

## POLYMORPHISM IN EXON 9 OF HEAT SHOCK TRANSCRIPTION FACTOR 4 (*HSF4*) GENE IN EXOTIC AND INDIGENOUS BREEDS OF DOGS

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**Abstract:** Mutations in *HSF4* gene have been reported to cause both human autosomal dominant and recessive cataracts and studies in mice had shown that *HSF4* was required for normal fibre cell differentiation during lens development. Thirty eight samples from dogs of different breeds (30 exotic and eight indigenous) were screened for polymorphism in *HSF4* gene, as compared to the reference from NCBI GeneID: 489766, which was reported to be responsible for hereditary cataract in few purebred dogs. On analyzing the fragment of size 430 bp upon sequencing the following variations were found - G deletion in three positions (2905, 2942 and 2944bp) in intron 8 and two SNPs T>C transitions at 3121 and 3124 bp in exon 9 of 430 bp product. No difference was identified between the exotic and indigenous breeds for the fragment analysed. The result showed that *HSF4* gene was polymorphic and further studies would help us understand the role of *HSF4* polymorphism in various conditions canine species.

**Keywords:** *HSF4* gene, Single Nucleotide Polymorphism, Purebred dogs.

### Introduction

Heat shock transcription factor 4 (*HSF4*) belongs to a family of heat shock transcription factors that regulate the expression of heat shock proteins in response to different stresses, such as oxidants, heavy metals, elevated temperatures and bacterial and viral infections. Different mutations in *HSF4* gene have been reported to cause both human autosomal dominant and recessive cataracts and studies in mice had shown that *HSF4* was required for normal fibre cell differentiation during lens development. Disruption of the gene leads to the development of cataracts *via*. multiple pathways, including the down-regulation or loss of post-translational modification of different crystalline proteins. Hence in a preliminary analysis the polymorphism of the *HSF4* gene between the indigenous and exotic dogs was attempted.

Hejtmancik (2008) revealed that 26 of the 39 mapped loci for isolated congenital or infantile cataracts had been associated with mutations in specific genes; thirteen have mutations in crystalline, about a quarter have mutations in connexions, with the remaining loci divided among the genes *HSF4*, aquaporin-0, and beaded filament structural protein-2. Candidate gene approach study by Mellersh *et al.* (2006) to investigate 20 genes known to be associated with cataract in humans, for their potential association with the development of hereditary cataract in dogs, revealed that mutations in the *HSF4* gene caused HC in dogs. The majority of primary HCs would be seem to be inherited *via* a simple autosomal recessive gene and occurs early in life. It is not congenital but appears at a few weeks to months in age, progressing to total by 2 to 3 years of age. The two canine mutations that have been identified both reside in exon 9 of *HSF4* gene. The objective of this study was to assess the Polymorphism in exon 9 of heat shock transcription factor 4 (*HSF4*) gene between exotic and indigenous breeds of dogs.

### **Materials and methods**

Blood samples were collected from Ophthalmology Unit of Madras veterinary teaching hospital and Private Clinics from 30 dogs of exotic breeds (Labrador-12, Spitz-4, Pug-3, Terrier-2, Dachshund-1, Doberman-1, Bronze dogue de Bordeaux-1, Boxer-1, Beagle-1, Dalmatian-1, Lhasa apso-1, Rottweiler-1, Golden Reteiever-1) and 8 dogs of Indigenous breeds (Rajapalayam-2, Kanni-2, Chippiparai-2 and Kombai-2). DNA was isolated using standard Phenol-Chloroform extraction procedure (Sambrook *et al.*, 1989).

The *HSF4* exon 9 sequence was downloaded from NCBI Reference Sequence: NC\_006587.3 (GeneID: 489766) and one set of primers to amplify *HSF4* exon 9 were designed using Primer3 (v. 0.4.0). PCR protocol was standardised for the products of size 430 bp. A partial region of Intron 8, complete Exon 9 and partial Intron 9 was amplified using the designed primers and PCR products sent for sequencing. The sequenced results (both forward and reverse reads) were obtained aligned and analysed using SeqMan program of LASERGENE software (DNASTAR Inc., USA).

### **Results and discussion**

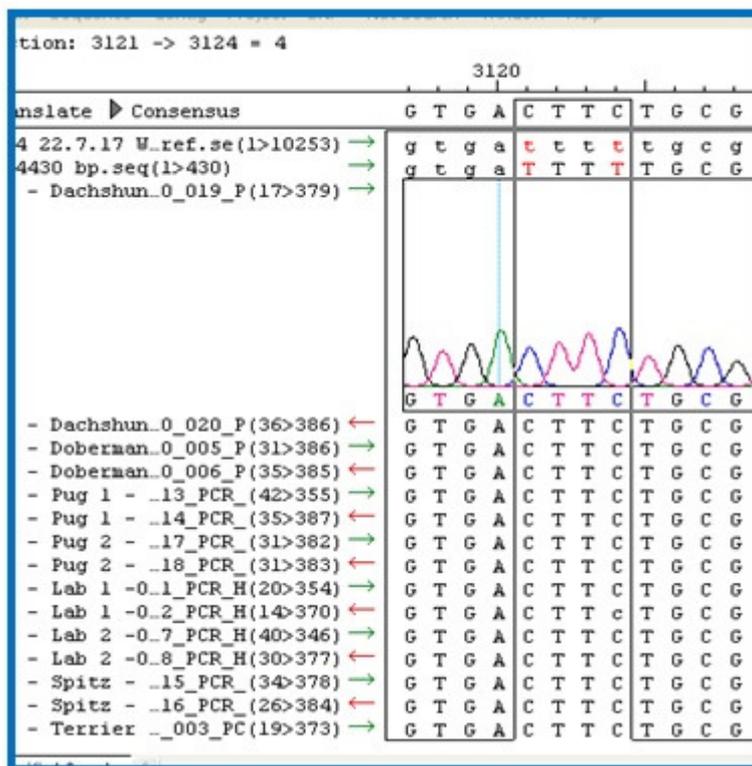
The reference sequence to which the sequence was compared was from Boxer breed of female sex. The sequence size of 430 bp was obtained which covered 99 per cent of the fragment region of reference sequence was assembled and screened to identify the polymorphism in this fragment. On analyzing the fragment region, G deletion in three positions (2905, 2942 and 2944bp) in intron 8 and two SNPs T>C transitions were identified

in two positions (3121 and 3124 bp) in exon 9 of 430 bp product. The chromatograms showing variations SNPs (3121 and 3124 bp T>C transitions) and, in exotic breeds and indigenous breeds of dogs are presented in Figure 1 and 2. As these transitions were present in Exon 9 analysis using Vaxa software was done which identified both the mutations to be synonymous and no change in the amino acid was seen.

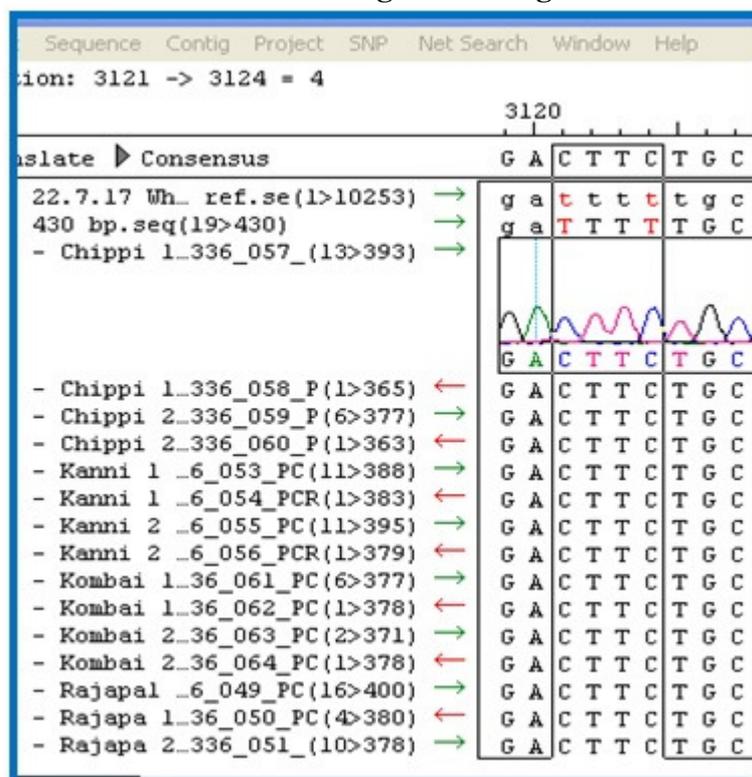
These two SNPs were present in all the samples both exotic as well as indigenous breeds. This was in concurrence with the results of Muller *et al.* (2008) who reported a same set of two T>C synonymous transitions just three base pairs apart in the two breeds (Dachshund and Entlebucher Mountain breeds). It can be speculated that these could be the same mutations as reported in our study as there is a nine years of time interval between the former and the present study and the sequence might have undergone some changes which could have caused this base pair difference. The SNPs of *HSF4* gene reported by Mellersh *et al.* (2006) was not identified in this study. The three deletions in intron 8 has not been reported in any of the earlier studies.

The SNPs, all T>C transition in exon 9 of *HSF4* gene were synonymous and T nucleotide was completely replaced by C in all dogs. It was understood that *HSF4* gene was polymorphic in all breeds of dogs studied. Also there was high homology among the breeds of dogs for exon 9 indicating the conservation of this region during evolution. Further analysis based on the traits affected by the *HSF4* gene should be studied to understand the association of these SNPs with any traits.

**Figure 1 Chromatogram showing SNPs (3122, 3124 T>C Transition) in exon 9 of *HSF4* gene in exotic breeds of dogs**



**Figure 2 Chromatogram showing SNPs (3122, 3124 T>C Transition) in exon 9 of *HSF4* gene in indigenous breeds of dogs**



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