Update on research into retinal degeneration in dachshunds

Latest advice for breeders

Dachshund Breed Conference
29.11.2009

Keiko Miyadera, DVM
Registration to the Japan Kennel Club

Number of dogs per breed

- Miniature dachshund
- Chihuahua
- Toy poodle
- Shiz tzu
- Yorkshire terrier
- Pembroke corgi
- Papillon
- Labrador retriever
- Pomeranian
- Golden Retriever

All breeds
Common diseases in dachshunds

780 diagnoses, 1996-2005
Veterinary Medical Center, University of Tokyo, Japan

- IVDP 41%
- Others 38%
- Myelomalacia 3%
- Epilepsy 3%
- Hydrocephalus 3%
- Pelvic fracture 2%
- Panniculitis 2%
- Perineal hernia 2%
- Progressive retinal atrophy 3%
- Congenital cardiac disorder 1%
- Mammary gland tumor 1%
- Lymphoma 1%
Progressive retinal atrophy, PRA

- **Inherited disease** caused by a mutation in a certain gene
- Gradual **degeneration of the retina**
- Progressive loss of vision → **blindness**

The owner notices...

Dilated pupils
Decreased activity

Strong reflection from the eye
Bumping into objects

![Normal](image1.png) ![PRA](image2.png)
Retina

- Photoreceptors
  - **Rod**: night vision
  - **Cone**: day vision
- **RPE**
  (retinal pigment epithelium)
Clinical feature of PRA is variable

- **Age of onset**
  - Early-onset
  - Late-onset

- **Rate Progression**
  - Fast
  - Slow

- **Mode of inheritance**
  - Autosomal recessive
  - Autosomal dominant
  - X-linked recessive

- **Primary target of the retina**
  - Rod photoreceptor
  - Cone photoreceptor
  - RPE
...but uniform within a breed.

- **Age of onset**
  - Early-onset (6 months)
  - Late-onset

- **Rate Progression**
  - Fast (blind <2 years)
  - Slow

- **Mode of inheritance**
  - Autosomal recessive
  - Autosomal dominant
  - X-linked recessive

- **Primary target of the retina**
  - Rod photoreceptor
  - Cone photoreceptor
  - RPE

- **PRA in miniature dachshunds**
  - cone-rod dystrophy 1 (cord1)
Which dog breed gets PRA?

Akita
American Eskimo
Australian cattle dog
Australian kelpie
Australian shepherd
Basenji
Beagle
Belgian sheepdog
Belgian tervuren
Bernese mountain dog
Border collie
Boykin spaniel
Briard
Brussels griffon
Bull mastiff
Chesapeake Bay retriever
Chinese crested
Chinese Shar Pei
Cocker Spaniel, American
Collie
Coton de Tulear
Dachshund
English Cocker Spaniel
English setter
English springer spaniel
Entlebacher
Finnish laphund
German shepherd
German shorthaired pointer
Glen of Imaal terrier
Golden retriever
Gordon setter
Great Dane
Great Pyrenees, Pyrenean
mountain dog
Greyhound
Havanese
Irish setter
Irish wolfhound
Italian greyhound
Kuvasz
Labrador retriever
Lowchen
Maltese
Mastiff
Miniature pinscher
Miniature schnauzer
Norfolk terrier
Norwegian elkhound
Nova Scotia duck tolling
retriever
Old English sheepdog
Papillon
Pekingese
Pit bull terrier
Poodle
Portuguese water dog
Rottweiler
Samoyed
Schipperke
Schnauzer
Shetland sheepdog
Shih tzu
Siberian husky
Staffordshire terrier
Tibetan spaniel
Tibetan Terrier
Weimaraner
Welsh corgi Cardigan
Welsh springer spaniel
West highland white terrier
Yorkshire terrier
# Breed-specific PRA with known mutation

<table>
<thead>
<tr>
<th>Onset</th>
<th>Age</th>
<th>PRA type</th>
<th>Breed</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early onset</td>
<td>1.3 m</td>
<td>cone-rod dystrophy</td>
<td>Standard wirehaired dachshund</td>
<td>NPHP4</td>
</tr>
<tr>
<td></td>
<td>2-7 m</td>
<td>cd</td>
<td>Alaskan malamute</td>
<td>CNGB3</td>
</tr>
<tr>
<td></td>
<td>4 m</td>
<td>rcd1</td>
<td>Irish setter</td>
<td>PDE6B</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>rcd1a</td>
<td>Sloughi</td>
<td>PDE6B</td>
</tr>
<tr>
<td></td>
<td>4 m</td>
<td>rcd2</td>
<td>Collie</td>
<td>RD3</td>
</tr>
<tr>
<td></td>
<td>6 m</td>
<td>cord1</td>
<td>Miniature dachshund</td>
<td>RPGRI1</td>
</tr>
<tr>
<td></td>
<td>1 y</td>
<td>rcd3</td>
<td>Cardigan Welsh corgi</td>
<td>PDE6A</td>
</tr>
<tr>
<td></td>
<td>1.5 y</td>
<td>pd</td>
<td>Miniature schnauzer</td>
<td>PDC</td>
</tr>
<tr>
<td></td>
<td>1.5 y</td>
<td>csnb</td>
<td>Briad</td>
<td>RPE65</td>
</tr>
<tr>
<td>Late onset</td>
<td>2-3 y</td>
<td>XL PRA1</td>
<td>Samoyed, Siberian husky</td>
<td>RPGR</td>
</tr>
<tr>
<td></td>
<td>2.5-8 y</td>
<td>prcd</td>
<td>18 breeds (Labrador, poodle)</td>
<td>PRCD</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>AD PRA</td>
<td>English/bull mastiff</td>
<td>RHO</td>
</tr>
</tbody>
</table>
DNA test — *RPGRIP1* mutation

1. DNA extraction
   - Cheek swab or blood samples
2. Amplification
   - Exon2 of *RPGRIP1* (PCR)
3. Visualisation
   - Gel electrophoresis

**Visualisation: Gel Electrophoresis**

- **A**  
  - RPGRIP1+/+  
  - Clear

- **B**  
  - RPGRIP1-/-  
  - Affected

- **C**  
  - RPGRIP1+/+  
  - Carrier

**Mutant allele, -**

- ...ACAGGATGAAAAAAGGAAGCAACAGGATGAGATCAAA...

**Normal allele, +**

- ...ACAGGATGAGATCAAA...
**RPGRIP1** test results did not always match the PRA status

<table>
<thead>
<tr>
<th></th>
<th>Clear</th>
<th>Carrier</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control dogs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>87 (44%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>81 (40%)</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Clear</th>
<th>Carrier</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRA cases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>47 (80%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 (10%)</td>
<td>6 (10%)</td>
<td></td>
</tr>
</tbody>
</table>

Miniature dachshunds
Pets from Japan and UK

- 59 PRA cases
- 200 apparently normal controls
PRA onset is variable

- Early onset
  - 6 cases (10%)
- Late onset
  - 47 cases (80%)

Number of dogs

Age of PRA onset (years)

- Clear
- Carrier
- Affected

Early onset

Late onset
Clinical feature varied among *RPGRIP1*-/- dogs

### PRA cases
- Clear
- Carrier
- Affected

<table>
<thead>
<tr>
<th></th>
<th>Clear</th>
<th>Carrier</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

- 6 (10%)
- 6 (10%)
- 47 (80%)
Electroretinography (ERG)

- Electrical response of the retina to light stimulation
- The earliest sign of retinal degeneration
- Diagnosis of subclinical PRA
Decreased retinal response in apparently normal dogs

- Clear
- Carrier
- Affected

MLD10 (0.4y)
MLD6 (3.3y)
MLD13 (2.2y)
MLD1
9.2~10.6y
MLD2
2.7y
MLD7
9.4y
MLD5
9.4y
MLD11
3.8y
MLD6
7.5y
MLD18
9.8y
MLD19
9.8y
MLD13
3.7y
MLD15
11.6y
MLD21
<=5y
MLD3
7.8y
MLD9
6.6y
MLD4
2.4~5y
MLD12
4.3y
MLD14
3.3y
MLD10
4.7y
MLD8
4.2y
Progression of retinal degeneration in a family of $RPGRI\!P1^{-/-}$ dogs with varied...

Score

Normal
Abnormal ERG
Abnormal fundus
Blind

Age (year)
Cord1 in miniature dachshunds

1. The age of onset is variable
   - 3 months – 15 years

2. The mutation (RPGRIP1) does not always match with the actual eye condition
   - Some healthy dogs may be DNA tested as “affected”
   - Some PRA cases may be missed out in the DNA test
Does *RPGRIP1* mutation cause retinal degeneration?

— Likely.

• Even the dog looks **normal** to the owner, “**affected**” dogs show **reduced retinal response** at ERG

Why is the PRA onset different (**early, late, never**) among “**affected**” dogs?

— Additional factor modifies the effect of *RPGRIP1* mutation.

1) Environment

2) **Other genetic factor**
Search for a genetic modifier

- Different clinical group of “affected” dogs

<table>
<thead>
<tr>
<th>Cases</th>
<th>vs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-onset PRA</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Early-onset PRA</td>
<td></td>
<td>Late-onset PRA</td>
</tr>
<tr>
<td>Early-onset PRA</td>
<td></td>
<td>Normal + Late-onset PRA</td>
</tr>
</tbody>
</table>

- Genetic markers across the whole genome
- 22,000 single nucleotide polymorphisms (SNPs)
From **locus** to **gene** to **mutation**

**Mapped locus**
- 2,000,000 base pair
- 13 genes

Look for a sequence change that is
*in all the cases and not in the controls*

**Method**
- Massively parallel high through-put sequencing (underway...)

Conclusion

- At least **two genes** are involved in **early-onset PRA**
- **DNA test** availability
  1) *RPGRIP1* mutation — **Yes** (Animal Health Trust)
  2) Second locus — **Not yet** (sequencing underway)
- 1) + 2) → **Early-onset PRA**
- 1) only → **Late-onset PRA**
- Reducing *RPGRIP1* mutation reduces PRA
- Prevalence of the *RPGRIP1* mutation could be high
How do we make use of the DNA test to reduce *RPGRIP1* mutation?

**RPGRIP1 genotype**

<table>
<thead>
<tr>
<th>Dam</th>
<th>Sire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Carrier</td>
<td>Carrier</td>
</tr>
<tr>
<td>Affected</td>
<td>Carrier</td>
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<td>Carrier</td>
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</table>

**Reduce risk of early/late-onset PRA**

- **Avoid pairs that may produce “affected” puppies**

**Conserve genetic diversity**

- **Use variable dogs for breeding**
- **Allow pairs that produce “carrier” puppies**

**Use at least one “clear” parent**
Important facts

DNA test screens only the known mutation
- *RPGRIP1* DNA test “clear” ≠ free from any PRA
- Unknown mutation may cause PRA
- Get regular eye checks for the sires/dams

A dog is not only about a disease that can be DNA tested
- Other health issues
- Temperament
- (Looks)
Treatment – Gene therapy

PRA in Briard

- Early-onset, mutation in the RPE65 gene
- Normal RPE65 introduced to the retina using a virus vector
- Recovery of visual function (3 years +)

Acland et al. Molecular Therapy (2005).
Acknowledgements

University of Cambridge
David Sargan
Jesús Aguirre-Hernández

University of Tokyo
Kumiko Kato
Kyohei Morimoto
Clinical staffs

Animal Health Trust
Cathryn Mellersh
Keith Barnett
Nigel Holmes
Mike Boursnell

Dachshund owners & breeders
Call for participants

- Dachshund
  - diagnosed with PRA
  - the DNA test result for the \textit{RPGRIp1} mutation did not match the actual eye condition

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Received 22 May 2009 | Accepted 28 October 2009 | Published 11 November 2009

\textbf{Phenotypic variation and genotype-phenotype discordance in canine cone-rod dystrophy with an \textit{RPGRIp1} mutation}

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http://www.molvis.org/molvis/v15/a246/