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

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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.

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).

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.

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 underestimated 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).

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 Genome Editing) to a full application and filed an International (PCT) Application on the invention (WO2017/152023).

REFERENCES