CRISPR Gene Editing of the Major Cat Allergen, Fel d 1

Nicole Brackett[1], Julia Riedy[1], Mazhar Adli[2], Anna Pomés[1], Martin Chapman[1]
[1] INDOOR Biotechnologies, Inc., Charlottesville, VA, USA; [2] Northwestern University, Chicago, IL, USA

BACKGROUND

Domestic cat (*Felis domesticus*) is the most common source of inhaled allergens derived from mammals. Cat allergy affects >10% of the population and sensitization to cat is often associated with asthma. More than 90% of cat allergic patients have IgE antibodies to the major cat allergen, Fel d 1 (**Figure 1**), which account for 60-90% of the anti-cat IgE. The goals of this study were to identify conserved regions of the Fel d 1 genes and to delete Fel d 1 from feline cells using CRISPR-Cas9 as an approach that could ultimately be used to generate Fel d 1-free cats.

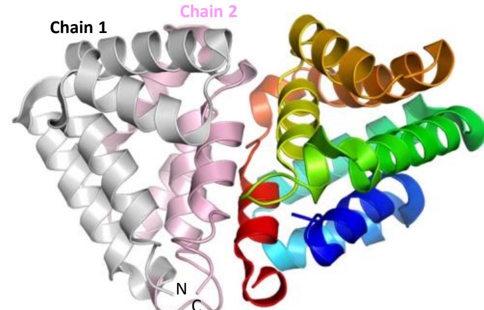

Figure 1: Fel d 1 is a dimer of heterodimers. The molecule on the left is a fusion of the monomers chain 1 (grey) and chain 2 (pink) that form a heterodimer in the natural allergen. The equivalent molecule is shown on the right, from the N-terminus (blue) to C-terminus (red).[1]

METHODS

DNA was extracted from >50 cats to sequence the genes encoding Fel d 1 chains 1 and 2. Fel d 1 sequence homology was evaluated, and conserved regions of the genes were selected as CRISPR target sequences. Guide RNAs (sgRNA) with sequences complementary to the Fel d 1 target DNA were synthesized, and sgRNA/Cas9 complexes were delivered to cat cells (CCL-94, ATCC) using lipid-based transfection. Successful CRISPR editing of Fel d 1 was assessed with DNA sequencing and T7E1 mismatch detection (**Figure 2**).

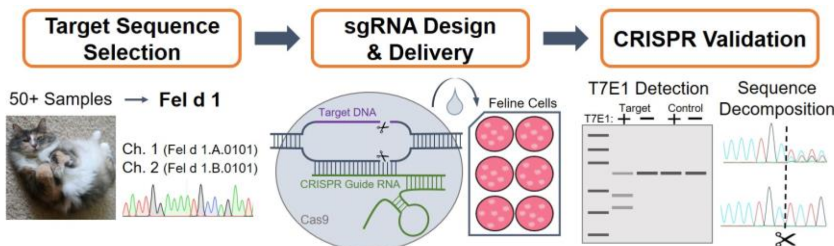

Figure 2: Workflow of experimental approach to delete the Fel d 1 genes using CRISPR-Cas9.

RESULTS

Sequence analysis of Fel d 1 chains 1 and 2 from >50 cats identified >25 unique amino acid substitutions at frequencies ranging from 2-98%, resulting in Fel d 1 polymorphisms with 92-99% identity (**Figure 3**). At least 16 novel natural variants were predicted and multiple conserved regions in the genes suitable for CRISPR editing were revealed.

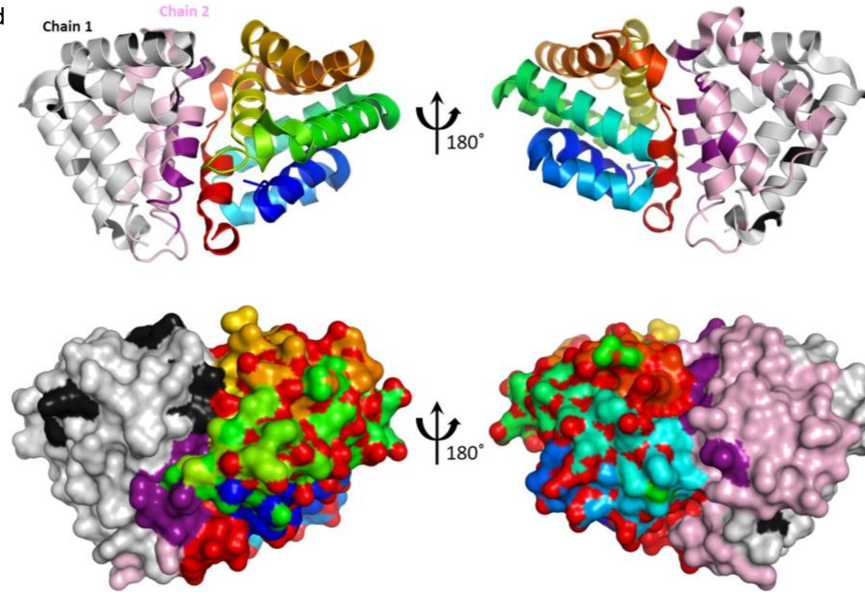

Figure 3: Fel d 1 ribbon (top) and surface (bottom) structures with chain 1 and chain 2 mutations denoted in black and dark purple, respectively.

Ten sgRNAs targeted to conserved regions in chain 1 (C1G1 - C1G6) or chain 2 (C2G1 - C2G4) were evaluated. Decomposition of control and CRISPR-edited Fel d 1 sequences found CRISPR editing efficiencies of up to 55%, while T7E1 mismatch detection showed editing efficiencies ranging from 5-45% for the panel of sgRNAs (**Figure 4A**). Two efficient sgRNAs, C1G1 and C2G1, were identified. T7E1 likely under-estimated the editing efficiency of C2G1 due to low variability of indel (insertion/deletion) distribution (**Figure 4B**), while the T7E1 results for C1G1 were comparable to the sequence analysis findings (**Figure 4C**). Preliminary T7E1 analysis of predicted potential off-target cleavage sites found no evidence of off-target CRISPR editing due to C1G1 or C2G1.

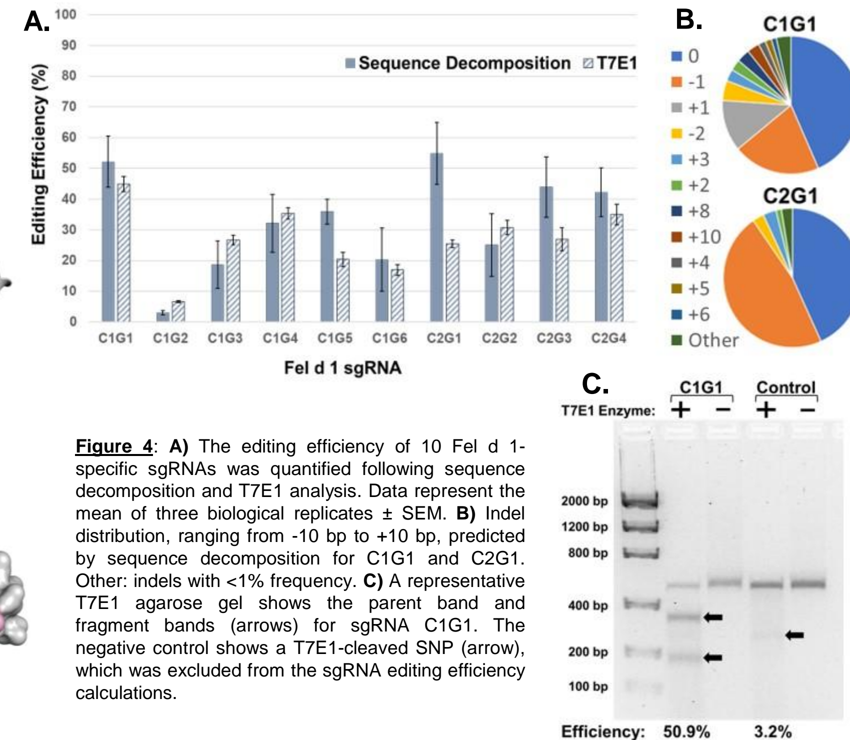

Figure 4: **A)** The editing efficiency of 10 Fel d 1-specific sgRNAs was quantified following sequence decomposition and T7E1 analysis. Data represent the mean of three biological replicates ± SEM. **B)** Indel distribution, ranging from -10 bp to +10 bp, predicted by sequence decomposition for C1G1 and C2G1. Other: indels with <1% frequency. **C)** A representative T7E1 agarose gel shows the parent band and fragment bands (arrows) for sgRNA C1G1. The negative control shows a T7E1-cleaved SNP (arrow), which was excluded from the sgRNA editing efficiency calculations.

CONCLUSIONS

The major cat allergen, Fel d 1, is a viable target for CRISPR gene editing. The results indicate that CRISPR-Cas9 is a valuable tool for deleting Fel d 1 in feline cells and suggest that CRISPR will serve as a viable approach for editing Fel d 1 in cats, which may significantly benefit cat allergic individuals by reducing their symptoms.

INTELLECTUAL PROPERTY

In 2017 Indoor Biotechnologies converted its preliminary U.S. patent application on CRISPR gene editing of Fel d 1 (Fel d 1 Knockouts and Associated Compositions and Methods Based on CRISPR-CAS9 Genomic Editing) to a full application and filed an International (PCT) Application on the invention (WO2017/152023).

REFERENCES

1. Kaiser L, et al. Structural characterization of the tetrameric form of the major cat allergen Fel d 1. J Mol Biol. 2007 Jul 20;370(4):714-27.