®

Abstract of Analysis for the Feline gene Fel D1

Microbac Laboratories was contracted by Allerca to study mutations in the feline gene Fel D1 to discern what differences exist between this gene in Allerca cats versus the gene in standard cats. Fel D1 is the major allergen in cats and is comprised of two distinct chains. Chain 1 is comprised of four regions (exons) in the gene, but only three are used at any one time. Exons 3 and 4 are always present but Exons 1(Leader B) and 2(Leader A) are alternately used depending on which cell type the gene is being expressed in. Leader A seems to be expressed in both salivary and skin cells whereas Leader B seems to be expressed preferentially in salivary glands. Chain 2 is comprised of three coding regions with no alternate coding region involved.

Sequence analysis of the genes coding for the Fel D1 protein yielded multiple mutations across the genes and were difficult to compare due to the ambiguity of DNA coding for amino acids. When the DNA sequences were converted into protein sequences the patterns were much more apparent and comparisons were carried out using only the protein sequences so that individual mutations to the protein itself could be studied. There are several mutations in Fel D1 that are naturally occurring and had previously been reported in literature. Based on the commonality of the naturally occurring mutations and sequence differences between the Allerca cats and standard cats it appears that chain 1 is not responsible for the hypoallergenic effects of the Allerca cats. The only mutations/differences observed in the Allerca cats for Fel D1 chain1 are documented in the literature as naturally occurring. Analysis of chain 2 however showed several mutations that were not common between the Allerca and standard cats, and were not previously documented in literature. These mutations show that there is a difference between the hypoallergenic Allerca cats and the standard cats. Offspring of the Allerca cats also show these same mutation patterns and sequence comparisons confirm this.

Extraction and preparation of the genomic DNA was carried out by first swabbing the mouths of each cat then extracting the DNA from the swabs using DNeasy tissue prep kits manufactured by Qiagen. Allerca also supplied an initial sample of previously prepared genomic DNA from *Felis domesticus*, which was obtained from EMD Biosciences catalog #69325-3, to perform the initial setup steps for this analysis and to test each batch of primers to ensure that they amplified the specific regions they were designed for. Analysis of the Fel D1 gene and its component chains was carried out using the polymerase chain reaction (PCR) and subsequent sequencing of the PCR products. Primers that were initially used were published for Fel D1 but these proved to be inappropriate for the analysis being performed due to large GC-rich areas within the non-coding regions between the exons of the intact gene that caused the PCR reactions to fail. New primers

specific for each exon or closely grouped exons of the gene were then designed to avoid these GC-rich regions. Primers were synthesized by Sigma-Genosys. The PCRs were carried out using a Stratagene Robocycler 96 and a high fidelity polymerase from Roche Applied Sciences. The products were resolved in 1.0% agarose gels using ethidium bromide. PCR products were then purified using GENE clean turbo kits from Qbiogene to remove excess primers and nucleotides before being sent for sequencing. All sequencing for this project was carried out by ACGT Inc. and data returned to Microbac for further analysis. The NCBi database was used to blast and compare the sequences between the Allerca cats and the standard cats and to generate the data comparisons.

In summary, the analysis performed for Allerca, shows that there is a definite difference in the Fel D1 gene derived from Allerca cats and the Fel D1 gene derived from standard or normal cats. The control sources for the standard cats were swabs taken directly from known non-Allerca cats and control feline genomic DNA obtained through EMD Biosciences, both of which when compared with the Allerca cats show mutations outside those that are known to occur naturally. When compared to the NCBi database all sequences matched to the Fel D1 major cat allergen and support the data that the Allerca cats are different from the standard cats in the Fel D1 region of their genome.

Analysis performed by Robert Brooks, Biotechnology Manager

James Nokes, Laboratory Director