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Type 1 Diabetes Resource (TIDR)
Mouse Husbandry and Generation Core
"Core B"

Personnel:

Dr. Ed Leiter, Ph.D., P.I.-25% effort

Dr. Cathy Lutz, Ph.D., co-P.I.-5% effort

Racheal Wallace, TIDR Manager-50% effort

Peter Reifsnyder, B.S., Senior Research Assistant-100% effort

Pamela Stanley, Vivarium Technician-100% effort

Core Operation

- AMDCC investigator contacts Ms. Wallace with a request
- Dr. Leiter and Ms. Wallace consult with investigator regarding the proposed Core service
- Importation of stocks or constructs are scheduled.

Mouse stocks

Importation

IVF Rederivation into full barrier

store cryopreserved embryos

- speed congenics
- combination with *Ins2*^{Akita}, etc.
- transgenesis

ES Cell Lines. DNA

Importation

"Knock-out" generation or transgenic construction

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Phenotyping*

“New” Diabetes Platform Strains†

All *Ins2*^{Akita} Stocks
B6-in distribution
FVB-in development
DBA/2-just arrived
129/Tac-importation pending

$Lepr^{db}$ Stocks
FVB.BKS-$Lepr^{db1J}$

⬇

available now in small quantities

Protocol: T1DR-MGHC-07-01

Estimate10-15 months to distribute mice and to complete cryopreservation.

† contact Racheal Wallace to register interest for any of these stocks

*no phenotyping projects initiated pending new vivarium space assignment.

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Projects in progress

Protocol: T1DR-MGHC-2007-02

Kakoki (UNC)-B6-Bdkrb1/2 KO.*Ins2*$^{\mathit{Akita}}$

Tandem KO stock imported and combined with Akita mutation; colony being developed with some mice available to ship.

Estimate 3-9 months we will be providing mice of desired quantity, genotypes, sex and age to principal investigator and cryopreservation should be complete. When project will actually complete will depend on the principle investigator’s demand for mice.

Protocol: T1DR-MGHC-2007-03

Kakoki (UNC)-B6-Bdkrb2 KO.*Ins2*$^{\mathit{Akita}}$

KO stock imported and combined with Akita mutation; colony being developed for AMDCC and public distribution

Estimate10-15 months to provide mice to AMDDC members and the public, as well as completing cryopreservation. When project will actually complete will depend on demand for mice by AMDCC members.

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Projects in progress

Protocol: T1DR-MGHC-2007-05

Levi (UCHSC)-FXR-deficient DBA/2J

First backcross from F1 to produce speed congenics on both backgrounds now in progress

Should be able to provide principal investigator with each new congenic stock in 18-24 months. Total estimate time to project completion 30-36 months.

Protocol: T1DR-MGHC-2007-07

Maeda (UNC)-B6/N-Lias-HL knock-in

B6/N ES cell line imported; construct verified and drug-resistant transfectants selected. Blastocyst injections into B6-Tyr^{c} planned for end of Sept. pending verification of inserted gene.

Estimate 10-12 months to establish targeted mutation lines to be evaluated on a C57BL/6J background. 8-10 months to produce mice containing targeted mutation and cre transgene for distribution to principal investigator. 8-10 months to produce mice containing targeted mutation, cre and $Ins2^{Akita}$ or ApoE deficient. Distribution and cryopreservation will take another 6-12 months. Total estimated time to completion: 40-44 months

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Projects in progress

Protocol: T1DR-MGHC-2007-08

Levi (UCHSC)-FXR-deficient FVB/NJ

First backcross from F1 to produce speed congenics on both backgrounds now in progress

Should be able to provide principal investigator with each new congenic stock in 18-24 months. Total estimate time to project completion 30-36 months.

Protocol: T1DR-MGHC-2007-09

Feldman (UM)-BKS-Lepr^{db}.Sod2^{lox}. Nes-Cre

B6.*Sod2*^{lox} stock imported; F1 with BKS-*m*+/+*db* completed, first backcross to BKS-*m*+/+*db* in progress. Separate F1 combining Lepr^{db} and Nes-Cre done; first backcross pending.

Should be able to provide principal investigator with mice in 18-24 months. Estimate project completion 30-36 months.

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Projects in progress

T1DR-MGHC-2007-11

Daneshgari (Cleveland Clinic)-B6-Tg(SM22-Cre/ESR1).SOD2^{lox}

SM22CreER stock has arrived in importation. IVF rederivation scheduled for Oct.

Should be able to provide principal investigator with mice in 8-12 months. Estimate project completion 18-24 months.

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Projects awaiting start-up materials

Protocol: T1DR-MGHC-2007-04

Takahashi/Smithies (UNC)- 129B6F1-Nos3 KO. Ins2^{Akita}

Estimate 10-14 months from receipt of mice.

Protocol: T1DR-MGHC-2007-06

Brosius (U Mich.) - B6;129-Jak2^{floxed} knockin to GtRosa26 locus

Estimate 9-12 months to establish targeted knockin lines to be evaluated for additional work-up.

Protocol: T1DR-MGHC-2007-10

Sharma (Jefferson) - FVB. Tg(Nox4-SM22α). Ins2^{Akita}

Estimate 18-24 months from receipt of transgenic construct

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Projects awaiting start-up materials

T1DR-MGHC-2007-12

Harris/Matthews (Vanderbilt) - 129/Sv.Nos3^{floxed}. Tek-cre. Ins2^{Akita}

Estimate 38-44 months upon receipt of project start up materials. 18-24 month to create a congenic 129-Tg(Tek-cre) stock. 12-18 months to make and evaluate transgenic eNOS lines on a 129 background. 6-8 months to have sufficient congenic 129-eNOS, Tg(Tek-cre) mice to ship principal investigator. Project completion estimated 38-44 months upon receipt of project start up materials.

T1DR-MGHC-2007-13

Fisher (NYU) - B6-LDLR KO .Tg(TRE/hAR). Tg(H2-K^{d}/rtTA)

Estimate 28-34 months upon receipt of project start up materials

Protocol: T1DR-MGHC-2007-14

Smithies (UNC) - Conditional bradykinin B1 and B2 receptor null: Akita diabetic mouse

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Approved Projects
MGHC waiting to be contacted

Coffman (Duke) - Inducible (TET) expression of stabilized HIFs (Hypoxia Inducible Factors)

Abel (U. of Utah) - Inducible (TAM-cre) Netrin Akita

Bottinger (MSSM) - Cd36 transgenic

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Question of Controls

Bradykinin receptor, beta 1,2-Chromosome 12
All combinations coisogenic on B6

- **Bdkrb1/2 -/-, Akita -/+ (Diabetic)**
- **Bdkrb1/2 -/-, Akita +/+**
- **Bdkrb1/2 -/+, Akita -/+ (Diabetic)**
- **Bdkrb1/2 -/+, Akita +/+**
- **Bdkrb1/2 +/+, Akita -/+ (Diabetic)**
- **Bdkrb1/2 +/+, Akita +/+**
- **Males vs. Females**

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Consideration of controls

There are cryptic Cre excission sites in the mouse genome

Ref: Semprini S, *et al*., Cryptic loxP sites in mammalian genomes: genome-wide distribution and relevance for the efficiency of BAC/PAC recombineering techniques. Nucleic Acids Res 2007;35(5):1402-10.

Promoter specific Cre expression in the absence of ploxed genes can effect cell function

Lee JY,et al., RIP-Cre revisited, evidence for impairments of pancreatic beta-cell function. J Biol Chem 2006;281(5):2649-53.

Leiter EH,et al., Unexpected functional consequences of xenogeneic transgene expression in beta cells of NOD mice. Diabetes, Obesity, and Metabolism 2007;9((Suppl2)):in press.

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A Project Generating Multiple Genotypic Classes: What Controls?

Stock 1:BKS-Sod2lox/lox, m+/+db	x	Stock 2: BKS-Nes-Cre+/-, m+/+db

1/4 -> Sod2-lox/+.cre/+.db/+ x BKS.Sod2-lox/lox.db/+

1/8 -> Sod2-lox/lox.cre/+.db/+ x BKS.Sod2-lox/lox.db/+

1/8 -> Sod2-lox/lox.cre/+.db/db (Diabetic)

1/4-> Sod2-lox/lox.cre/+.db/+

1/8-> Sod2-lox/lox.cre/+.+/+

1/8 -> Sod2-lox/lox.+/+.db/db (Diabetic)

1/4-> Sod2-lox/lox.+/+.db/+

1/8-> Sod2-lox/lox.+/+.+/+

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A Project Generating Multiple Genotypic Classes: What Controls?

Stock 1:BKS-Sod2lox/lox, m+/+db	x	Stock 2: BKS-Nes-Cre+/-, m+/+db

Sod2-lox	Nes-cre	db	
Hom	Hemi	Hom	(Diabetic)
Hom	Hemi	wt	
Hom	wt	Hom	(Diabetic)
Het	Hemi	Hom	(Diabetic)
Het	Hemi	wt	
wt	Hemi	Hom	(Diabetic)
wt	Hemi	wt	
wt	wt	Hom	(BKS.db/db) (Diabetic)
wt	wt	wt	

- Males vs. Females

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The Aging Phenome Project: TJL Shock-Ellison Aging Center

Goal: to study aging in 32 inbred strains with enough mice to detect a 10% difference in lifespan.

Measure as many aging phenotypes as budget will allow.

Use haplotype analysis and structural modeling to understand aging.

Experimental Design

Longitudinal study: 32 males and 64 females/strain for lifespan

Non-invasive measures every 6 months

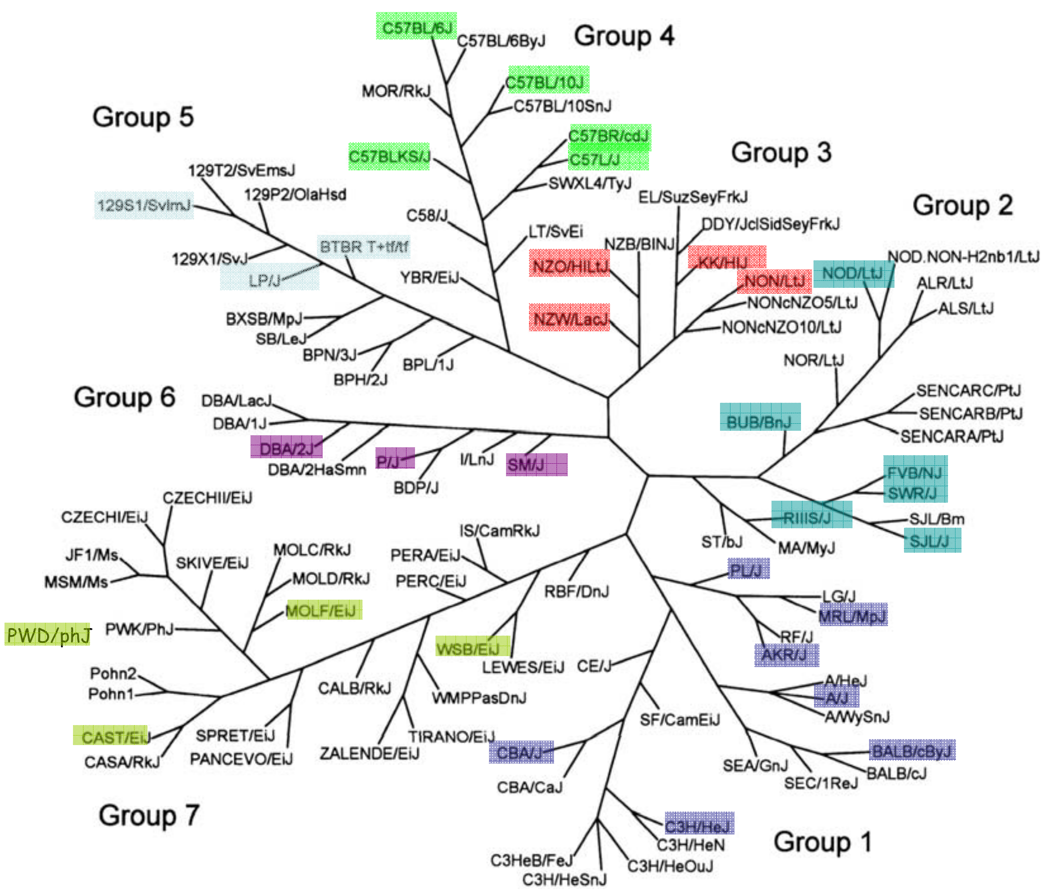
Cross-sectional study:15 males and 15 females/strain

Invasive tests and necropsy at 6, 12, and 20 months

Complete pathology at 12 and 20 months

Experimental design: *Strains*

Mouse Family Tree



Petko M. Petkov et al. Genome Res. 2004; 14: 1806-1811

32 inbred strains

129S1/SvIm	BUB/Bn
A/J	C57BL/10
AKR	C57BLKS
BALB/cBy	C57BR/cd
BTBR-T +tf	C57L
C3H/He	CBA
C57BL/6	▶ KK/HI
CAST/Ei	LP
DBA/2	MRL
FVB/N	NZO/H1Lt
MOLF/Ei	P
NOD.B10-H2b	PL
▶**NON/Lt**	RIIIS
NZW/Lac	SJL
PWD/Ph	SM
WSB/Ei	SWR

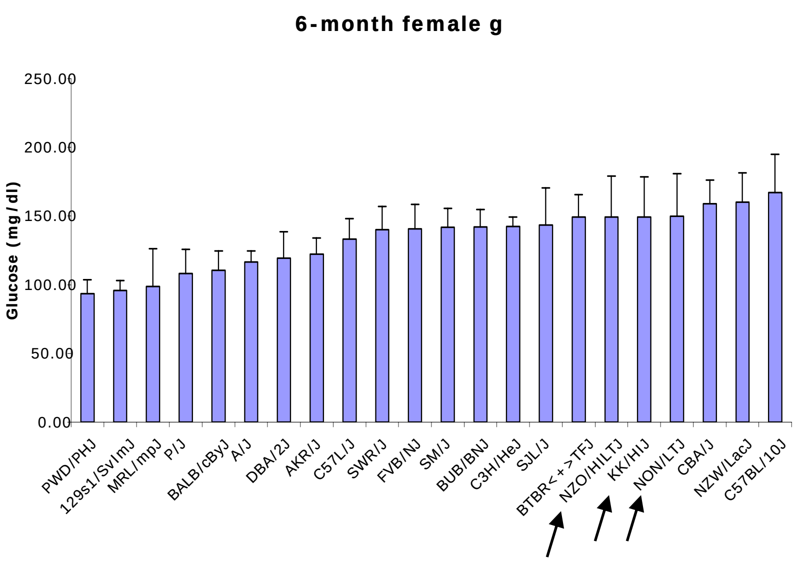
Strain names in bold are the strains sequenced by Perlegen Sciences.

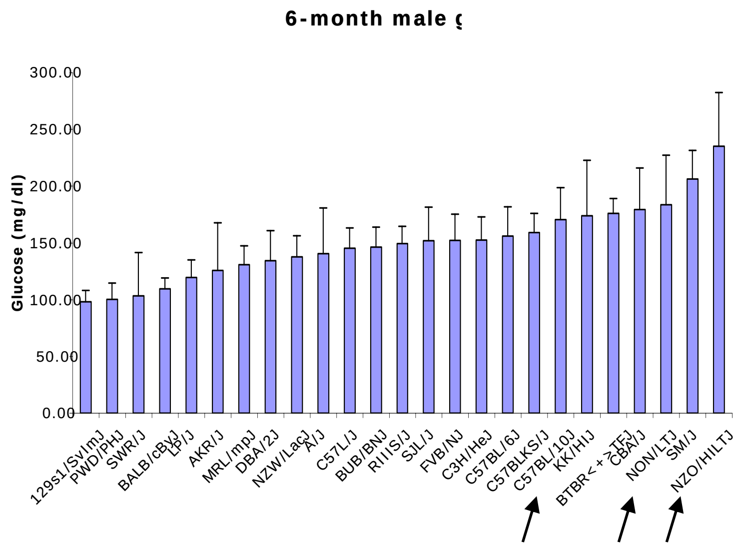
Glucose levels in inbred strains

6, 12-month old mice

Fasting for 4 hours

Glucose in blood





Male Survival Curves

Survival Fraction

Group 1
- A/J
- AKR
- BALB
- C3H
- CBA
- MRL
- PL

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Group 2
- BUB
- FVB
- NOD
- RIIIS
- SJL
- SWR

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Group 3
- KK
- NON
- NZO
- NZW

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Group 4
- B10
- B6
- BLKS
- BR/CDJ
- C57L

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Group 5
- 129S1
- BTBR
- LP

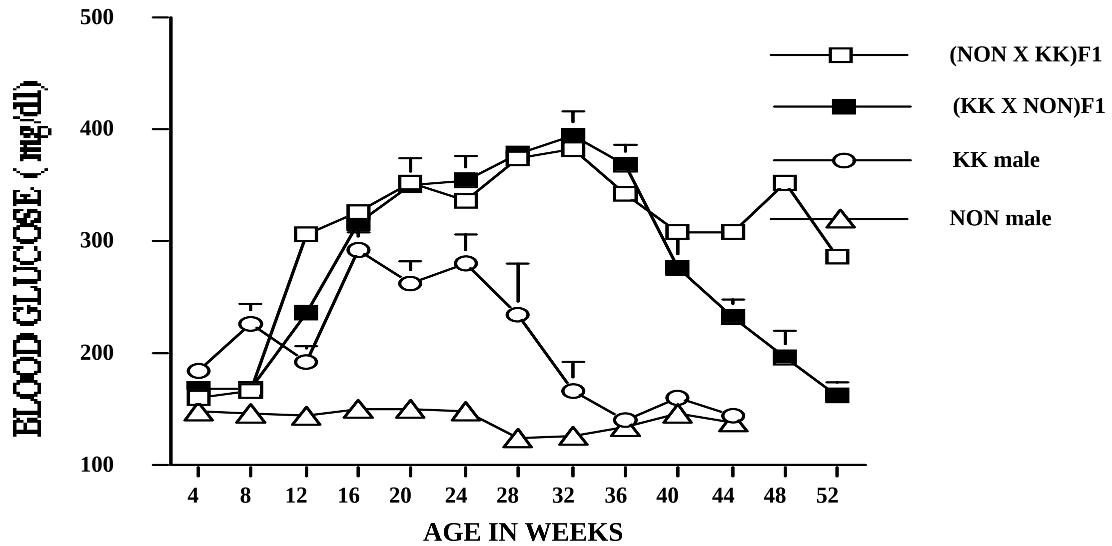
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

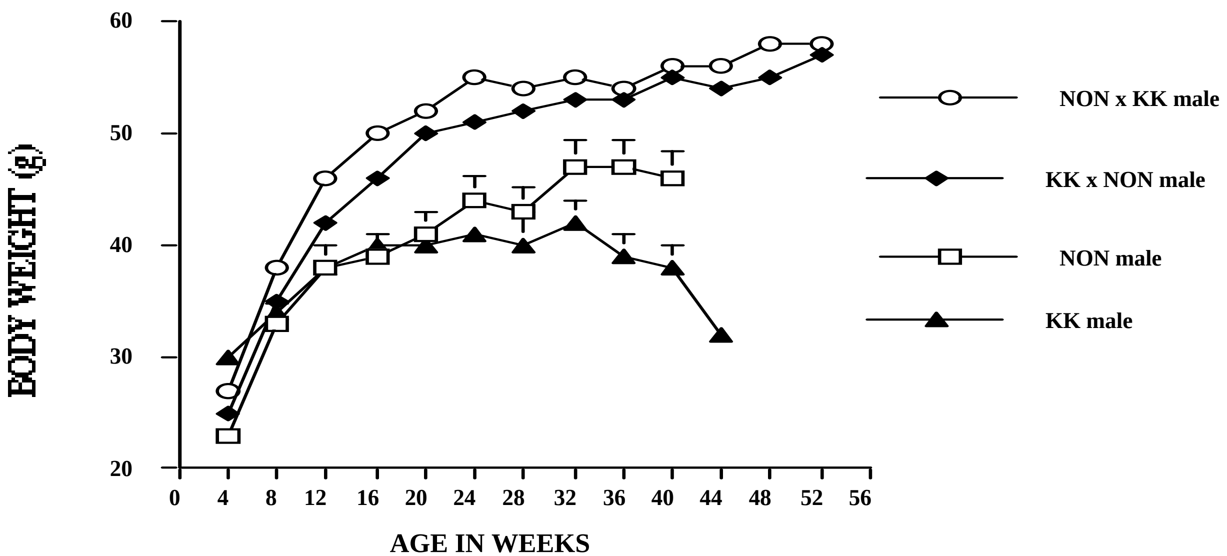
Group 6,7
- D2
- PWD
- SM
- WSB

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Days

NON Genome Exacerbates Diabesity in F1 Males





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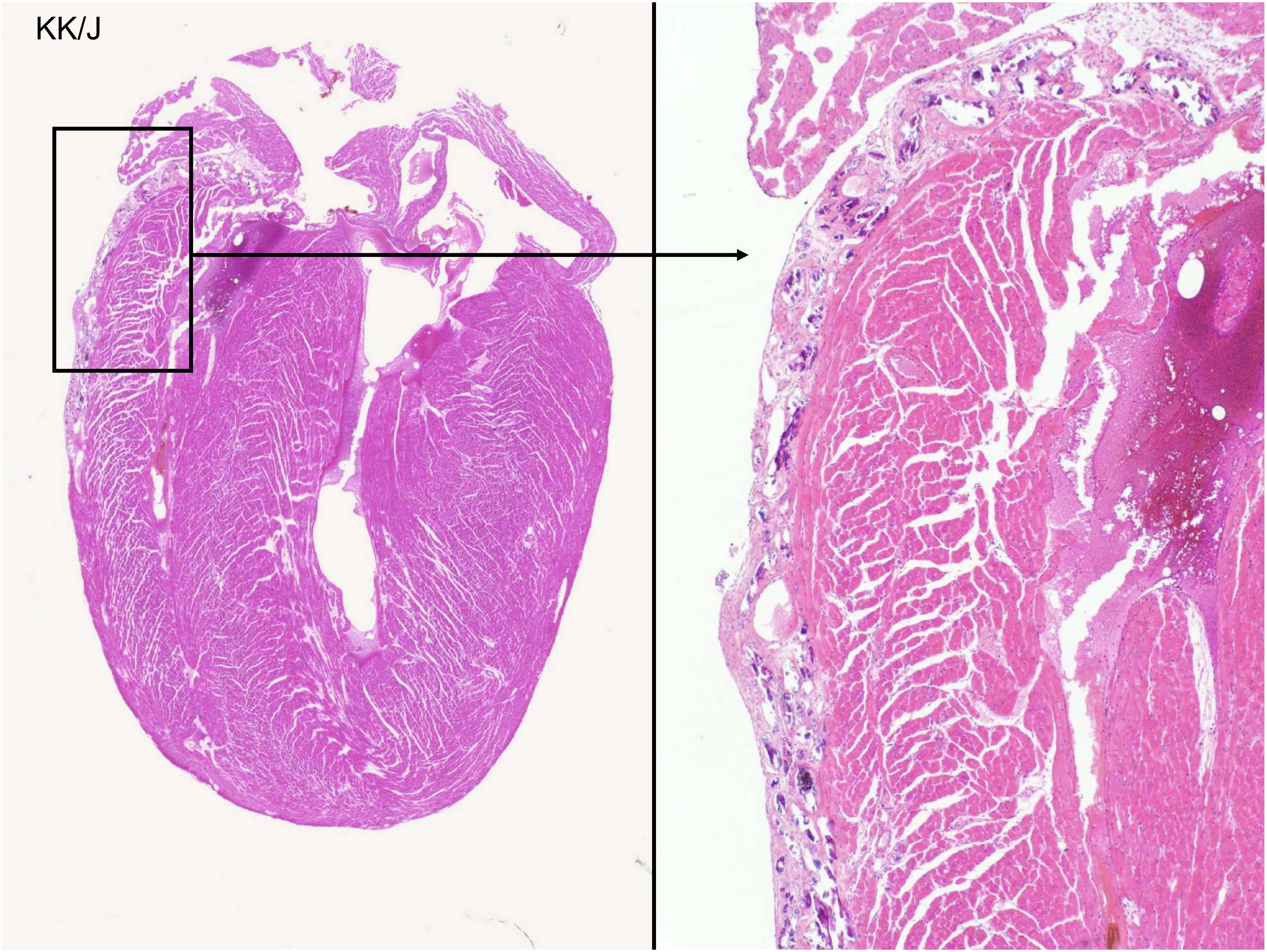
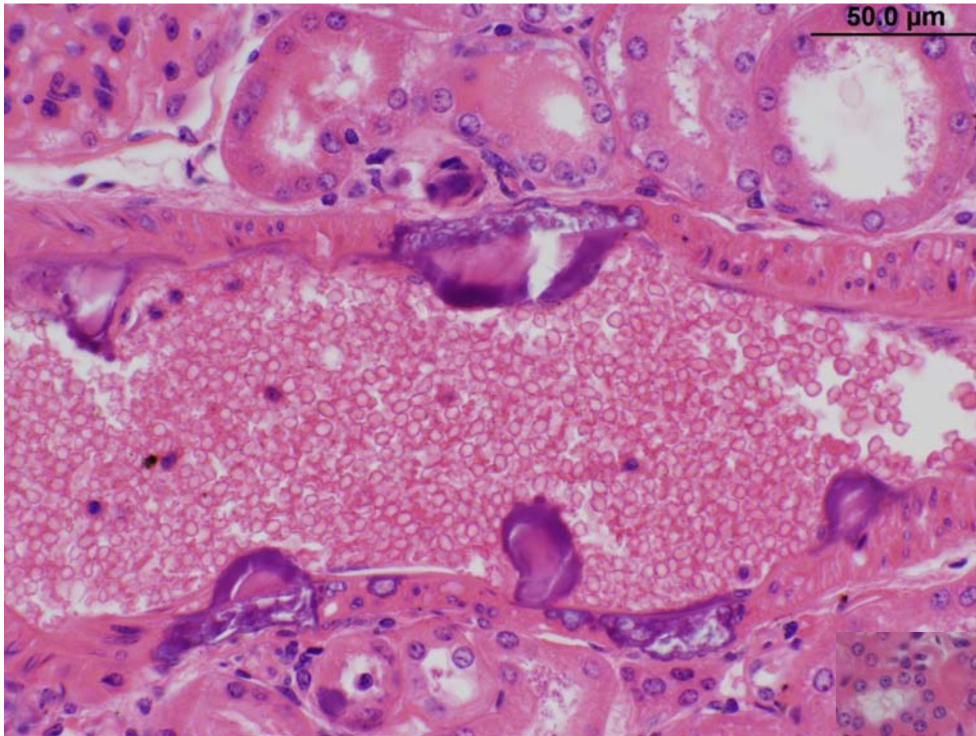
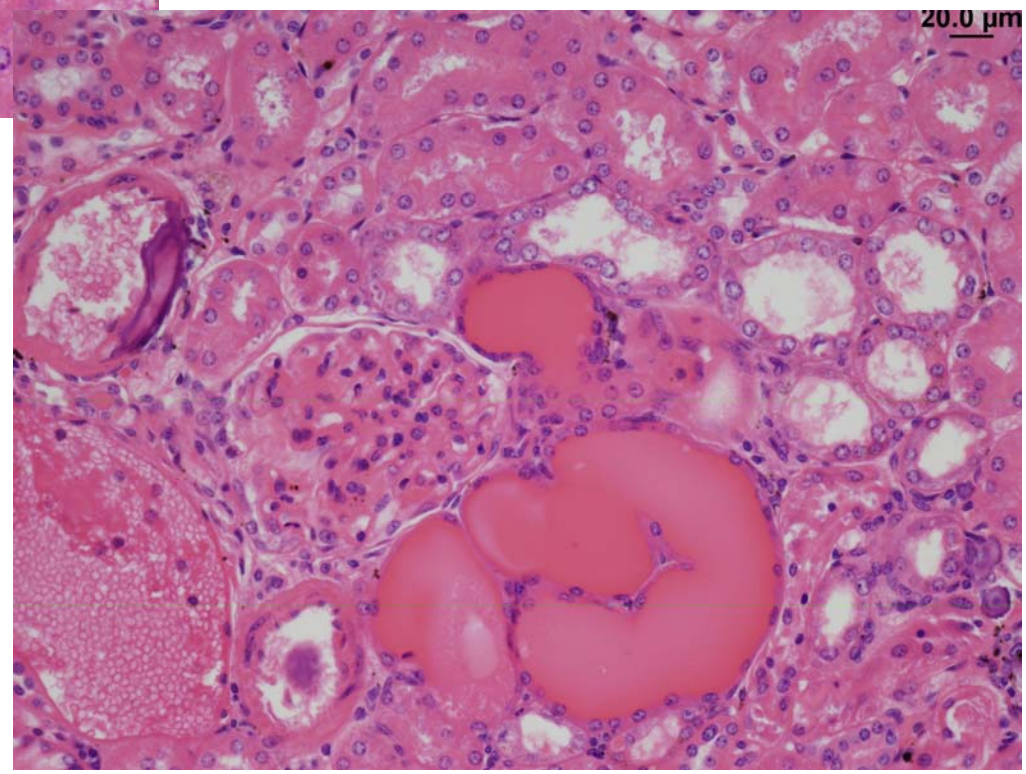


KK/J


50.0 µm

20.0 µm

Lamr1 functional retroposon causes right ventricular dysplasia in mice

Yoshihiro Asano[1], Seiji Takashima[1], Masanori Asakura[1], Yasunori Shintani[1], Yulin Liao[1], Tetsuo Minamino[1], Hiroshi Asanuma[1], Shoji Sanada[1], Jiyoong Kim[2], Akiko Ogai[2], Tomi Fukushima[1], Yumiko Oikawa[1], Yasushi Okazaki[3], Yasufumi Kaneda[4], Manabu Sato[4], Jun-ichi Miyazaki[5], Soichiro Kitamura[2], Hitonobu Tomoike[2], Masafumi Kitakaze[2] & Masatsugu Hori[1]

Arrhythmogenic right ventricular dysplasia (ARVD) is a hereditary cardiomyopathy that causes sudden death in the young. We found a line of mice with inherited right ventricular dysplasia (RVD) caused by a mutation of the gene laminin receptor 1 (*Lamr1*). This locus contained an intron-processed retroposon that was transcribed in the mice with RVD. Introduction of a mutated *Lamr1* gene into normal mice by breeding or by direct injection caused susceptibility to RVD, which was similar to that seen in the RVD mice. An *in vitro* study of cardiomyocytes expressing the product of mutated *Lamr1* showed early cell death accompanied by alteration of the chromatin architecture. We found that heterochromatin protein 1 (HP1) bound specifically to mutant LAMR1. HP1 is a dynamic regulator of heterochromatin sites, suggesting that mutant LAMR1 impairs a crucial process of transcriptional regulation. Indeed, mutant LAMR1 caused specific changes to gene expression in cardiomyocytes, as detected by gene chip analysis. Thus, we concluded that products of the *Lamr1* retroposon interact with HP1 to cause degeneration of cardiomyocytes. This mechanism may also contribute to the etiology of human ARVD.

Nature Genetics, 2004;36(2):123-30.