Evaluation of three canine γ-crystallins (CRYGB, CRYGC, and CRYGS) as candidates for hereditary cataracts in the dachshund

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Purpose: We analyzed the γ-crystallin genes CRYGB, CRYGC, and CRYGS in the dog and tested single nucleotide polymorphisms (SNPs) for linkage and association with primary noncongenital cataract (CAT) in the dachshund, a popular dog breed. The crystallin genes may be involved in the pathogenesis of canine CAT as shown in humans and mice.

Methods: We sequenced all exons and their flanking intronic regions of the CRYGB, CRYGC, and CRYGS genes and in addition, the complete cDNA of these three genes using lens tissue from CAT-affected and unaffected dogs of several breeds. After examining BLASTN analyses, we compared the gene structure with the predicted genes in the current dog genome assembly and the orthologs of humans and mice.

Results: The search for SNPs within these crystallin genes revealed a total of five polymorphisms. As both CAT-affected and unaffected dogs shared identical haplotypes, there was no cosegregation of the SNP alleles with the affected animals. Expression did not differ among CAT-affected and unaffected dogs.

Conclusions: The polymorphisms reported for CRYGB, CRYGC, and CRYGS can be excluded as causative mutations for the CAT phenotype in the wire- and smooth-haired dachshund. The canine cataract gene orthologs described here may serve as a valuable resource for further studies in other dog breeds to develop a canine model. Many different dog breeds are affected by CAT. The use of the SNPs presented in this paper can facilitate the screening of more dog breeds.

Primary hereditary cataracts are common in purebred dogs, affecting over 120 breeds. Cataracts frequently cause visual impairment and are a major cause of blindness in dogs [1-6]. Inheritance of noncongenital cataracts has been demonstrated in several dog breeds, e.g., the golden and labrador retrievers [7,8], German shepherd [9], West Highland white terrier [10], American cocker spaniel [11], Tibetan terrier [12], Afghan hound [13], standard poodle [14,15], and the Entlebucher mountain dog [16]. As the dachshund is a breed predisposed to primary noncongenital cataract (CAT), it is assumed that these cataracts are also hereditary [2,4].

Dachshunds are bred in three coat varieties (long-haired, smooth-haired, and wire-haired) and three different sizes (standard, dwarf, and rabbit). Dwarf- and rabbit-sized dachshunds are referred to in this investigation as miniature dachshunds. The prevalence of CAT in the long-haired dachshund in North America is 2.10% [6]. In Germany, the prevalence has been estimated to be 3.21% for the long-haired, 1.87% for the smooth-haired, and 4.80% for the wire-haired dachshund [17]. In an animal threshold model, the heritabilities for CAT were 0.39±0.06 (wire-haired), 0.08±011 (long-haired), and 0.72±0.28 (short-haired) [17].

The transparency and high refractive index of the eye lens is achieved by a regular arrangement of the lens fiber cells and by a high concentration and the supramolecular organization of the lens-specific proteins, the crystallins, within each fiber cell [18].

The crystallin proteins are the major structural components of the eye lens that constitute 80-90% of its soluble proteins. These proteins are divided into three classes, α-, β-, and γ-crystallins, which form two protein superfamilies: the α-crystallin superfamily and the β-/γ-crystallin superfamily [19,20]. The common characteristic of the β-/γ-superfamily is a unique folding structure, the Greek-key motif. Each of the β- and γ-crystallins has two domains, with each domain being composed of two extremely stable protein structures, the so-called Greek-key structural motifs. These structures allow a dense packing of proteins in the ocular lens [21,22]. Any structural alterations of these proteins can disturb the highly ordered tissue architecture and can lead to opacity. Because of that, the genes that encode these proteins are obvious candidate genes for cataracts.

The γ-crystallins are encoded by the CRYG genes. Six members of the CRYG family (CRYGA-CRYGF) are located in a cluster on mouse chromosome 1 [23-25] and on human chromosome 2, respectively [26-28]. The seventh CRYG gene, CRYGS, maps to mouse chromosome 16 and human chromosome 3 [29,30]. Also in the dog, the CRYG genes are located in a cluster on dog chromosome (CFA) 37 with the exception of CRYGS, which maps to CFA34 [31]. In mice, mutations identified in different CRYG genes are known to cause dominant or recessive cataracts [32-38]. Also in humans, several hereditary cataracts have been shown to be caused by mutations in the CRYG genes [39-43]. In this report, we provide the complete sequence and the genomic sequences of all ex-
ons of \textit{CRYGB}, \textit{CRYGC}, and \textit{CRYGS}. In addition, we test single nucleotide polymorphisms for linkage and association in dachshunds and compare expression of mRNA in lenses of dogs affected by primary cataract and an unaffected control dog.

**METHODS**

\textit{Animals, phenotypic data, and DNA specimens:} Ophthalmological data for the dachshunds were provided by the Dortmunder Kreis (DOK), which is the German panel of the European Eye Scheme for diagnosis of inherited eye diseases in animals. The German Dachshund Club 1888 e.V. (DTK) supplied pedigree data and we identified pedigrees with multiple CAT-affected dogs. For the present analysis, we chose 24 dogs from four different dachshund families. Most of the animals (14 dogs) came from a standard-sized wire-haired family, whereas seven wire-haired miniature dachshunds were from two different families. The other three dogs were smooth-haired standard-sized dachshunds. Altogether, this study included 17 CAT-affected dachshunds. The signs of CAT in these dogs differed in regard to stage. In seven dogs an immature cataract was diagnosed, in eight dogs an incipient cataract, and in two dogs a mature cataract. Most of the affected dogs included in the analysis had an opacification localized in the cortex of the lens (82.4%). Lens opacity was additionally found in the capsule (one dog) and the nucleus (five dogs). In one dog only the nucleus was affected; two dogs showed only capsular opacifications. Both eyes were affected in ten animals, while alterations were found only in the lens of the left eye in the other seven. Most of the dogs (about 70%) were examined two to three times. CAT was first diagnosed at a mean age of 3.67±2.14 years. At least one unaffected dog was investigated from each family. The seven unaffected dogs were between 5.1 and 9.7 years old at the last ophthalmological examination.

We also tested four unaffected dogs from other breeds (dalmatian, German shepherd mix, Hanoverian hound, great dane) as control animals.

Two milliliters of heparinized blood were obtained from each dog, and DNA was extracted using QIAamp 96 DNA Blood kit (Qiagen, Hilden, Germany).

For cDNA analysis of the three genes, we used lens tissue of seven dogs of six different breeds (mixed breed, German shepherd dog, dachshund mix, Jack Russell terrier, Tibetan terrier, and Yorkshire terrier). Six of the seven dogs underwent cataract surgery; one dog (mixed breed) with normal vision.

\begin{table}

\begin{tabular}{|c|c|c|c|c|}
\hline
Gene & Target & Primer & Sequence (5’ – 3’) of primers & \textbf{Annealing temperature (°C)} & \textbf{Product size (bp)} \\
\hline
\textbf{CRYGB} & Exon 1 and Exon 2 & CRYGB_Ex1_Ex2_F & TGGTTTAAATGGCCTTGGAG & 58 & 929 \\
& & CRYGB_Ex1_Ex2_R & AAGCAAGCACCACAGAGTC & & \\
& Exon 3 & CRYGB_Ex3_F & TGGGAAGCAAACCTAGACTCC & 58 & 577 \\
& & CRYGB_Ex3_R & TCCCCCCTAGAGACAGTTTTTC & & \\
\textbf{CRYGC} & Exon 1 and 5’ UTR & CRYGC_Ex1_F & CACTAAGAATCCAAATAAAAAGCAAC & 58 & 390 \\
& & CRYGC_Ex1_R & CGTAGAAGGTGATCTCAGCAAG & & \\
& Exon2 & CRYGC_Ex2_F & AAGGTAGGCGGGAGTACAG & 58 & 494 \\
& & CRYGC_Ex2_R & CGTGGCTTGTGCATTTGGTC & & \\
& Exon 3 and 3’ UTR & CRYGC_Ex3_F & ACCACAGCGCATCCTGAGCTC & 58 & 577 \\
& & CRYGC_Ex3_R & CATTTCATTTTCGAGGCTTC & & \\
\textbf{CRYGS} & Exon 1 and 5’ UTR & CRYGS_Ex1_F & TCAATAGCCCTCTAAATACGACTGACT & 58 & 290 \\
& & CRYGS_Ex1_R & GCACATGGAAAGGAGGAAAC & & \\
& Exon2 & CRYGS_Ex2_F & GCCAGAGGATAGGTTGTGT & 58 & 495 \\
& & CRYGS_Ex2_R & GGGAGGAGTGGGAAAG & & \\
& Exon 3 and 3’ UTR & CRYGS_Ex3_F & CATGCTGTTCCTGCGAGTT & 58 & 468 \\
& & CRYGS_Ex3_R & AGCCATTACAGTCACACAGT & & \\
\hline
\textbf{CRYGB} & cDNA CRYGBå & CRYGB_F & CATGGAAGAAGATCACTTCCTTAC & 58 & 567 \\
& & CRYGB_R & TTGATTTCTAAAAGGAAACAGTG & & \\
\textbf{CRYGC} & cDNA CRYGC** & CRYGC_F & GCCAGTGGCAGCTGACT & 603 & \\
& & CRYGC_R & TTAGGTCAGACTGACTGAAAT & & \\
\textbf{CRYGS} & CRYGS_F & ACCATCTATGCAACAAATGG & & 645 \\
& & CRYGS_R & GCCAATTGTTTTITATTTAGT & & \\
\hline
\end{tabular}

\caption{γ-Crystallin PCR primers}

Shown are the PCR primers for the amplification of genomic canine \textit{CRYGB}, \textit{CRYGC}, and \textit{CRYGS} exons with their flanking intronic regions and PCR primers for the amplification of the cDNA of the canine \textit{CRYGB}, \textit{CRYGC}, and \textit{CRYGS} genes. The asterisk indicates that the forward primer is located 1 bp upstream of the start codon and the reverse primer is located 38 bp downstream of the stop codon. The double asterisk indicates that the forward primer is located 39 bp upstream of the start codon and the reverse primer is located 39 bp downstream of the stop codon. The sharp (hash mark) indicates that the forward primer is located 8 bp upstream of the start codon and the reverse primer is located 90 bp downstream of the stop codon.

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lenses was used as reference. The cataract surgery was done using the phacoemulsification method with ultrasound. After removal from eye, the lens tissue was conserved using RNA-later solution (Qiagen). The RNA was extracted from dog lens tissue using the Nucleospin RNA II-Kit (Macherey-Nagel, Düren, Germany) and transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany).

Structural analysis of the canine CRYGB, CRYGC, and CRYGS gene: We searched the dog-expressed sequence tag (EST) archive for ESTs by cross-species BLAST searches with the corresponding human reference mRNA sequences for CRYGB (NM_005210), CRYGC (NM_020989), and CRYGS (NM_017541). We found a canine EST (DN866034) isolated from dog lens tissue with 88% identity to the human CRYGB mRNA sequence. A significant match to this canine EST was identified on canine chromosome 37 (NW_876304.1|Cfa37_WGA83_2) by means of BLASTN searches of this canine EST against the dog genome assembly (Dog genome assembly 2.1).

For CRYGC we found a canine EST (DN867687) with 87% identity to the human mRNA sequence. A significant match to this canine EST was identified on canine chromosome 37 (NW_876304.1|Cfa37_WGA83_2) [44].

We also found a canine EST (DN867380) isolated from beagle lens tissue with 90% identity to the human CRYGS mRNA sequence. A significant match to this canine EST was identified on canine chromosome 34 (NW_876301.1|Cfa34_WGA80_2) by means of BLASTN searches of this canine EST against the dog genome assembly (Dog genome assembly 2.1). The genomic structure of the canine CRYGB, CRYGC, and CRYGS genes were determined with the Spidey mRNA-to-genomic alignment program. We verified the canine ESTs by sequencing the cDNA of all three genes isolated from the lens of seven dogs. The primers were designed in such a way that the open reading frames of the three genes were amplified. All reverse primers were located downstream from the stop codons of the three genes. The forward primers included the start codon (CRYGB and CRYGS) or were located a few bases upstream of the start codon (CRYGC; Table 1).

Mutation analysis: For evaluation of CRYGB, CRYGC, and CRYGS as candidate genes for CAT in the dachshund, we sequenced all exons and their flanking intronic regions of the three genes for the animals mentioned above (Table 1). All PCRs were performed in 50 µl reactions using 50 pmol of each primer, 100 µM dNTPs, 2 U Taq-DNA-Polymerase (Q-BIOgene, Heidelberg, Germany) in the reaction buffer supplied by the manufacturer, and 10X PCR Enhancer (Invitrogen, Karlsruhe, Germany) for 2 µl template DNA or cDNA, respectively. The PCR conditions were as follows: 95 °C for 4 min followed by 34 cycles of 94 °C for 30 s, annealing temperature of 58 °C for 45 s, 72 °C for 45 s, and 4 °C for 10 min. All PCR products were cleaned using the Nucleo-Fast PCR purification kit (Macherey-Nagel) and directly sequenced with the DYEnamic ET Terminator kit (Amersham Biosciences, Freiburg, Germany) and a MegaBACE 1000-capillary sequencer (Amersham Biosciences). PCR primers were generated with the Primer3 program based on the canine ESTs (DN866034, DN867687, and DN867380) and the canine genomic sequences (NW_876304.1|Cfa37_WGA83_2, NW_876304.1|Cfa37_WGA83_2, NW_876301.1|Cfa34_WGA80_2, and NW_876301.1|Cfa34_WGA80_2). Sequence data were analyzed with Sequencher 4.7 (GeneCodes, Ann Arbor, MI).

| Table 2. SNP analysis |
|----------------------|
| **Position of gene (Mb)** | **SNP** | **HET (%)** | **PIC (%)** | **Z-mean** | **Z-mean** | **P** | **χ²** | **P** | **P** | **χ²** | **P** |
| CRYGB on CFA37 19.440-19.442 | LOC488947:g.2537C>A | 45.8 | 31.7 | 0.32 | 0.4 | 0.16 | 0.2 | 0.02 | 0.88 | 3.17 | 0.20 |
| | LOC488947:g.4348T>C | 26.1 | 24.6 | 0.32 | 0.4 | 0.16 | 0.2 | 0.23 | 0.63 | 2.83 | 0.24 |
| CRYGC on CFA37 19.431-19.433 | DN867687:c.364C>T | 16.7 | 19.5 | 0.32 | 0.4 | 0.16 | 0.2 | 0.52 | 0.47 | 0.50 | 0.78 |
| | DN867687:c.379C>T | 25.0 | 23.9 | 0.32 | 0.4 | 0.16 | 0.2 | 0.32 | 0.57 | 2.88 | 0.24 |
| CRYGS on CFA34 22.166-22.172 | DN867380:c.*7G>A | 33.3 | 30.5 | 0.22 | 0.4 | 0.15 | 0.2 | 0.13 | 0.71 | 2.91 | 0.23 |

Shown are heterozygosity (HET), polymorphism information content (PIC), chromosome-wide multipoint test statistics Z-mean and LOD Score, their error probabilities (p Z-mean, p LOD Score), χ²-tests for allele and genotype distribution of the case-control analysis and their corresponding error probabilities (P) for the SNPs in the dachshund families. According to the SNP nomenclature, the asterisk indicates the position of a changed nucleotide 3' of the translation stop codon.
Nonparametric linkage and association analyses: A non-parametric multipoint linkage analysis was employed for the four dachshund families. This analysis was based on allele sharing by identical-by-descent methods and the MERLIN 1.0.1 software [45]. Haplotypes were estimated using MERLIN 1.0.1 using the option “best”. A case-control analysis based on $\chi^2$ tests for genotypes, alleles, and trends of the most prevalent allele was also performed for the dachshund families. The CASECONTROL and ALLELE procedures of SAS were used for association tests, tests for Hardy-Weinberg equilibrium of genotype frequencies, and the estimation of allele frequencies.

Figure 1. γ-Crystallin protein alignment. Shown are the alignment of the canine CRYGB protein (175 amino acids), the canine CRYGC protein (174 amino acids), and the canine CRYGS protein (178 amino acids) derived from our sequenced cDNA with the known orthologous protein sequences. The sequences were derived from GenBank entries with the following accession numbers: NP_005201 (human CRYGB), NP_658906 (mouse CRYGB), NP_066269 (human CRYGC), NP_031801 (mouse CRYGC), NP_060011 (human CRYGS) and NP_034097 (mouse CRYGS). Residues identical to the dog are indicated by asterisks. The three exons are labeled by different colors. All exons included only complete triplets.

Figure 2. γ-Crystallin cDNA analysis. Bands of cDNA PCR products of the lens tissue of two dogs affected by CAT and an unaffected dog for each gene (CRYGB, 567 bp; CRYGC, 603 bp; and CRYGS, 645 bp) on an agarose gel. In the gel, band 1=mixed breed, unaffected; band 2=dachshund mix, affected; band 3=German shepherd, affected. The cDNAs of the other four dogs did not differ in sequence and product size.
RESULTS & DISCUSSION

Position of CRYGB, CRYGC, and CRYGS on canine chromosomes: The canine ESTs for CRYGB (DN866034) and CRYGS (DN867380), which were found by cross-species BLAST searches with the corresponding human reference mRNA sequences, mapped to the same positions as the annotated canine genes for CRYGB (LOC488497) and CRYGS (LOC607506; Table 2). For CRYGC, no annotated canine gene is available but the CRYGE EST (DN86768) mapped between canine CRYGB (LOC488497) and CRYGD (LOC488495). This assumed position of CRYGC in dogs agrees with all other investigated species [23-25]. A recent study tried to obtain sequence tagged sites (STS) for CRYGE and other candidate genes for primary cataracts in the dog [31].

The crystallin genes are similar genes that show high sequence homologies to each other. We assume that the problems of the previous study to place the putative CRYGB amplicon to the predicted canine CRYGB location was due to assortment of the wrong sequence (AACN010184836) for primer design of the CRYGB amplicon.

Genomic organization of canine CRYGB: The canine CRYGB gene was found to contain all three exons and two introns that are present in the orthologous human gene, and the canine exon/intron boundaries conformed perfectly to the GT/AG rule. The sizes of all three exons of the canine CRYGB gene were identical to those of the human CRYGB gene. Analysis of the 648 bp of the canine CRYGB EST (DN866034) revealed an open reading frame of 528 bp predicting a protein of 175 amino acids. The canine CRYGB protein displayed 84.6% similarity to the human CRYGB protein and 91.4% similarity to the mouse CRYGB protein (Figure 1).

The canine EST contained 29 nucleotides before the start codon in exon 1, and 91 nucleotides after the stop codon in exon 3. The polyadenylation signal AAUAAA was located 37 bp downstream of the stop codon.

The cDNA sequences of lens tissue of the seven dogs perfectly matched the sequences of the canine ESTs. Only the 5' and the 3' ends were shorter due to primer position.

The gene structure described here is in contrast to the annotated structure of the canine CRYGB gene (LOC488497), which was derived by automated computational analysis (Dog genome assembly 2.1). Under this accession number, the canine CRYGB gene has five exons, with exon 3 corresponding to human exon 4 corresponding to human exon 2, and exon 5 corresponding to human exon 3. The canine EST and our sequenced CRYGB cDNA contained only the predicted canine exons 3, 4, and 5. No canine EST was found for the predicted exons 1 and 2.

The γ-crystallin genes are considered to be highly conserved genes, which are similar among the different species. In all mammals examined to date, the γ-crystallin genes have a three-exon-structure: a short first exon, which encodes the start codon, and the short NH2-terminal “arm”. The other two exons encode the two structural domains, each of which contains...
contains two Greek key motifs [21,22,46]. We could not verify the predicted canine exons 1 and 2 as described in LOC488497. Due to the fact that the gene structure of LOC488497 was derived by automated computational analysis, it is possible that the predicted exons 1 and 2 do not exist.

**Genomic organization of canine CRYGC:** The canine CRYGC gene contained all three exons and two introns that are present in the orthologous human gene. The sizes of all three exons of the canine CRYGC gene were identical to the human CRYGC gene. The analysis of the 669 bp of the canine CRYGC EST (DN867687) revealed an open reading frame of 525 bp, predicting a protein of 174 amino acids. As the canine EST contained 89 nucleotides before the start codon in exon 1 and 55 nucleotides after the stop codon in exon 3, we assumed that the 5'- and 3'-UTR of the canine CRYGC were included [44]. The canine CRYGC protein displayed 87.4% similarity to the mouse CRYGC and 87.9% similarity to the human CRYGC protein (Figure 1).

**Genomic organization of canine CRYGS:** The canine CRYGS gene also had three exons interrupted by two introns as does the orthologous human gene. The canine exon/intron boundaries conformed perfectly to the GT/AG rule, and the sizes of all three exons of the canine CRYGS gene were identical to those of the human CRYGS gene. Analysis of the 680 bp of the canine CRYGS EST (DN867380) revealed an open reading frame of 537 bp, predicting a protein of 178 amino acids. The canine CRYGS protein displayed 93.3% similarity to the human CRYGS protein and 87.1% similarity to the mouse CRYGS protein (Figure 1). The canine CRYGS EST contained 39 nucleotides before the start codon in exon 1 and 104 nucleotides after the stop codon in exon 3. The polyadenylation signal AAUAAA was located 76 bp downstream of the stop codon.

LOC607506 lists seven isoforms of the canine CRYGS gene which were derived by automated computational analysis (Dog genome assembly 2.1). The isoform with the transcript ID XM_844792.1 agrees with the results of our study. The other isoforms have additional exons, which were not confirmed in our analysis, or have different exon sizes that do not fit into the canine EST.

To confirm the results of the structure analyses of the three genes, it would be necessary to produce a full-length cDNA using RACE methods. For this purpose it is necessary to obtain complete RNA with intact 3' and 5' ends. It was possible to gain the cDNA sequence of all three genes without the 5' ends. The cDNA sequences of all dogs perfectly matched to the full RNA with intact 3' and 5' ends. It was possible using RACE methods. For this purpose it is necessary to obtain complete RNA with intact 3' and 5' ends.

**Polymorphisms within the canine CRYGB, CRYGC, and CRYGS gene:** The search for sequence variations within the three genes revealed a total of five SNPs as shown in Table 3. Of these five SNPs, two were located in the exon sequence of CRYGC while another was located in the exon sequence of CRYGB. The exonic SNP of CRYGB was T>C transition in exon 3, which changes a GTT triplet to a GTC triplet. Both triplets code for valine and thus do not alter the amino acid sequence of CRYGB. In the CRYGC gene, a C/T transition at position 112 of exon 3 was observed only in the wire-haired dachshunds. This transition changes a CTC triplet into a TGC triplet and thus causes an amino acid change from arginine (R) to cysteine (C). This means a change from a charged alkaline amino acid to a neutral amino acid with a nonpolar side chain. Multi species protein sequence comparisons between human (R; accession number NP_066269), mice (R; accession number NP_031801), rat (R; accession number XP_343583), and cattle (R; accession number NP_001013613) showed that this position was not variable between the known orthologous CRYGB proteins.

The second exonic SNP of CRYGC was found at position 127 of exon 3. This C/T SNP changes a CTG triplet to a TTG triplet, which has no effect on the amino acid sequence of CRYGC. Except for DN867687:c.364C>T, all other SNPs were at saddle iliac polymorphic in all six examined breeds (Table 3). None of the polymorphisms affected the splice sites in the investigated genes.

**Linkage and association analyses for CRYGB, CRYGC, and CRYGS:** Table 2 shows the results of the nonparametric multipoint linkage analysis in the dachshunds. All SNP alleles were in Hardy-Weinberg equilibrium. The highest Z-mean value was 0.32 and the highest LOD score was 0.16, while the error probabilities ranged from 0.2 to 0.4. The maximum achievable Z-mean was 29.85 and the corresponding value for the LOD score was 5.52. These values indicated that the pedigrees used had enough power to detect significant linkage. There were also no significant results from the case-control χ²-tests for the dachshund families. The χ² test statistics for allelic distributions between cases and controls ranged from 0.02 to 0.52 and their error probabilities from 0.88 to 0.47. Similar results were obtained for the distributions of genotypes between cases and controls (χ² from 0.50 to 3.17 with error probabilities from 0.78 to 0.20).

Therefore, it is unlikely that the CRYGB, CRYGC, and CRYGS genes are involved in the pathogenesis of CAT in these wire- and smooth-haired dachshunds.

None of the five polymorphisms identified in this study proved to be a causal mutation for CAT in canine CRYGB, CRYGC, and CRYGS exons and exon/intron junctions in the CAT-affected wire- and smooth-haired dachshunds from our pedigrees. In addition, expression analysis of these genes in six affected dogs and a control dog did not reveal any differences in the bands on an agarose gel. So it seems unlikely that a mutation outside of the genomic regions analyzed here possibly affects CRYGB, CRYGC or CRYGS expression. However, the SNPs identified here may be useful to test CRYGB, CRYGC, and CRYGS as candidate genes in other dog breeds.
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