Mouse Genetics:
Determining gene function
Determining gene function

- Mutagenesis approaches
  - Gene-driven, phenotype-driven
- Phenotyping
  - Challenges: standardisation
  - European Programmes, the clinic concept
  - Informatics
- Otitis Media: mutagenesis and phenotyping for the provision of novel disease models
Challenges for the study of disease in the 21st century

• Characterise the function of every gene in the mammalian genome

• Generate mutations in every gene in the mouse genome

• Characterise the phenotype of every mutant mice

• Identify models of human disease
Challenges for the study of disease in the 21\textsuperscript{st} century

- Characterise the function of every gene in the mammalian genome
- Generate mutations in every gene in the mouse genome
- Characterise the phenotype of every mutant mice
- Identify models of human disease
# Mutagenesis in the Mouse

## Phenotype-driven and gene-driven approaches

<table>
<thead>
<tr>
<th>Gene Driven</th>
<th>Phenotype Driven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene traps</td>
<td>EthylNitrosourea, ENU -</td>
</tr>
<tr>
<td>Gene targeting</td>
<td>unbiased chemical mutagenesis</td>
</tr>
<tr>
<td>Gene driven ENU</td>
<td></td>
</tr>
<tr>
<td>RNAi</td>
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**EUCOMM, Europe**
- European Conditional Mouse Mutagenesis

**KOMP, US**
- Knock-out Mouse Project

An International Centre for Mouse Genetics

Mammalian Genetics Unit
## Mutagenesis in the Mouse
### Phenotype-driven and gene-driven

<table>
<thead>
<tr>
<th>Gene Driven</th>
<th>Start with a known locus</th>
<th>Often make <em>a priori</em> assumptions about function of gene</th>
<th>Unpredictable phenotypes</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Phenotype Driven</th>
<th>Requires identification of mutated gene</th>
<th>No assumptions about underlying pathways</th>
<th>Phenotype is the starting point</th>
</tr>
</thead>
</table>
Transgenics - overexpression/ inappropriate expression/shRNA

- Construction of transgene driven by promoter X
- Collection of fertilised eggs from donor mouse
- Injection of transgene into fertilised egg
- Transfer of embryos into foster mother, analysis of offspring for transgenic DNA and expression in tissue X

Transgenics - overexpression/ inappropriate expression/shRNA
Knock out technology - targeted conditional mutations

Drug selection

Injection into blastocyst

“Floxed” gene

ES cell gene locus

Homologous recombination

ES cell

Chimeric offspring

Germline offspring
Knock out technology - targeted conditional mutations

Mouse carrying a Cre recombinase gene controlled by a tissue-specific promoter X

Mouse carrying conditional (floxed) alleles of gene Y

cross

geneY<sub>cond</sub>

Gene Y is inactivated by Cre in tissue X
Mutagenesis in the Mouse
Phenotype-driven and gene-driven approaches

Gene Driven
- Gene traps
- Gene targeting
- Gene driven ENU
- RNAi

Phenotype Driven
- EthylNitrosourea, ENU - unbiased chemical mutagenesis
ENU mutagenesis

- ENU, ethylnitrosourea (alkylating agent) - mutagenesis of male spermatogonial stem cells
- Specific locus mutation rate of \( \geq 1 \) in 1,000 gametes
- Every 1,000 mice carry a new ENU hit at any locus
- A point mutagen
  - Can deliver the full range of mutational effects - hypomorphs, gain-of-function, dominant negative
Harwell ENU Programme 1
Genome-wide screen for dominant mutations

ENU

♂ ♂
X

♀

F1 progeny

SCREENS
Male mice are injected with the mutagen ENU.

Mutagenized males are mated to wild-type females.

Wild-type oocytes × Sperm carrying mutations.

G1 Screening for dominant mutations.
An International Centre for Mouse Genetics

Mammalian Genetics Unit

Screen for dominant phenotype

Mutagenise BALB/c males

Mate with C3H/He females

Visible anomalies

SHIRPA testing

Behavioural testing

Blood Biochemistry

New screens collaborators

Data into Mutabase

Inheritance Testing

Low resolution mapping (IVF used for backcrosses into C3H)

Archive of embryos & sperm

Detailed analysis of selected mutants

New screens collaborators

Behavioural testing

Blood Biochemistry

New screens collaborators

Data into Mutabase

Inheritance Testing

Low resolution mapping (IVF used for backcrosses into C3H)
Harwell ENU programme 1

Focus on:
- Neurological
- Behavioural
- Circadian
- Deafness
- Vision
- Diabetes
- Kidney stones
- Alcohol preference

- 35,000 mice weaned, scored for visible phenotype
- 15,000 mice SHIRPA, 10,500 mice LMA, 10,500 PPI, 7,000 vision screens, 2,000 clinical chemistry...
- 1,500 abnormal phenotypes
- 376 inheritance tested
  - > 200 inherited mutations
- > 700 new mutations generated
- > 100 mapped, > 40 cloned

Nolan et al. Nature Genetics 2000
Phenotype Classes

- Pigment 31
- Skin and hair texture 21
- Growth 31
- Craniofacial 12
- Digits/limbs 3
- Tail 4
- Clinical chemistry 13
  - Type II diabetes, dyslipidemias, bone & liver disease
- Vestibular/Deafness 21
- Eye/Vision 24
- Neurological/Behavioural 56
Detection of visible mutations

- Dominant spotting
- Batface
- Microphthalmia
- Nanomouse
<table>
<thead>
<tr>
<th>Line #</th>
<th>Mutant Name</th>
<th>Phenotype</th>
<th>Chr</th>
<th>Gene</th>
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<td>bare patches, Bpa</td>
<td>X</td>
<td>Nsdhl</td>
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<td>GENA37</td>
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<td>GENA51</td>
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<td>left-right asymmetry</td>
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<td>Dnahc11</td>
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<td>MUT1494</td>
<td>Crash</td>
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<td>DMS101</td>
<td>Chuzhoi</td>
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<td>to be reported</td>
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<td>RECB4</td>
<td>Neural tube closure</td>
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<td>PLAY68</td>
<td>Long circadian period</td>
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<td>to be reported</td>
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<tr>
<td>Cth</td>
<td>Cloth ears</td>
<td>15</td>
<td>Scn8a</td>
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</tr>
</tbody>
</table>
Male mice are injected with the mutagen ENU.

G0

G1 male mice mated to wild-type females

G1

Option one: intercross G2s

G2

Option two: backcross G2 females to G1 father

G3 screening for recessive mutations

G3
Mutagenesis in the Mouse
Phenotype-driven and gene-driven approaches

Gene Driven
- Gene traps
- Gene targeting
- Gene driven ENU
- RNAi

Phenotype Driven
- EthylNitrosourea, ENU - unbiased chemical mutagenesis

EUCOMM, Europe
European Conditional Mouse Mutagenesis
KOMP, US
Knock-out Mouse Project
Transforming the functional annotation of the mouse and human genomes
ENU gene-driven screens
Creation of parallel archives of DNA and sperm
A step change in genetics

• Whole genome association (WGA) studies are identifying many genomic regions carrying variation associated with common diseases e.g. obesity, diabetes, neurological disease…

• Variation causing disease in the human population occurs in both genes and the surrounding non-coding sequence

• ENU emulates this variation
  • Targets both coding and non-coding sequences

• The Harwell ENU archive is a rich source of mutations that can be used to explore the effects of variation within and in neighbouring regions of genes
ENU gene-driven screens
Creation of parallel archives of DNA and sperm

Coghill et al. Nature Genetics 2002

DNA archive
Sperm archive

Mutant identification

10,000 DNAs

Recovery and examination of mutants

ENU
C3H X BALB/c
Probabilities of finding n or more mutant alleles in varying numbers of DNAs from offspring of ENU mutagenised male mice

Assumptions
1. Mutation rate of 1/1000 per locus
2. Mutation detection rate of 90%
Harwell Gene-Driven Screens

- 51 genes screened
- 122 mutations found in 137.05 Mb
- 1 mutation every 1Mb
- 1 mutation per 100 bp, over 10,000 genomes
- 25 million mouse mutations in archive
Putative functional human SNPs

200kb region 2000 ENU mutations

135 ENU mutations in coding sequences - up to 75 functional

50 ENU mutations in non-coding conserved sequences

Select 5-10 mutations in non-coding conserved sequences coincident with functional human SNPs

Select 5-10 mutations in relevant genes and functional domains

Rederive mice and phenotype
Next Generation Sequencing of the Harwell ENU Archive - First Steps

- Sequence exons from 1000 genes in 6000 animals
- Include non-coding conserved sequences
- Generate a database of point mutations - a new mutation resource for exploring gene function in the mammalian genome
Challenges for the study of disease in the 21st century

• Characterise the function of every gene in the mammalian genome

• Generate mutations in every gene in the mouse genome

• Characterise the phenotype of every mutant mice

• Identify models of human disease
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European Mouse Programmes

- **EUCOMM - European Conditional Mouse Mutagenesis**
  - Developing mouse mutants for most of the genes in the mouse genome

- **EUMORPHIA - European Mouse Phenotyping**
  - Development and standardisation of mouse phenotyping platforms

- **EUMODIC - European Mouse Disease Clinic**
  - Undertake a major pilot programme to utilise standardised phenotyping platforms for the analysis of mouse mutants from EUCOMM

- **EMMA - European Mouse Mutant Archive**
  - Archiving and dissemination of mice
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The European Conditional Mouse Mutagenesis Program

Helmholtz Center, Munich, Germany
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Wellcome Trust Sanger Institute, Hinxton, UK
A. Bradley (coordinator), W. Skarnes, P. Liu

University Frankfurt, Germany
H. von Melchner

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P. Chambon

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N. Rosentahl

Medical Research Council, Harwell, UK
S. Brown

National Research Council, Monterotondo, Italy
G. Tocchini-Valentini

German Resource Center of Genome Research (RZPD), Heidelberg
B. Korn
European Mouse Programmes

• EUCOMM 2006-2010
  • European Conditional Mouse Mutagenesis program
  • 8,000 gene targeted null/conditional ES lines (BL/6N)
  • ES library archived at Helmholtz Centre, Munich for distribution
  • 320 mouse lines generated and re-archived for distribution to the community
  • 20 new Cre-expressing mouse strains generated

• Complementary programmes
  • Canada - NorCOMM and US - KOMP
Challenges for the study of disease in the 21st century

• Characterise the function of every gene in the mammalian genome

• Generate mutations in every gene in the mouse genome

• Characterise the phenotype of every mutant mice

• Identify models of human disease
The Challenges of Phenotyping
Specific Data Point

Systems Analysis

Mutant/Genetic Background

Environment

Phenotypes

Test 1
Test 2
Test 3
Test 4
Test 5
Test 6
Test 7
Test 8
Challenges of Phenotyping

• Phenotype discovery - characterising disease models through a systems approach

• Undertaking broad based, systematic phenotype screens of medical relevance

• Without broad based screens, we will not understand systems

• Identifying efficient primary, first-pass indicators of interesting disease models

• Ensuring utility of models and engagement/take-up from the community and further characterisation beyond the major mouse centres
Challenges of Phenotyping

- Develop robust and broad-based comprehensive phenotyping platforms able to deliver phenotypic information for all body systems
- Standardising phenotyping protocols so that we can share and compare phenotype data from mouse genetics centres throughout the world
- Ensure appropriate informatics developments in protocol descriptions, data exchange standards, ontologies
The importance of standardisation

• Better reproducibility of test outcome

• Better comparability of test outcome

• Sharing of phenome results
Building a European Model
European Mouse Programmes

- **EUCOMM - European Conditional Mouse Mutagenesis**
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EUMORPHIA

• Development and standardisation of mouse phenotyping platforms

• EMPReSS, European Mouse Phenotyping Resource for Standardised Screens
Phenotyping - Workpackages

- Standardisation - animal handling
- Clinical Chemistry/Haematology
- Renal systems
- Central, peripheral nervous system, muscle
- Behaviour and cognition
- Imaging
- Necropsy, pathology, histology

European Mouse Phenotyping Resource for Standardised Screens - EMPReSS

- Cardiovascular
- Hormonal/metabolic
- Allergy and infection
- Sensory systems
- Pulmonary
- Cancer
- Bone, Cartilage
- Expression analysis
EMPReSS

- European Mouse Phenotyping Resource for Standardised Screens
- The EMPReSS provides a platform for the systematic and standardised primary characterisation of mouse mutant models
- It is a comprehensive database of validated SOPs for systematic screens and tests that allows us to describe the phenotype of a mouse
- http://www.eumorphia.org/
- http://empress.har.mrc.ac.uk/

Nature Genetics, Nov. 2005
Phenotyping - Workpackages

- Standardisation - animal handling
- Clinical Chemistry/Haematology
- Renal systems
- Central, peripheral nervous system, muscle
- Behaviour and cognition
- Imaging
- Necropsy, pathology, histology
- First-line phenotyping
- Cardiovascular
- Hormonal/metabolic
- Allergy and infection
- Sensory systems
- Pulmonary
- Cancer
- Bone, Cartilage
- Expression analysis
Integrating and ordering tests in different systems - neurobehaviour and sensory

<table>
<thead>
<tr>
<th>Week</th>
<th>Tests</th>
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<td>8</td>
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<td>Clickbox</td>
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<td>Trunk curl</td>
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<td>Limb grasping</td>
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<td>Toe pinch</td>
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<td>Rotarod</td>
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<td>9</td>
<td>ASR/PPI</td>
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<tr>
<td>10</td>
<td>Swim test</td>
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Reliability, Robustness and Reproducibility

% Centre Time

CNR  GSF  MRC  ICS

Open field Test

- C57BL/6J
- C3HeB/FeJ
- BALB/cByJ
- 129S2/SvPas
Integrating and ordering tests in different systems - neurobehaviour and sensory

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</table>

- **Open field** (week 8)
- **Modified SHIRPA** (week 8)
- **Grip strength** (week 8)
- **Rotarod** (week 8)
- **Y maze** (week 9)
- **PPI and ASR** (week 9)
- **Tail Flick** (week 9)
- **Tail Suspension** (week 10)
- **Swim Test** (week 10)
Reliability, Robustness and Reproducibility

Rotarod - mean latency

**Mean Latency (Sec)**

- C57BL/6J
- C3HeB/FeJ
- BALB/cByJ
- 129S2/SvPas

**Institutions:**
- CNR
- GSF
- MRC
- ICS
- EMBL
Reliability, Robustness and Reproducibility

Rotarod - mean latency

![Graph showing mean latency for CNR, GSF, and ICS]

Second Validation in 3 centres
- modified foam cover on rod
- eliminating training phase and reducing trials

- C57BL/6J
- C3HeB/FeJ
- BALB/cByJ
- 129S2/SvPas
EMPReSS

- European Mouse Phenotyping Resource of Standardised Screens
- 150 standard operating procedures (SOPs) and associated annexes

Browse
- Phenotype Platform
- Ontology Term
- Free Text Search
- EMPReSS Slim Pipeline

http://empress.har.mrc.ac.uk
Harwell Mouse Clinic
Mary Lyon Centre
Phenotyping Platforms

- Dysmorphology and Development
- Neurological
- Sensory
- Behaviour
- Metabolism
- Cardiovascular
- Imaging
- Pathology

Mary Lyon Centre

Primary
- Primary
- Extended
Secondary

An International Centre for Mouse Genetics

Mammalian Genetics Unit
Phenotyping Platforms

Detection of visible mutations

Dysmorphology

Primary

Weaning and adult surveys

Weight

Batface

Nanomouse
Phenotyping - Neuro/Behaviour/Sensory

Open field (week 8)

Modified SHIRPA (week 8)

Grip strength (week 8)

Y maze (week 9)

PPI and ASR (week 9)

Tail Flick (week 9)

Tail Suspension (week 10)

Swim Test (week 10)
Phenotyping - Neuro/Behaviour/Sensory

- Behaviour
  - Circadian Rhythm
  - MoRaG
  - Morris Water Maze
- Sensory
  - Acoustic Brainstem Response
- Secondary
- Primary
  - Extended

An International Centre for Mouse Genetics

Mammalian Genetics Unit
Phenotyping - Metabolism

- Metabolism
- Clinical Chemistry
- Primary
- Extended
- Simplified (24h) Metabolic Cages
- Simplified IPGTT

Graphs showing metabolic data:
- Intercross females (age 35 days)
- Simplified IPGTT
- Metabolism
- Clinical Chemistry
Phenotyping - Cardiovascular

Cardiovascular

Blood pressure - tail cuff

MRI - developmental screens
Phenotyping - Imaging and Pathology

**Imaging**
- X-ray
- pQCT
- MRI

**Pathology**
- Necropsy
- H & E
- IHC

Primary

Secondary
Harwell ENU programme

**BL/6 x C3H**

- **Dominant G1**
  - 400 mice per month

**BL/6 x C3H**

- **Recessive G3**
  - 12 pedigrees per month

**Neurological**

- Behavioural
- Circadian
- Development
- Deafness
- Diabetes

**Development**

- Deafness
- Diabetes
- Vision
- Cardiovascular

Mary Lyon Centre

An International Centre for Mouse Genetics
Mutagenesis Pipeline

- Development
- Hearing
- Metabolism
- Behaviour
- Anatomy
- Imprinting
- Dysmorphology
- Immunology
- Diabetes
- Immunity
- Vision
- Axon Regeneration
- Osteology
- Locomotion
- Neurodegeneration
- Hepatology

An International Centre for Mouse Genetics

Mammalian Genetics Unit
European Mouse Programmes

- **EUCOMM**
  - Developing mouse mutants for most of the genes in the mouse genome

- **EUMORPHIA**
  - Development and standardisation of mouse phenotyping platforms

- **EUMODIC - European Mouse Disease Clinic**
  - Undertake a major pilot programme to utilise standardised phenotyping platforms for the analysis of mouse mutants from EUCOMM

- **EMMA**
  - Archiving and dissemination of mice and data
Beyond Eumorphia - EUMODIC

650 mouse lines

Primary Phenotyping EMPReSSslim

Mouse clinics
GSF, Munich
ICS, Strasbourg
MRC, Harwell
Sanger, Hinxton

EuroPhenome Database
EMPRess slim primary phenotyping screen

**Pipeline 1**
- **Morphology Metabolism**
  - Dysemorphology (9 weeks)
  - Vacant (10 weeks)
  - Simplified IPGTT (13 weeks)

**Cardiovascular**
- Non-invasive Blood Pressure (11 weeks)
- Calorimetry (12 weeks)
- Heart weight (15/16 weeks)

**Bone**
- DEXA (24 weeks)
- X-ray (3)

**Neuro-behavioural & Sensory**
- Open field (9 weeks)
- Modified SHIRPA (10 weeks)
- Rotarod (13 weeks)
- Acoustic Startle & PPI (12 weeks)
- Hot plate (13 weeks)
- Ophthalmoscope & Slit Lamp

**Haematology & clinical chemistry**
- Clinical Chemistry (15 weeks)
- Hematology
- ANI* (14 weeks terminal blood)
- FACS analysis of peripheral blood cells
- Immunoglobulin concentration

**Allergy/Immune**
- Number of males
- Number of females

*ANII*
Beyond Eumorphia - EUMODIC

650 mouse lines

Primary Phenotyping
*EMPReSSslim*

Mouse clinics
- GSF, Munich
- ICS, Strasbourg
- MRC, Harwell
- Sanger, Hinxton

Secondary Phenotyping

EuroPhenome Database

Specialist Centres
EUMODIC progress

- EMPReSS SOPs harmonised and output parameters agreed between clinics
- All screens established and tested at clinics
- Comparison of baseline phenotypes of BL/6J, BL/6N, 129P2/Ola, 129SvEv underway
  - Defining BL/6J - BL/6N phenotyping differences
- Mutant phenotyping beginning
  - 110 BL/6 lines injected by the four clinics
- Target of 100 lines phenotyped by end 2008
Informatics Requirements

• Common protocol description
  – Develop common formats e.g. Phenotyping Procedure XML to accommodate SOP information and link these to ontologies

• Standard for data exchange
  – Develop XML schema that will allow structured exchange of phenotype data and metadata

• Improved phenotype description
  – Develop increasingly sophisticated ontological structures (vocabularies) to describe phenotypes

Coordination and Sustainability of International Mouse Informatics Resources

An international project to integrate mouse phenotype data
EMPReSS – Future

- **MIMPP** **Minimal Information for Mouse Phenotyping Procedures**
  - PPML (Phenotyping Procedure XML) – single XML framework
    - Phenotyping procedures to be exchanged with other databases
    - Searching across databases
  - Annotation with controlled vocabularies (PATO & MP) for describing phenotypes
EuroPhenome

- Online Mouse Phenotyping Resource
  - Version 1: Pilot Data obtained on background strains (C57BL/6J, C3H/HeBFeJ, BALB/cByJ and 129/SvPas) by researchers in EUMORPHIA project
  - Version 2: Data from 4 primary phenotyping centres in EUMODIC (baseline and mutant data)

- Open Source MySQL Relational Database

- Integrated access to data from other resources or databases e.g. EUCOMM and Ensembl
Data Capture

- PDML (Phenotyping Data XML)
- Nightly XML file upload from phenotyping centres
- Validation with EMPReSS e.g. Parameters/units/bounds

http://www.europhenome.org
Mutagenesis and Phenotyping

Novel disease models

Otitis Media - chronic middle ear inflammatory disease
Otitis Media

- 65% of children have at least one episode of OM
- A large fraction will develop recurrent or chronic OM
- Genetic factors play a major role in chronic OM in the human population but no genes have been cloned
Middle Ear Cavity (MEC) and Eustachian Tube (ET) structure
ENU Mutagenesis - Harwell

- New mouse models of human genetic deafness
- ENU - chemical mutagen - point mutations
- 30,000 mutant mice screened for auditory and vestibular dominant mutations

**Auditory Function**
- Click-box or Preyer Reflex test

**Vestibular Function**
- Circling/head tossing
- Contact righting test
- Negative geotaxis test
# ENU Mutagenesis - Harwell

<table>
<thead>
<tr>
<th>Name</th>
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<td>Circler</td>
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<td>Deaf and circling</td>
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<tr>
<td>Cloth-ears</td>
<td>Deaf</td>
<td>15</td>
</tr>
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<td>Loopy</td>
<td>Deaf and circling</td>
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54 vestibular phenotypes identified
28 deafness phenotypes identified
80% of phenotypes inherited
### ENU Mutagenesis - Harwell

**54 vestibular phenotypes identified**

**28 deafness phenotypes identified**

**80% of phenotypes inherited**

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**Circling and Deafness Mutations**
The mouse mutant Junbo is a single gene model of OM

- Identified from the ENU mutagenesis programme as a highly penetrant mutant showing hearing loss
- No sensorineural hearing loss
- Displays a conductive deafness due to development of a chronic suppurative OM

180DAB Jbo/+ pathology

- In adults, Jbo/+ displays a chronic suppurative OM with polyp formation and mucoperiostial fibrosis
- OM was often associated with perforation of the eardrum
Summary - pathology of Junbo mice

• Comprehensive pathology phenotyping failed to reveal significant organ pathology outside of the middle ear

• No significant differences between Junbo and wild-type mice in T-dependent and T-independent responses or immature and mature neutrophil levels

• Development of pathology from pre-weaning to adult mirrors many of the features of human OM
Junbo is a mutation in the *Evi1* gene.
**Mechanism:** *Evi1* mutations may disturb the balance between TGFβ and p38 MAPK pathways

- *Evi1* mutations may lead to increased MKP1 expression and reduced mucin production.

Diagram:
- Haemophilus influenzae
  - TLR2
  - TβRII/I
  - p38
  - Smad3
  - MKP1 expression
  - MUC5AC expression
  - MUCIN production
The mouse mutant *Jeff* is a single gene model for OM

- Identified from the ENU mutagenesis programme as a highly penetrant dominant mutant showing hearing loss
- Displays a conductive deafness due to development of a chronic proliferative and suppurative OM

*Hardisty et al. JARO 4: 130-138 (2003)*
Jeff mice have a chronic suppurative otitis media.

Thickened mucoperiosteal lining and polyps in the middle ear in Jeff/+ mice with otitis media.
**Jeff is a mutation in the Fbxo11 gene**

**F-box**

75-115

**CASH**

334-470

**Q491L Jeff**

**CASH**

624-753

**S244L Mutt**

754-819

**ZnF**

**Jeff - non-conservative Glutamine to Leucine mutation**

<table>
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<tr>
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<td>Jeff Fbxo11</td>
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<td>F. rubripes</td>
<td>NALAGIQIRTNSCP</td>
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Expression of *Fbxo11* in middle ear

- E18.5 middle ear epithelia
- 4DAB middle ear epithelia
- 13DAB middle ear epithelia
F-box proteins are specificity factors for SCF ubiquitin ligases

- 74 mouse genes encoding F-box motifs
- 3 subsets:
  - FBXL - containing leucine-rich repeats
  - FBXW - containing WD40 motifs
  - FBXO - contain an F-box and another identifiable motif
Fbxo11 interacts with Spectrin βII

- **IP: Flag Fbxo11**
  - cos7

- **IP: Spectrin βII**
  - cos7

- **IP: SPβII**
  - Brain

- **WB: hFbxo11**

- **WB: Spectrin βII**

- **WB: hFbxo11**

- **WB: SPβII**

- **SPβII**
Spectrin βII is an essential adaptor protein for Tgfβ signalling via Smad3/Smad4

Disruption of Transforming Growth Factor-β signalling in ELF β-spectrin-deficient mice
Tang et al. Science 2003

Junbo
Ubiquitination?

Spectrin βII

Evi1

Smad3

Mucins?

Hypoxia?

Fbxo11

+Nf

p53

Neddylation

Fbxo11: apoptosis inhibitor

Gain-of-function: ?

Anti-proliferative; pro-apoptotic
Suppressor of inflammatory response

+/

+/

+/

An International Centre for Mouse Genetics
Hypoxia in Junbo mice

In vivo pimonidazole labelling of Jbo/+ and wild-type mice
Fbxo11

Ubiquitination?

Neddylation

Spectrin βII

p53

Evi1

Interaction between HIF-1α and SMAD3

Smad3

Mucins?

PHD2

HIF-1α

Anti-proliferative; pro-apoptotic
Suppressor of inflammatory response

Fbxo11: apoptosis inhibitor

Gain-of-function: ?

Therapeutic opportunity: PHD2 inhibitors and HIF-1α inhibitors?
Hypoxia, Hif-1α and OM

• Middle ear in OM is susceptible to hypoxia

• Key mediator of hypoxic response is HIF-1α

• Jeff and Junbo mutations may dysregulate protective mechanisms that enable middle ear to respond to inflammatory response under hypoxic conditions

• Smad3 is a co-factor of HIF-1α and HIF-1α is pro-inflammatory - dysregulation of Smad3 may compound the already raised levels of HIF-1α in the hypoxic middle ear, leading to runaway chronic inflammation
Association of *FBXO11* with chronic otitis media with effusion (COME) and recurrent otitis media (ROM)

Segade et al. *Arch Otolaryngol.* 2006

- Minnesota COME/ROM study - 142 families with multiple affected individuals
- 13 SNPs genotyped across 99kb region of *Fbxo11*
- 1 SNP showed nominal evidence of association to COME/ROM (p=0.02)
- Association of SNP confirmed using Pedigree Equilibrium Test; haplotypes incorporating this SNP also showed evidence of association (p=0.03 to 0.1)
Conclusions

• *Jeff* and *Junbo* are models for human OM, carrying mutations in the *Fbxo11* and *Evi1* genes respectively

• Association studies indicate a role for *Fbxo11* in genetic susceptibility for chronic OM in humans

• *Fbxo11* and *Evi1* cause OM through effects on signalling via *Smad3*, providing a common mechanistic route for the development of chronic OM that may involve dysregulation of hypoxic responses in the middle ear

• The effect of the *Jeff* mutation on p53 activity and p53’s role in OM remains to be determined

• The elaboration of genetic pathways provides new candidate genes for association studies
Acknowledgements

MRC Mammalian Genetics Unit, Harwell
Rachel Hardisty-Hughes  Michael Cheeseman
Hilda Tateossian Jiewu Yang
Nick Parkinson Sue Morse

Zuzanna Lalanne Rosario Romero
Alice Middleton Richard Gale
Debra Brooker Fran Mackenzie
Martin Fray Pete Glenister
Anne-Marie Woodward Sian Polley
Ivana Barbaric Hsun-Tien Tsai
Neil Dear Tertius Hough

GSK
Jackie Hunter
Challenges for the study of disease in the 21st century

- Characterise the function of every gene in the mammalian genome
- Generate mutations in every gene in the mouse genome
- Characterise the phenotype of every mutant mice
- Identify models of human disease