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Scriptwriter Name: Sulakshana Karkala

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Title: Mouse Model of Acute to Chronic Kidney Disease Transition Induced by Renal Ischemia/Reperfusion Injury

Authors and Affiliations:

Akira Oda, Débora Malta Cerqueira, Ayako Kinoshita, Jacqueline Ho, Sunder Sims-Lucas, Takuto Chiba

Department of Pediatrics, University of Pittsburgh Medical Center Children's Hospital of Pittsburgh (UPMC CHP), University of Pittsburgh

Corresponding Authors:

Takuto Chiba (chibat@pitt.edu)

Email Addresses for All Authors:

Akira Oda (ODAA@pitt.edu)

Débora Malta Cerqueira (debora.maltacssantos@chp.edu)

Ayako Kinoshita (AYS114@pitt.edu)

Jacqueline Ho (jacqueline.ho2@chp.edu)

Sunder Sims-Lucas (sunder.sims-lucas@chp.edu)

Takuto Chiba (chibat@pitt.edu)

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

The interview section will be held in a room on the 6th floor of our research building, and the protocol section will take place in a procedure room on the 4th floor of the same building. The two locations are approximately a 5-minute walk apart. When entering the procedure room in the animal facility, all participants are required to wear a disposable gown.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 20

Number of Shots: 41

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Takuto Chiba:** Our research focuses on renal ischemia–reperfusion injury, investigating how it drives chronic kidney disease development and progression.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What technologies are currently used to advance research in your field?~~

- 1.2. **Takuto Chiba:** We use a mouse ischemic CKD model, inducing unilateral renal IRI followed by delayed contralateral nephrectomy to study disease progression.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer's Note: 1.1-1.2 combined together

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Takuto Chiba:** This protocol enables researchers to assess kidney surgery outcomes, demonstrating high survival rates and improved reproducibility through a dorsal surgical approach.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer's Note: 1.3-1.5 combined together

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Takuto Chiba:** A major challenge is the lack of established therapies capable of preventing or halting progression from acute kidney injury to chronic kidney disease.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Takuto Chiba:** Future studies can use this ischemic CKD model to evaluate novel therapeutics and investigate gene function using targeted knockout mice.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.6. **Takuto Chiba, Research Assistant Professor ,University of Pittsburgh**: (authors will present their testimonial statements live)

- 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~The key takeaway of my video is the high survival rate that our protocol provides. Chronic disease models often have high mortality rates, which significantly reduce the number of samples available at the end of the study. Our protocol can bypass this problem by using delayed contralateral nephrectomy~~

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Takuto Chiba, Research Assistant Professor ,University of Pittsburgh**: (authors will present their testimonial statements live)

- 1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~I am a huge fan of JOVE. Visual demonstrations can really make complicated experimental procedures much easier to understand. I hope our video article will help new researchers in nephrology explore and test their interest using our CKD model.~~

Videographer's Note: 1.6-1.7 combined together

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh

Protocol

2. Mouse Model Preparation for Ischemia–Reperfusion Injury Surgery

Demonstrator: Akira Oda

NOTE: Note: The section headings were added during the post-shoot stage. The shot numbers (2.1–2.20) have been kept continuous to reflect the original sequence captured by the videographer and to avoid any confusion.

- 2.1. To begin, autoclave all surgical instruments prior to use [1]. When performing surgeries on multiple animals, remove blood and debris from the instruments between procedures [2], then sterilize them for 10 to 15 seconds using a hot bead sterilizer [3-TXT].
 - 2.1.1. WIDE: Talent placing surgical instruments into an autoclave.
 - 2.1.2. Talent wiping blood and debris from used instruments.
 - 2.1.3. Talent placing instruments into a hot bead sterilizer. **TXT: Allow the instruments to cool before reuse**
- 2.2. Set up a water-circulating heating pad on the surgical table [1] and position an infrared lamp above the surgical field [2].
 - 2.2.1. Talent placing and adjusting the water-circulating heating pad.
 - 2.2.2. Talent positioning the infrared lamp over the surgical area.
- 2.3. Transfer an anesthetized mouse to the heated surgical pad [1]. Administer Ethiqx XR extended-release buprenorphine at 3.25 milligrams per kilogram subcutaneously for postoperative analgesia [2].
 - 2.3.1. Talent moving the anesthetized mouse onto the heating pad.
 - 2.3.2. Talent administering a subcutaneous injection of analgesic.
- 2.4. Place the mouse in the prone position on the surgical pad [1] and secure the limbs with tape to minimize movement [2].
 - 2.4.1. Talent positioning the mouse prone on the pad.
 - 2.4.2. Talent taping the limbs to stabilize the mouse.
- 2.5. Shave the dorsal hair around the intended incision site using clippers [1].
 - 2.5.1. Talent shaving the dorsal surgical area with clippers.

- 2.6. Apply ophthalmic ointment to the eyes to prevent drying during the procedure [1].
 - 2.6.1. Talent applying ointment to both eyes of the mouse.
- 2.7. Insert a rectal probe [1], secure it with tape [2], and connect it to a thermometer for continuous monitoring [3].
 - 2.7.1. Talent inserting the rectal temperature probe.
Videographer's Note: 2.7.1.1-2.7.3 combined together as 2.7.1
 - 2.7.2. Talent taping the probe in place.
 - 2.7.3. Talent connecting the probe to a thermometer.
- 2.8. Disinfect the surgical area by alternating 10 percent povidone iodine and 70 percent ethanol [1], then cover the area with a sterile drape [2].
 - 2.8.1. Talent disinfecting the skin with alternating antiseptic solutions.
 - 2.8.2. Talent placing a sterile drape over the surgical field.

3. Unilateral Renal Ischemia–Reperfusion Injury in Mice

- 2.9. To begin the unilateral renal ischemia reperfusion injury procedure, make a midline dorsal skin incision of 3 to 4 centimeters spanning the kidney level [1]. Separate the skin from the subcutaneous layers using tissue-separating scissors [2].
 - 2.9.1. WIDE: Talent making a midline dorsal skin incision.
Videographer's Note: 2.9.1-2.9.2 combined together as 2.9.1
 - 2.9.2. Talent bluntly separating skin from underlying tissue.
- 2.10. Gently insert a sterile gloved finger between the abdominal wall and the surgical table [1] and slide along the abdominal wall to push the kidney dorsally until it becomes visible through the subcutaneous tissue [2].
 - 2.10.1. Talent inserting a gloved finger beneath the abdominal wall.
Videographer's Note: 2.10.1-2.10.2 combined together as 2.10.1
 - 2.10.2. Talent displacing the kidney until it is visible.
- 2.11. At the level where the kidney is visualized, approximately 1 centimeter lateral to the spine on the left side, make a longitudinal muscle incision of 1 to 1.5 centimeters [1]. Then gently exteriorize the kidney through the dorsal incision [2]. If a thin membrane obstructs access, bluntly dissect it [3].
Videographer's Note: 2.11.1-2.11.3 combined together as 2.11.1
 - 2.11.1. Talent making a small longitudinal muscle incision.
 - 2.11.2. Talent exteriorizing the kidney through the incision.
 - 2.11.3. Talent bluntly dissecting obstructing membrane.

- 2.12. While stabilizing the kidney with non-serrated forceps [1], bluntly dissect the perihilar fat and connective tissue around the upper and lower renal pedicle to create a canal for vascular clamp placement [2].

Videographer's Note: 2.12.1-2.12.2 combined together as 2.12.1

2.12.1. Talent stabilizing the kidney with forceps.

2.12.2. Talent dissecting perihilar fat to expose the pedicle.

- 2.13. Confirm that the core body temperature is between 36.8 and 37.2 degrees Celsius [1]. Then apply a non-traumatic vascular clamp across the renal artery and vein as a bundle [2].

Videographer's Note: 2.13.1-2.14.1 combined together as 2.13.1

2.13.1. Talent checking body temperature on the thermometer display.

2.13.2. Talent applying a vascular clamp to the renal pedicle.

- 2.14. Pull the skin to cover the kidney to keep it close to core body temperature [1].

2.14.1. Talent repositioning skin over the exteriorized kidney.

- 2.15. Maintain core body temperature between 36.8 and 37.2 degrees Celsius by adjusting the heat pump and infrared lamp as needed [1]. After 30 minutes of ischemia, remove the vascular clamp [2] and return the kidney to the abdominal cavity using a cotton swab [3].

2.15.1. Talent adjusting heating pad and infrared lamp settings.

2.15.2. Talent removing the vascular clamp.

Videographer's Note: 2.15.2-2.15.3 combined together as 2.15.2. Use only take 3

2.15.3. Talent gently repositioning the kidney with a cotton swab.

- 2.16. Close the muscle layer with 5-0 absorbable sutures [1] and close the skin with wound clips [2]. Allow the mouse to recover in a clean cage after discontinuing anesthesia [3].

2.16.1. Talent suturing the muscle layer.

2.16.2. Talent applying wound clips to the skin.

2.16.3. Talent placing the mouse into a clean recovery cage.

4. Contralateral Nephrectomy in Mice

- 2.17. To perform contralateral nephrectomy, make a dorsal skin and subcutaneous incision to expose the right kidney of a prepared, anesthetized animal [1]. Create a longitudinal muscle incision of 1 to 1.5 centimeters approximately 1 centimeter lateral to the spine [2].

Videographer's Note: 2.17.1-2.17.2 combined together as 2.17.1

2.17.1. Talent making a dorsal incision on the right side.

2.17.2. Talent incising the muscle layer.

2.18. While holding the kidney with non-serrated forceps [1], bluntly dissect the perihilar fat and connective tissue to expose the renal pedicle [2].

2.18.1. Talent stabilizing the kidney.

Videographer's Note: Do not use any footage before camera is repositioned in the shot

2.18.2. Talent dissecting tissue around the renal pedicle.

2.19. Carefully separate the adrenal gland from the kidney while leaving the adrenal gland intact [1]. Then ligate the renal pedicle with a small ligating clip and 4-0 silk sutures [2]. Transect the vessels on the renal side [3], then transect the ureter and remove the kidney [4].

Videographer's Note: 2.19.1-2.19.4 combined together as 2.19.1

2.19.1. Talent separating the adrenal gland from the kidney.

2.19.2. Talent placing a ligating clip and sutures on the renal pedicle.

2.19.3. Talent transecting renal vessels.

2.19.4. Talent transecting the ureter and removing the kidney.

2.20. Close the incision by suturing the muscle and clipping the skin [1].

2.20.1. Talent closing the incision layers.

Results

3. Results

- 3.1. Low-magnification images revealed progressive kidney atrophy at 28 and 42 days after renal ischemia-reperfusion injury [1].
 - 3.1.1. LAB MEDIA: Figure 2A. *Video editor: Sequentially show the three panels labeled “Uninjured,” “d28,” and “d42,”*
- 3.2. Masson’s trichrome staining showed extensive interstitial fibrosis in the cortex and medulla at 28 and 42 days after renal ischemia-reperfusion injury [1].
 - 3.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the “d28” and “d42” panels*
- 3.3. Serum blood urea nitrogen levels and Serum creatinine levels were significantly elevated at 28 and 42 days after injury [1].
 - 3.3.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the bars for 28 and 42 days in both BUN and Creatinine graphs*
- 3.4. Expression of the CKD (C-K-D) marker genes *Acta2* (Act-Ah-Two) and *Col1a1* (Kohl-One-A-One) was significantly upregulated at days 28 and 42 after injury [1].
 - 3.4.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the bars for 28 and 42 days in both Acta2 and Col1a1 graphs*

Pronunciation Guide:

Ischemia–Reperfusion

Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemia>

IPA: /ɪ'ski:mia/

Phonetic Spelling: ih-skee-mee-uh

Nephrectomy

Pronunciation link: <https://www.merriam-webster.com/dictionary/nephrectomy>

IPA: /nə'frɛktəmi/

Phonetic Spelling: nuh-frek-tuh-mee

Pediatrics

Pronunciation link: <https://www.merriam-webster.com/dictionary/pediatrics>

IPA: /,pi:di'ætrɪks/

Phonetic Spelling: pee-dee-a-triks

Autoclave

Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>

IPA: /'ɔ:toʊ'kleɪv/

Phonetic Spelling: aw-toe-klayv

Subcutaneously

Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneously>

IPA: /,sʌbkju:'teɪniəsli/

Phonetic Spelling: sub-kyoo-tay-nee-uhs-lee

Analgesia

Pronunciation link: <https://www.merriam-webster.com/dictionary/analgesia>

IPA: /,ænəl'dʒi:ziə/

Phonetic Spelling: an-uhl-jee-zee-uh

Ophthalmic

Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>

IPA: /ɑ:f'θæl.mɪk/

Phonetic Spelling: ahf-thal-mik

Povidone

Pronunciation link: <https://www.merriam-webster.com/dictionary/povidone>

IPA: /'pɑ:və'doʊn/

Phonetic Spelling: pah-vuh-dohn

Ethanol

Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>

IPA: /'εθə,nɔ:l/

Phonetic Spelling: eh-thuh-nawl

Perihilar

Pronunciation link: No confirmed link found

IPA: /,pəri'hætɪlə/

Phonetic Spelling: peh-ree-hy-lur

Pedicle

Pronunciation link: <https://www.merriam-webster.com/dictionary/pedicle>

IPA: /'pɛdɪkəl/

Phonetic Spelling: peh·duh·kuhl

Ischemia

Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemia>

IPA: /ɪ'ski:miə/

Phonetic Spelling: ih·skee·mee·uh

Transect

Pronunciation link: <https://www.merriam-webster.com/dictionary/transect>

IPA: /træn'sɛkt/

Phonetic Spelling: tran·sekt

Ureter

Pronunciation link: <https://www.merriam-webster.com/dictionary/ureter>

IPA: /jʊ'ri:tər/

Phonetic Spelling: yuh·ree·tur

Atrophy

Pronunciation link: <https://www.merriam-webster.com/dictionary/atrophy>

IPA: /'ætrəfi/

Phonetic Spelling: a·truh·fee

Masson's

Pronunciation link: <https://www.merriam-webster.com/dictionary/Masson>

IPA: /'mæsən/

Phonetic Spelling: ma·son

Trichrome

Pronunciation link: <https://www.merriam-webster.com/dictionary/trichrome>

IPA: /'traɪ,kroʊm/

Phonetic Spelling: try·kroh·m

Interstitial

Pronunciation link: <https://www.merriam-webster.com/dictionary/interstitial>

IPA: /,ɪntər'stiʃəl/

Phonetic Spelling: in·ter·stish·uhl

Fibrosis

Pronunciation link: <https://www.merriam-webster.com/dictionary/fibrosis>

IPA: /faɪ'broʊsɪs/

Phonetic Spelling: fy·broh·suhs

Creatinine

Pronunciation link: <https://www.merriam-webster.com/dictionary/creatinine>

IPA: /kri'ætə,ni:n/

Phonetic Spelling: kree·a·tuh·neen

Upregulated

Pronunciation link: <https://www.merriam-webster.com/dictionary/upregulate>

FINAL SCRIPT: APPROVED FOR FILMING



IPA: /ˌʌpˈrɛɡjəˌleɪtɪd/

Phonetic Spelling: up·reg·yuh·lay·tuhd