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Molecular investigation of *SILV* and *SLC45A2* genes associated with congenital defect of deafness and blindness in dog breeds of Tamil Nadu

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Abstract
The study was conducted to develop a genomic tool to identify puppies having congenital deafness and blindness in their early ages. The present study was aimed at identifying mutations present in the genes that may cause congenital deafness and blindness. *SILV* and *SLC45A2* are the two coat color genes expected to have association with the congenital defects of dogs. The exon 10 of *SILV* gene revealed three different set genotypes of AA, AB and BB with the base pairs of 550, 550 and 200 and 200 bp respectively in Rajapalayam breeds of Dogs. The results of present study clearly indicates that all deaf animals with white colour coat expressed AA genotype, whereas white coat coloured silver eyed normal hearing dogs had AB genotype and the BB genotypes are present only in normal ealthy dogs. The exon7 of *SLC45A2* gene had no amplified PCR product for the 7WDP primers, but a 750bp fragments was observed for 7SDP primers in all samples analyzed and it clearly indicates that SLC45A2 gene has not associated with deafness and blindness. Variations in the amplicon size between normal and healthy dogs are identified for puppies susceptible for the deafness and blindness.

Keywords: *SILV*, *SLC45A2*, congenital

Introduction
Dog (*Canis lupus familiaris*), the first animal domesticated by man and most widely kept as a guard, hunter and pet, belongs to subspecies of gray wolf (*Canis lupus*). India is rich in canine genetic resource, mainly utilized for guarding the farm and farm house and for shepherding the livestock species during grazing, migration and hunting.

Rajapalayam is one of the few Indian bred pedigree dogs that has been developed in the country and is almost on the verge of extinction now. Most of the dog breeders are unknowingly favoring for a white (albino) dog, characterized by the pink nose and the lack of pigmentation. In the past, puppies of solid colour in Rajapalayam breed were usually culled from the litters since the owners preferred the pure white dogs. Later it was found that dogs with complete white colour have a high incidence of deafness and blindness. Since the breed was mainly used for hunting, a dog with poor hearing and vision is not favoured by pet owners.

The deafness has been reported in many dog breeds to be associated with the presence of *SILV* and *MITF* genes (Strain, 2011) [14] or genes with particular mutation (SINE insertion). White coat color is often related to blue eyes and dogs with blue eyes are either blind or had a higher prevalence of deafness than pigmented eyed dogs. (Famula *et al*., 2000) [3]. Little (1957) [6] have identified more than 20 loci that affect coat color in dogs. The association of blindness and deafness was studied by Juraschko *et al*., (2003) [4] in German Dalmatian dogs and observed that the puppies born with one or two blue eyes had higher prevalence of deafness compared to normal eyed puppies.

Since molecular screening for the presence of mutation or changes in the genotype in Rajapalaym breed of dog in relation to other Tamil Nadu breeds of dog will be useful to detect the inheritance pattern among the dog breeds, the study was aimed at identifying genes associated with deafness and color of coat and eyes in dogs.

Material and Methods
Blood samples from 30 healthy dogs and 20 vision and hearing impaired dogs of Rajapalayam breed were collected in EDTA vials following standard procedure. The total DNA was extracted from blood following conventional phenol chloroform method (Sambrook and

Russell, 2001) [10]. Exon 10 and Exon 11 of *SILV* gene and exon seven of *SLC45A2* gene were amplified using primers given in table 1.

Table 1: Sequence of primers used for PCR amplification and amplicon size of *SILV* and *SLC45A2* genes

Genes	Primers	Amplicon size
Exon 10 *SILV*	F: TGG CGG GGA GCA GAC A R: AAG AAT GAG CAG TGG CAA GAG	550 bp, 200 bp
Exon 11 *SILV*	F: CAG TTT CTC CTT TAT TCT CCC A R: CCT CGG CAA ATC ACA GCA	200 bp
Exon 7 of *SLC45A2*	F:CAG TTT CTT GGT GAC TGT AAA GC R1:GAC TTC AGC CTC AAC AAT CTG R2:CTC TGC TCA GTC ACC GAC G	750 bp

The PCR mixture contained 100 ng isolated DNA as template, 100 pmol each of forward and reverse primers, 200 µmol dNTPs, 10% DMSO, and 3.5 IU TaqDNA polymerase in 20 µL final reaction volume. The protocol followed for each PCR is given in table 2.

Table 2: PCR protocol for SILV and SLC45A2 genes

Initial denaturation	94 °C for 1 min
Denaturation	94 °C for 30s
Annealing	63 °C for 10s (*SILV* exon 10 57 °C for 10s (*SILV* exon 11) 60 °C for 10s (*SLC45A2* exon 10)
Extension	72 °C for 30s
Final extension	72 °C for 7min

The amplicons were visualized in two per cent agarose gel using ethidium bromide stain.

Results and Discussion

PCR amplification of SILV gene

Two exons of *SILV* gene were amplified and the amplicon were visualized on gel electrophoresis. It was observed that exon ten of *SILV* gene showed three different genotypes *viz*, two homozygotes AA (550 bp) and BB (200bp) and a heterozygote AB (550 bp and 200 bp) genotype in Rajapalayam breeds of Dogs (Figure 1A). The homozygote AA genotype was observed only in deaf and blind dogs whereas the heterozygotes were apparently healthy but expressed silver colored eye. However, the exon 11 showed no variations in the amplicon size (200bp) and it was shown in Figure 1B.

The results of preset study clearly indicated that normal dogs had only 200bp band (BB genotype), deaf animals showed only 550 bands (AA genotype) and silver eyed normal hearing dogs showed both 200 and 550 bands (AB genotype). Further, the results indicated a retrotransposon insertion in exon ten of *SILV*gene which might be responsible for abnormality in the animals studied. Amplicon of exon eleven of *SILV* gene had a size of 200bp, which is in par with the expected output as in healthy merle animals but showed no variation in healthy and diseased animal.

PCR amplification of SLC45A2

The exon7 of *SLC45A2* gene showed two different amplicon size of 750bp amplicons with 7SDP and no amplification with 7WDP reverse primers in all the samples analyzed. The deletion of 750 bp sequence was observed in both normal and healthy dogs indicated that the blindness might not be associated with deletion in exon 7 of *SLC4A2* gene (Figure 2).

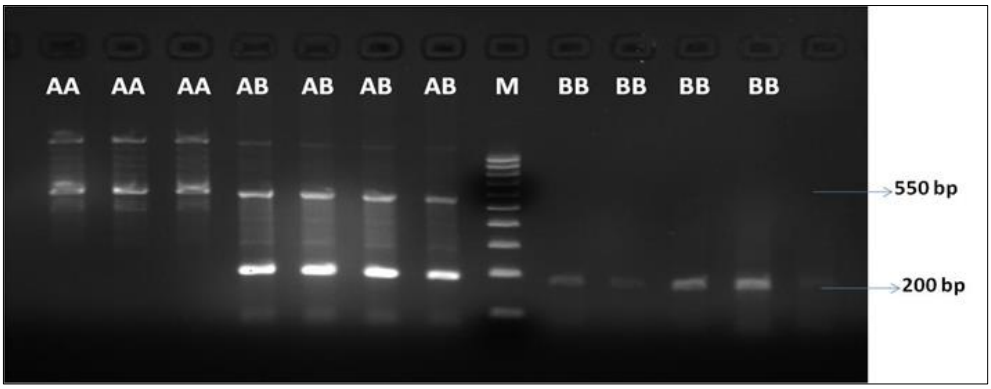
Clark *et al*., (2006) [2] identified and characterized the merle gene on dog chromosome 10. He found that the mutation responsible for merle is a 253 base pair short interspersed element (SINE) just before exon 11 that also includes a poly adenine tail. Phenotypic expression of merle required the presence of multiple adenine repeat of the SINE to be 90-100 adenine repeats in length. Dogs homozygous for merle (MM) are known as double merles and are white in color. Failure of expression of merle genotype in certain dogs is due to the absence of the poly A tail of 90-100 repeats and they are called cryptic merles. The heterozygote pattern of both 200 and 550 bands (AB genotype) was also noticed as small patches of normal phenotypic color with diluted pigmentation in both MM and Mm dogs of Dachshund (Sorsby and Day, 1953) [12]. Martina *et al* (2015) [7] classified the merle dogs as single or double merle and cryptic merles based on the genotypic pattern of *SILV* locus in Border collie, Shetland sheepdog, Australian Shepherd dog, and Chihuahua breed of dog. Schmutz *et al*., (2007) [11] observed that dogs that are homozygous for the merle mutation were deaf by breeder observation or BAER testing and also had ocular anomalies like microphthalmia, blue or heterochromatic iris and are partially or totally blind.

A study conducted by Platt *et al*., (2006) [9] revealed that deaf Border Collies had more white pigmentation or merle patterning than normal hearing collies. And those deaf dogs were found to have blue colored iris that impaired their vision. Significant association was found between coat color linked to *SILV* gene and deafness in all age groups. Little (1957) [6] explained that the Piebald locus (S locus) affects the pigment distribution in body of dogs. Strain (2004) [13] reported that the S locus is one of the most associated genes with congenital deafness in canines and extreme-white piebald allele (s^w) is associated with white pigmentation. He also suggests that dogs with a patch had a significant negative association with deafness and dogs with blue iris had positive association with vision and hearing loss.

Karlsson *et al*., (2007) [5] identified that the white dogs must contain piebald alleles in its recessive form since all alleles are recessive in nature. He also reported that the various alleles of piebald gene are present in chromosome number 20 as two mutations in *MITF* gene. One is a SINE insertion present in all piebald and extreme white piebald breeds, but absent in Irish spotting and solid colors and second one is a unique polymorphism present in solid colors. SINE insertion is found in all white and piebald breeds and not seen in irish spotting or solid breeds. Length of polymorphism was long in breeds with SINE insertion (35-36bp) and short (29-32) in solid color breeds. Murphy *et al* (2018) [8] concluded that the phenotypic consequence of the Merle SINE insertion directly depends upon oligo(dT) length resulting in insufficient PMEL and a pigment dilution spectrum, from dark grey to complete hypopigmentation.

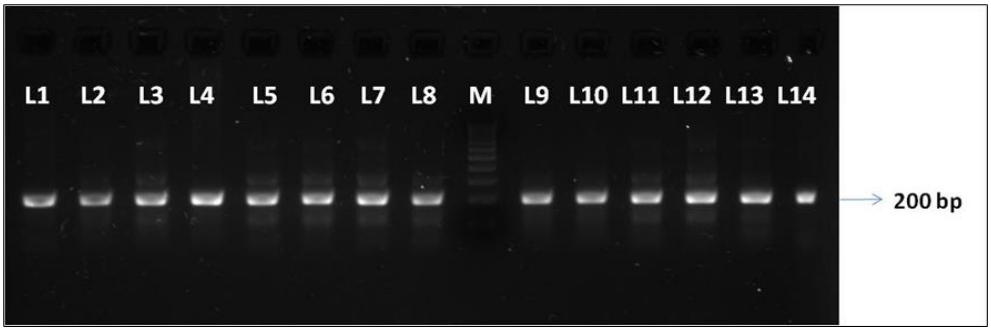
There exist significant association between *MITF* and congenital sensorineural deafness in Dalmatian dogs. The mutation responsible for the disease is suggested to be seen in the non-coding region of the *MITF* gene. This mutation has significant association with blindness as explained by Stritzel *et al*., (2009) [16]. Further, the *SLC45A2* gene deletion and Oculocutaneous albinism in Doberman Pinscher was studied (Winkler *et al*., 2014) [17]. The exon seven of *SLC45A2*gene showed two different amplicon size of 750bp and nil amplicons with 7SDP and 7WDP reverse primers respectively in all the samples analyzed. Results showed very clear bands corresponding to the genotype of the sample analyzed. This

indicated that the deletion of 750 bp was observed in both normal and healthy dogs indicate that the blindness is not associated with deletion in exon 7 of *SLC4A2* gene.*SLC45A2* gene showed no amplification for exon 7WDP but a 750 bp band was observed for exon 7SDP in all samples (Figure 2). Similarly the homozygous wild type was reported by Caduff *et al*. (2017) [1] from the genotyped 174 unrelated dogs of various breeds and they correlated one of the deletion in SLC45A2:c.1287delC in exon 6 might be the cause for the observed oculocutaneous albinism in the affected Bullmastiff. Strain (2009) [15] explained that one of the pigmentation genes associated with deafness that is merle have two alleles. M is dominant and produces dapple pigmentation in heterozygous condition, were as homozygous recessive dogs produces uniform pigmentation. Homozygous dominant individuals are often nearly all white, deaf, sterile and blind. Homozygous merle dogs are usually pale or completely white with ocular and hearing abnormalities.



Lanes AA are the homozygote with single 550bp in deaf and blind animals; lanes AB are the Heterozygote with 200bp and 550bp fragments in apparently healthy but with silver eyes; lanes BB are the homozygote with single 200bp fragments in healthy animals; Lane M, a 100-bp ladder

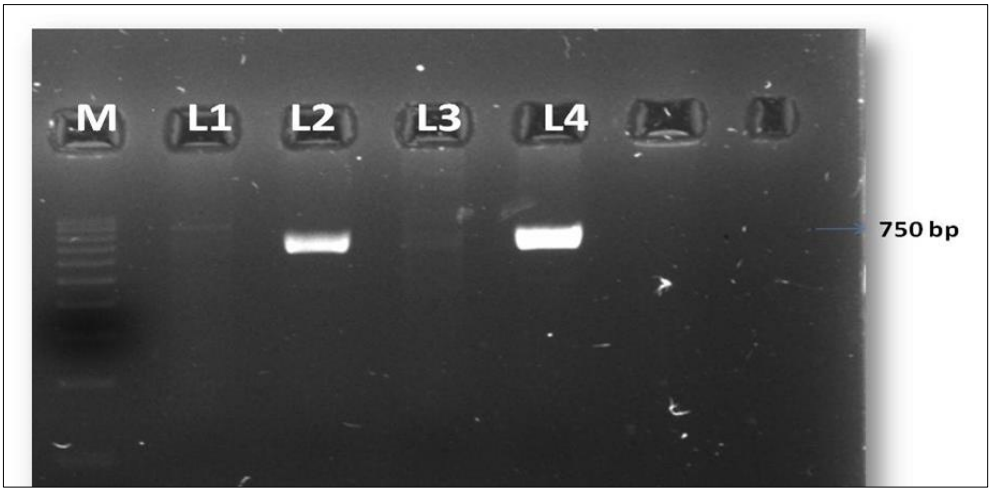
Fig 1A: Exon 10 of SILV gene



Lanes 1 to 14 are the homozygote with single 200 bp in all healthy and deaf animals; Lane M, a 100-bp ladder

Fig 1B: Exon 10 of SILV gene

Fig 1: Representative 2 per cent agarose gel electrophoresis of SILV Gene amplicon sizes.



Lane M, a 100-bp ladder; Lanes 1 and 3 with no amplification for the 7WDP reverse primer; lanes 2 and 4 with amplicon 750bp for the 7SDP reverse primer in all samples.

Fig 2: The amplified PCR product of exon 7 SLC45A2 Gene in 2 per cent agarose gel electrophoresis

Conclusion

The results indicated that the exon 10 of *SILV* might be associated with congenital deafness and blindness in Rajapalayam breed of dogs. But no association could be identified between other exon 10 of SILV gene and exon 10 of *SLC45A2*gene. But since the sample size is less, more samples need to be analysed to confirm the association between the gene and coat color.

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