were amplified on the template of the genomic DNA from knockout rabbit (Fig. 3a), and no additional modified allele was detected in the PCR products by Sanger sequencing. The findings indicate that the rabbit obtained via genome editing was most likely heterozygous for the knockout allele.

Morbid obesity is a main phenotypic feature of a *LEPR* knockout. It is of interest that the birth weight of the knockout rabbit (104 g) was almost twice as higher as the normal birth weight of Soviet Chinchilla rabbits (approximately 60 g). The body weight of the *LEPR* knockout rabbit was further monitored and compared with the mean weight known for wild-type rabbits of the same breed from the literature [23] because comparisons with wild-type rabbits of the same litter were impossible. As is seen from Fig. 4b, the body weight of the *LEPR* knockout rabbit was substantially higher than the weight of wild-type rabbits of the same breed and age, suggesting morbid obesity.

Once sexual maturity was reached at 6 months of age, the *LEPR* knockout female was mated with a male of the same breed to obtain F1 rabbits. However, the female died from pneumonia during pregnancy. Nine implanted fetuses were found in the uterus upon its postmortem examination. Genotyping detected the knockout allele in five out of the nine fetuses (data not shown). Vertical transmission of the introduced genetic modification from a mother to offspring was thus demonstrated. It is known that a *LEPR* knockout can affect parturition activity and leads to stillbirths in animals [7]. We were unable to verify this experimentally because the female died. Still we can conclude that implantation of embryos is not affected by inactivation of one *LEPR* allele.

To summarize, a *LEPR* knockout rabbit was generated for the first time by introducing a deletion into the region of exon 10 with the use of the CRISPR/Cas9 system. It should be noted that a stop codon located closer to the 5' end of the transcript will be more likely to cause transcript degradation via the nonsense-mediated mRNA decay mechanism. Even if the aberrant transcript is expressed to a sufficiently high level, the protein product truncated to 450 amino acid residues will lack the transmembrane domain and the functional leptin-binding CRH2 domains (residues 428–641) [3], thus being incapable of anchoraging in the plasma membrane or acting as a soluble Lepr isoform. Thus, our model substantially differs from all earlier knockout models described in mammals in terms of the localization of the genetic defect introduced. The body weight of the *LEPR* knockout rabbit was found to be far higher than the average weight of wild-type rabbits of the same breed, suggesting morbid obesity. Our technique to generate genetically modified rabbits with a CRISPR/Cas9-mediated *LEPR* knockout will make it possible to produce a model of morbid obesity and metabolic alterations due to mutations of the leptin receptor gene.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All manipulations with animals were carried out in accordance with guidelines of the Ethics Committee of the Institute of Gene Biology. Experimental protocols were approved by the Ethics Committee of the Institute of Gene Biology (Protocol no. 10 dated February 15, 2023).

CONFLICT OF INTEREST

The authors have submitted a patent application (no. 2023134478 dated December 21, 2023, "Method to Generate Genetically Modified Rabbits with a *LEPR* Knockout with the Use of the CRISPR/Cas9 System").

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