Phenotypic Characterizations and Genetic Study of Progressive Rod-Cone Degeneration in Poodles in Thailand

Metita Sussadee1, 2 Janjira Phavaphutanon3 Chaiyan Kasornthorkbu4 Areerat Kongcharoen5 Preenun Jitasombuti6 Sudtisa Laopiem7 Aree Thayanuphat3*

Abstract

Progressive rod-cone degeneration (PRCD) is one of several groups of inherited progressive retinal atrophy in dogs. This study aims to describe the characterization of phenotypic appearance and demonstrate the responsible gene mutation, including analysis of the allele frequency of PRCD in Poodle dogs in Thailand. All 10 Poodles with clinical signs of PRCD were identified by a history of progressive vision loss, an abnormal obstacle test, and fundic appearance. Genetic testing of PRCD gene mutation using the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism technique was performed in all dogs. The result confirmed that all 10 dogs were affected. The affected Poodles had presented age at first examination at 8.9 ± 2.33 years. Typical findings of retinal changes were bilateral retinal degeneration with tapetal hyperreflectivity, retinal vessel attenuation and optic disc atrophy. The electroretinogram from 7 affected dogs revealed non-detectable wave amplitude and implicit time in the session of rod responses in all dogs whereas small wave amplitude of cone response was recorded in 4 dogs. Histopathological examination of an affected eye revealed the collapse of the nuclear and photoreceptor layers. A prevalence of carriers in 50 Poodles was 12% while the allele frequency of mutant allele was 0.1. Genetic finding of PRCD in Poodles in Thailand corresponds to the previous report whereas the age onset of dogs presented from this disease and the age at complete blindness are much later than previously reported.

Keywords: electroretinography, gene mutation, Poodle, progressive rod-cone degeneration

1Center for Agricultural Biotechnology, Kasetsart University, Kamphaengsaen Campus, Nakhonpathom 73140, Thailand
2Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand
3Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Nakhonpathom 73140, Thailand
4Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
5Veterinary Teaching Hospital, Faculty of Veterinary Science, Mahidol University, Nakhonpathom 73170, Thailand
6Department of Surgery and Theriogenology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
7Center of Veterinary Research and Service, Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen Campus, Nakhonpathom 73140, Thailand

*Correspondence: fvetatn@ku.ac.th

Introduction

Progressive rod-cone degeneration (PRCD) is the most common form of late onset progressive retinal atrophy (PRA) which has been described in more than 100 domestic dog breeds worldwide (Acland et al., 1998; Petersen-Jones, 2005; Andre et al., 2008). This autosomal recessive photoreceptor degeneration has revealed that the point mutation of guanine to adenine substitution in the second codon of the exon1 of PRCD gene of canine chromosome 9, a region orthologous to the telomeric end of human chromosome 17, was the cause of disease. The mutation resulted in amino acid change from cysteine to tyrosine (TGC→TAC) which was identified in humans with autosomal recessive retinitis pigmentosa (Zangerl et al., 2006). Expression studies of this gene indicated the mainly expression in the retina with equal expression in the retinal pigment epithelium, photoreceptor, and ganglion cell layers (Zangerl et al., 2006). Affected dogs and human patients showed similar clinical signs of night blindness and eventually followed by total blindness because the disease degeneration usually began as a rod abnormality before the cone. Diagnosis of PRCD is based on clinical signs, fundic examination, histopathology, electroretinography (ERG), and molecular genetic testing (Acland et al., 1998; Aguirre and Acland, 2006; Ofri, 2008).

There are many phenotypic variation of clinical diagnosis and there has never been an in depth study and survey on PRCD of dog breeds in Thailand. The allele frequency of PRCD gene in affected dog breeds has been published only in Czech Republic (Dostal et al., 2011). Moreover, the Poodle is among 18 dog breeds (Zangerl et al., 2006) that affected with PRCD resulting in blindness, and causing stress for their owners. This report describes Poodle dogs with clinical signs of PRCD speculated by a history of vision loss, scotopic and photopic obstacle tests, ocul relex tests, and fundic examination. Electroretinography and molecular testing of responsible gene mutation was performed. In addition, survey of allele frequency of the disease in Poodles in Thailand from May 2012-Jan 2013 was processed. These databases of disease diagnosis and genetic frequency will be beneficial for veterinarians and breeders for future diagnosis and proper awareness with this disease.

Materials and Methods

Animal selection: Seventy Poodle dogs were recruited for the present study. Ten normal dogs and ten dogs previously diagnosed with PRA based on ophthalmic examination and a history of visual impairment in dim light were included for characterization of PRCD gene mutation. For analysis of allele frequency, 50 Poodles at the age between 6 months to 12 years were randomly selected from dogs presented at Kasetsart University Veterinary Teaching Hospital with an unknown history.

Ophthalmic examination and vision test: Vision of 10 normal and 10 PRCD-suspected dogs was examined by a photopic/scotopic obstacle test. Complete ophthalmic examination was performed including menace response, dazzle reflex and pupillary light reflex. The anterior segment was examined using a portable slit lamp biomicroscope (SL-15, Kowa Optimed, Japan). Intraocular pressure was measured using a tonometer (TonoVet, iCare, Finland). Fundus pictures were captured with a binocular indirect ophthalmoscope (Vantage, Keeler Ltd, UK) in a dark room after electroretinographic examination.

Electroretinography: The ERGs were recorded from ten normal and seven PRCD-suspected dogs based on the owner allowance. All dogs were fasted for at least 10 hours application of topical anesthetic eye drops (0.5% tetracaine hydrochloride ophthalmic solution, Alcon, Belgium) and the artificial tear solution (Methocel 2%, OmniVision, Germany) between the corneal surface and the contact lens. The needle electrodes were inserted subcutaneously at 3 cm caudal to the lateral canthi and over the external occipital protuberance, as reference and ground, respectively. The signals from all electrodes were amplified with a bandpass filter by a preamplifier of the Handheld Multi-species ElectroRetinoGraph (HMsERG, Xenotec, USA). Placing electrodes was performed

Table 1 Sex, age onset noticed by the owners, age at first presentation, age at study and results of obstacle test at the study of PRCD-suspected Poodles

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Sex</th>
<th>Age onset (year)</th>
<th>Age at first presentation (years)</th>
<th>Age at the study (years)</th>
<th>Obstacle test at the study</th>
<th>Cataract stage at the study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scotopic</td>
<td>Photopic</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>11</td>
<td>13</td>
<td>15</td>
<td>Neg</td>
<td>Poor</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>Poor</td>
<td>Pos</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>Poor</td>
<td>Pos</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>Neg</td>
<td>Poor</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>Neg</td>
<td>Poor</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>Neg</td>
<td>Poor</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>Neg</td>
<td>Poor</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>10</td>
<td>11</td>
<td>13</td>
<td>Neg</td>
<td>Poor</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>Poor</td>
<td>Pos</td>
</tr>
</tbody>
</table>

Mean±SD 7.9±2.18 8.9±2.33 9.8±2.94

Neg means negative, Pos means positive, - means no cataract formation
under light room condition. The recording room was quiet and free from electrical noise. The HMsERG flash stimulator was placed close to, but not touching, the dog’s eye when making a test to minimize scatter rays. Impedance and baseline tests were performed before ERGs recording. These procedures would help for evaluation of noise levels in the environment. The ERGs were recorded automatically in dark condition using the standard protocol, Dog Diagnostic, recommended by the European College of Veterinary Ophthalmologists (Narfström et al., 2002).

**Histopathological examination:** Only the left eye of a PRCD-suspected dog (Dog 1) was enucleated with the owner permission. The eye was fixed in Bouin’s solution overnight. The tissue was washed 6-8 hours in cold running tap water and dehydrated in 70% ethanol. The eye was subsequently embedded into paraffin blocks and sectioned into 4-μm thickness on a semi-automatic microtome at the level of the optic nerve and stained with hematoxylin and eosin (H&E) stain. The tissue sections were microscopically examined with bright-light microscopy.

**DNA testing:** Individual genomic DNA of 10 normal and 10 dogs with clinical signs of PRCD was extracted from 2 ml of peripheral blood in EDTA using the Phenol/Chloroform protocol DNA extraction. DNA quality and concentration were determined by a Spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). The 346 bp DNA fragment of exon 1 of the PRCD locus was amplified using published primers sequence (Gentilini et al., 2009), forward (5’AGCCCTCTAAATCCAGTGG 3’) and reverse (5’GTGCTCTGATGGGAAACC3’). Amplification conditions were 94 °C (2 min), followed by 45 cycles at 94 °C (15 sec), 55 °C (20 sec), 68 °C (20 sec), and final extension at 72 °C (10 min). Agarose gel (1.5%) electrophoresis and the gels screened under UV light were performed to confirm the PCR products. Then the PCR product was digested with restriction enzyme in specific DNA sequence (GT/AC) using Rsal endonuclease (FastDigest, Thermo Fisher Scientific, USA). 2% agarose gel electrophoresis of digested solution was performed to verify the disease status. To confirm the specific gene amplification, the amplified PCR products of 10 affected Poodle dogs and 10 normal
Poodle dogs were purified with PCR-clean up kit and directly sequenced using a standard protocol. All sequenced DNA fragments were analyzed with the published sequence of PRCD gene (EMBL Accession number: DQ390331.1).

Analysis of allele frequency in 50 Poodles was performed using the same technique of DNA extraction and PCR-RFLP. The allele frequency was determined from the genotype as homozygous normal, heterozygous and homozygous mutant.

Results

Clinical Characterization of PRCD-suspected Poodles: All PRCD-suspected dogs were brought for ocular examination due to visual deficit. Both sexes were presented equally. Age onset of visual impairment noticing by the owners was 7.9 ± 2.18 (5-11) years (Table 1). Only one dog had completely loss of vision at first visit (Dog 1). This dog had a history of progressive loss of vision starting at night time. The other affected dogs remained partial vision, at least in the photopic condition. The mean age for all dogs at first examination was 8.9 ± 2.33 (6-13) years. Seven suspected dogs (Dog 1, 2, 3, 6, 7, 9 and 10) had visual problems for greater than or equal to 1 year before being brought for diagnosis. Only 3 affected dogs (Dog 4, 5 and 8) were promptly brought for ocular examination after noticing their dogs’ visual problem. Fundic appearance of all PRCD-suspected dogs included with tapetal hyperreflectivity, retinal vessel attenuation, and optic disc atrophy (Fig 1). Seven PRCD-suspected dogs had cataract at the incipient or immature stage at the study day. Five dogs (Dog 4, 5, 7, 9 and 10) had incipient cataract at the first presentation and showed immature stage at the study (Fig 2).

Electroretinography: ERG record consisted of 9 sessions of photoreceptor stimulation (Fig 3). ERGs of Dog 3 (10-year-old) and Dog 7 (8-year-old) showed a marked reduction of a- and b-wave amplitudes in all stimulation compared to ERG recordings in an 8-year-old normal Poodle. In the session of low light intensity stimulation (Fig 3 a-e), ERGs of Dog 3 showed extremely small in the amplitude and delay in the implicit time of b-wave while ERG waveform of Dog 7 was not recordable. Attenuated waveforms of both PRCD-suspected dogs were recorded when stimulated with high light intensity and flicker flash after 10 minutes of light adaptation. ERGs from Dog 5, 6, 9 were similar to Dog 7 while Dog 4 still had attenuated ERG waveform for rod response as Dog 3. Waveforms in Dog 1 revealed flat lines of all ERG recordings. (data not shown)

Histopathological examination: An enucleated eye from a PRCD-suspected dog (Dog 1) was microscopically examined under hematoxylin & eosin stain. The major lesions were confined to the retina. Thinning of the retina was profound, and characterized by collapse of the nuclear and photoreceptor layers, with loss of ganglion cell layer where few ganglion cells remained. The complete loss of the photoreceptor nuclei were noted (Fig 4).

Genetic testing: To identify the result, PCR product (346 bp) of wild type allele (TGC) was not digested by restriction endonuclease while mutant allele (TAC) was digested into two bands (219 bp and 127 bp) when separated in agarose gel electrophoresis. All patterns of DNA fragments showed the PRCD-PRA status; lane 1 and 2 were carrier and affected dog (Dog 1) respectively, lane 3, 4, 5 and 6 were normal dogs.

Figure 4 The retina adjacent to the optic nerve of an 8-year-old poodle dog (Dog 1) showed retinal degeneration characterized by thinning of the retina, collapse of the nuclear and photoreceptor layers (*), presence of gliosis (arrow), and few remaining ganglion cells (arrowhead); H & E, 40x.

Figure 5 The PCR products after digested with RsaI restriction enzyme that were separated in 2% agarose gel electrophoresis. The pattern of DNA fragments showed the PRCD-PRA status; lane 1 and 2 were carrier and affected dog (Dog 1) respectively, lane 3, 4, 5 and 6 were normal dogs.
12% (6/50) respectively. The allele frequency of normal and mutant allele was 0.9 and 0.1 respectively.

Discussion

The present study showed a variable onset (5-11 years) of PRCD in affected Poodles based on clinical observation. The range of the age onset was surprisingly much greater than previously reported, 3-5 years old (Petersen-Jones, 1998). Indeed, the entire age onset investigated in this study obtained from observation by the owners when their dogs had suffered from the progressing disease. Since the loss of vision only in dim light might be difficult to detect by the owners and the loss of vision in PRCD Poodles was slowly progressed, disease onset reported by the present study was possible much later than the actual onset. However visual impairment in dim light which was an early sign of the disease in Dog 3, 4, 10 was first recognized by the owners and confirmed by an experience veterinarian at the age of 9, 11, 7 years respectively. These results implied that much later age onset could be expected in clinical practice than the previous report.

Our observations revealed longer remained vision than the previous report which stated that complete blindness in Poodles usually occurred between 5-6 years (Acland et al., 1990). Only one dog (Dog 1) in the present study had completely loss of vision. Dog 3, 4 and 10 (10, 13 and 9 years of age respectively) remained normal vision in bright light and had visual impairment in dim light at study. The previous report was studied on dogs which most were maintained in the same laboratory animal facility. Variable of phenotypic severity between this study and the previous report might be determined by genetic and environmental modifiers (Aguirre and Acland, 2006). However, the rate of progression was believed to be faster in young affected dogs than in disease that developed later in life (Narström and Petersen-Jones, 2007).

ERG could be used to determine the progression of the diseases. Our ERG results corresponded well to stages of the disease. ERG was wildly used to detect PRA when cataract obscured fundus (Sandberg et al., 1986). However, for the electroretinographic diagnosis, normal parameters of ERG should be established. Because the results of recording can vary by many factors such as types of recording equipment, anesthetic protocols, environmental factors, intrinsic factors of breed, and age ranges of animals (Ofri, 2002).

In PRA, the retinal tissue slowly died and released toxic by-products of cell death that were absorbed by the lens, causing lens damage and cataract development (Babizhayev and Deyev, 1989). Cataract formation in the present study was observed in quite a high number (7/10 dogs) when compared to a previous reported which revealed the concurrent cataract in 44% of dogs with PRA. However, that report mainly studied in Miniature Schnauzer which was accounted for 50% of all PRA cases (Park et al., 2009). A study in Republic of Korea surveyed the age at cataract formation revealed the mean age onset of cataract in the small dogs breed at 8.3±3.9 years. These included the common presumed etiologies of breed predisposition (53.9%), aging (23.2%) and PRA (7.6%), (Park et al., 2009). However, the age onset in affected Poodles in this study was quite late and Poodle is a breed predisposed to cataract formation. Therefore the cataract formation in these Poodles might result from aging, hereditary, disease condition, or a combination of any causes.

The mutation allele frequency of Poodle (0.1), a popular breeds in Thailand, corresponds to the mutation allele of Miniature Poodle (0.2) which was reported in Czech Republic gene mutation in 17 domestic dog breeds (Dostal et al., 2011). This study revealed a correspondence of the phenotypic characterization observed from obstacle test, ophthalmoscopy, and electroretinography in retinal degeneration to genotypic characterization in Poodle dogs in Thailand. Clinical observation revealed that the age onset and remained vision was longer than expected. However, genetic defect of PRCD-PRA of Poodles in Thailand corresponded to the previous report (Zangerl et al., 2006). It is for the first time that the molecular testing has been applied to this disease in Thailand. Relatively high prevalence of the PRCD-carrier in Poodles (12%) indicated the beneficial of the genetic testing to identify PRCD-PRA status before breeding to avoid the transmission of affected allele to offspring.
Acknowledgements

The authors would like to thank Center for Agricultural Biotechnology, Kasetsart University and Center for Agricultural Biotechnology: (AG-BIO/PERDO-CHE) for financially supporting part of this study.

References


บทคัดย่อ

ลักษณะแสดงออกและการศึกษาทางพันธุกรรมของโรคจอประสาทตาเสื่อมในสุนัขพันธุ์พุดเดิลในประเทศไทย

เมทิดา สัสดี 1, 2 จันทร์จิรา ภวภูตานนท์ 3 ไชยยันต์ เกษรดอกบัว 4 อารีรัตน์ คงเจริญ 5 ปรีณัน จิตะสมบัติ 6 สุดธิษา เหล่าเปี่ยม 7 อารีย์ ทยานานุภัทร์ 3*

โรค progressive rod-cone degeneration (PRCD) เป็นรูปแบบหนึ่งของกลุ่มโรคจอประสาทตาเสื่อมทางพันธุกรรมในสุนัข การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาลักษณะที่แสดงออกและระบุยีนที่เกี่ยวข้องกับการกลายพันธุ์จอประสาทตาเสื่อมในสุนัขพันธุ์พุดเดิลในประเทศไทย โดยทำการศึกษาในสุนัขพันธุ์พุดเดิลจำนวน 10 ตัวที่มีประวัติการมีอาการละเอียดบางแบบค่อนข้างถี่ คือความผิดปกติในการเดินผ่านสิ่งกีดขวางและความผิดปกติจากการมองเห็น มีการระบุยีนกลายพันธุ์ด้วยเทคนิค PCR-RFLP บนเนื้อเยื่อตัวต่าง ๆ ผลการศึกษาทั้งหมดยืนยันการเป็นโรคของสุนัขทั้ง 10 ตัว สุนัขป่วยเริ่มเข้ารับการตรวจที่อายุเฉลี่ย 8.9 ± 2.33 ปี ลักษณะความผิดปกติของการมองเห็นทางแสงก็ส่งผลกระทบต่อการเดินผ่านสิ่งกีดขวางและมีความเสื่อมข้อมูลจอประสาทตา ผลการบันทึกคลื่นสัญญาณของเซลล์รับแสงชนิด rod ในสุนัขทั้ง 10 ตัว ไม่สามารถบันทึกคลื่น amplitude และ implicit time ได้ เมื่อกระตุ้นการทำงานของเซลล์รับแสงชนิด cone ในสุนัขป่วย 4 ตัว ผลการตรวจทางจุลทรรศน์ในสุนัขป่วย 1 ตัวพบความเสื่อมข้อมูลเซลล์รับแสง คลื่นจอประสาทตา 7 ตัว พบไม่สามารถบันทึกคลื่น amplitude และ implicit time ได้ เมื่อกระตุ้นการทำงานของเซลล์รับแสงชนิด rod ในสุนัขป่วยทุกตัว กะลายน้ำที่สามารถบันทึกคลื่น amplitude ได้เพียงเล็กน้อยเมื่อกระตุ้นการทำงานของเซลล์รับแสงชนิด cone ในสุนัขป่วย 4 ตัว ผลการตรวจทางจุลทรรศน์ในสุนัขป่วย 1 ตัวพบความเสื่อมข้อมูลเซลล์รับแสง คลื่นจอประสาทตา 7 ตัว พบไม่สามารถบันทึกคลื่น amplitude และ implicit time ได้ เมื่อกระตุ้นการทำงานของเซลล์รับแสงชนิด rod ในสุนัขป่วยทุกตัว กระลายน้ำที่สามารถบันทึกคลื่น amplitude ได้เพียงเล็กน้อยเมื่อกระตุ้นการทำงานของเซลล์รับแสงชนิด cone ในสุนัขป่วย 4 ตัว

คำสำคัญ: การบันทึกคลื่นจอประสาทตา ลักษณะพันธุ์ พุดเดิล โรคจอประสาทตาเสื่อมทางพันธุกรรม

1ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตก าแพงแสน จ. นครปฐม 73140
2ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตก าแพงแสน จ. นครปฐม 73140
3ภาควิชาเวชศาสตร์คลินิกสัตว์เลี้ยง คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตก าแพงแสน จ. นครปฐม 73140
4ภาควิชาวิทยาการสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน กรุงเทพฯ 10900
5โรงพยาบาลสัตว์ประชุภูมิ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล จ. นครปฐม 73170
6ภาควิชาวิทยาการสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ บางเขน กรุงเทพฯ 10900
7หน่วยงานชันสูตรโรคสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตก าแพงแสน จ. นครปฐม 73140
*ผู้รับผิดชอบบทความ E mail: fvetatn@ku.ac.th