Simultaneous Disruption of Five SerpinA1 Genes in Mice Using CRISPR/Cas9 to Generate the First Animal Model of Alpha-1 Antitrypsin Deficiency

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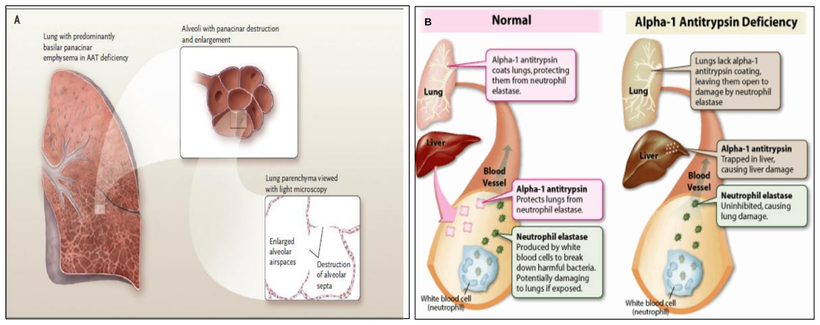
Background

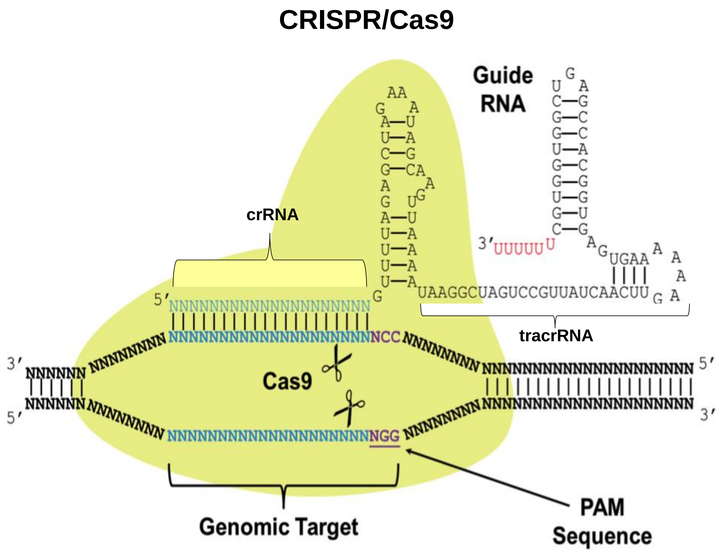
Alpha-1 antitrypsin (AAT) deficiency is a common autosomal co-dominant genetic disorder. This condition affects 1:2500 individuals of European ancestry, leading to the development of lung and liver disease. Within North American and Northern European populations, an estimated 4% of individuals are carriers of deficient genes for AAT. AAT deficiency presents with an emphysema-like phenotype in the lungs of older subjects. AAT deficient subjects can also suffer from liver disease of varying severity; however, lung disease is the principle cause of death. Belonging to the serpin family, AAT is a protease inhibitor predominantly synthesized in the liver. Upon secretion into the blood stream, AAT is directed towards the lungs where it inactivates excess neutrophil elastase, thereby preventing damage to the alveoli. Mutations of the SerpinA1 gene can lead to reduced serum levels of AAT and decreased protein functionality, allowing for unrestricted elastin breakdown, pulmonary inflammation and eventual emphysema. Currently, an animal model simulating the lung condition does not exist, which severely limits the development of innovative therapeutics.



(www.sandysandhaus.com/About_Alpha-1.html) (http://flipper.diff.org/app/items/2843)

Figure 1: (A) pathology of tissue with AAT deficiency from an alveolar perspective **(B)** normal and pathophysiological mechanisms of AAT.

Genomic Editing to Create Knockout



(http://2013.igem.org/Team:Paris_Bettencourt/Project/Detect)

Figure 2: shows the structure of the CRISPR/Cas system associated with a target sequence of genomic DNA. Diagram includes components of a single guide RNA (sgRNA) including tracrRNA and crRNA, Cas proteins, and a required down stream protospacer adjacent motif (PAM) sequence.

Using the innate adaptive immunity of the CRISPR system, we targeted specific loci within exon 2 of the SerpinA1 gene to effectively disrupt and silence the gene caused by a DNA repair mechanism known as Non-Homologous End Joining (NHEJ). With 5 copies of the SerpinA1 gene in the mouse genome, the CRISPR/Cas9 system offered a more effective method to achieve knockout of each individual gene.

Experimental Design

1. Design gRNA's that target coding regions within each SerpinA1 gene (5 copies per chromosome)
2. Test efficiency of gRNA constructs using a Single Strand Annealing Assay
3. Design primers to screen for AAT mutations (In/Del) using PCR assay
4. *In vitro:* Screen embryonic stem cells co-transfected with gRNA and CRISPR/Cas system
   In vivo: Screen microinjected transgenic zygotes (gRNA: 20ng/uL, Cas9 mRNA:50ng/uL)
5. Perform mouse specific AAT ELISA to validate knockout candidates
6. Sequence bands from PCR products of suspected knockout embryonic stem (ES) cells and transgenic mice
7. Perform neutrophil elastase inhibition assay to assess activity of AAT knockout (KO) serum vs. WT controls
8. Pulmonary challenge pilot study using novel AAT KO model:
   - LPS Challenge – 4 AAT KO males were age, gender matched with 4 C57BL6 controls. LPS given intratracheally (dose: 1 mg/kg). Plethysmography and Flexivent data collected over 1 week span.

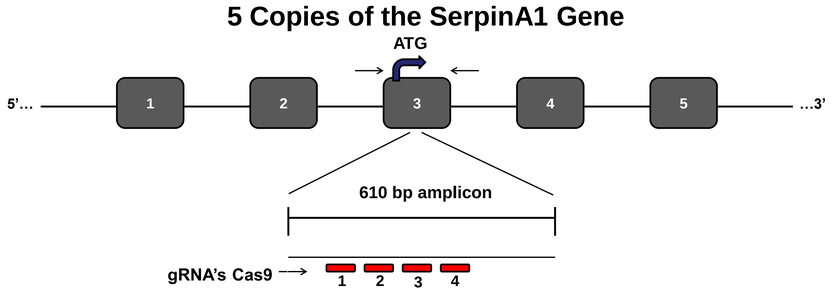


Figure 3: 5 sequential copies of the SerpinA1 gene exist in the mouse genome, 4 gRNA's were designed to cause In/Dels after NHEJ occurs. The figure shows the 610bp amplicon that results from amplifying wild type genomic DNA. From PCR amplification of samples, various deletion patterns were observed due to differing gRNA/CRISPR/Cas9 activity.

Results

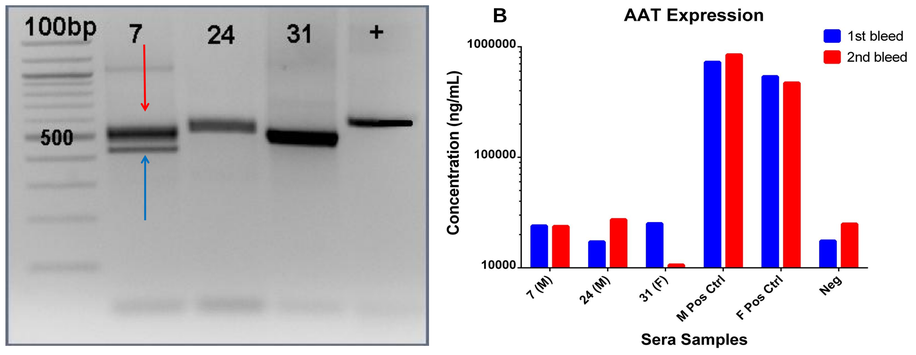


Figure 4: (A) PCR results of AAT KO mice from original n=39 microinjected C57BL6 mice. Positive control: gDNA from W/T C57BL6 mouse. 610bp amplicon denotes wild type genotype. #7, 24, 31 demonstrate various sized deletions caused by an endogenous NHEJ repair mechanism. **(B)** Results from mouse specific AAT ELISA . Positive controls: 1 male and 1 female C57BL6 W/T. Negative control: human serum.

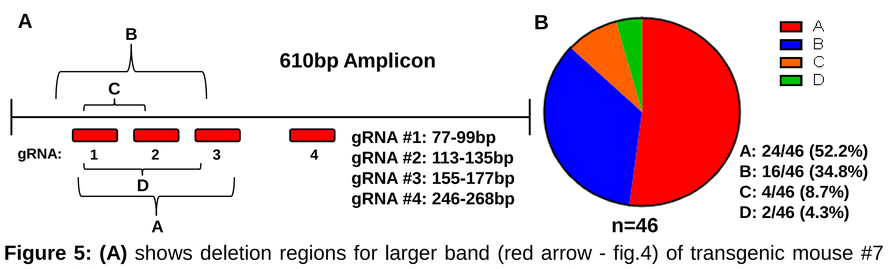


Figure 5: (A) shows deletion regions for larger band (red arrow - fig.4) of transgenic mouse #7 after PCR amplification, extraction, cloning and sequencing. Smaller band (blue arrow - fig.4) denotes 5th deletion pattern ranging from gRNA #1-4 (172bp deletion).**(B)** from an n=46, statistical distribution of deletion patterns of larger band (red arrow) from fig. 4

Neutrophil Elastase Inhibition Assay

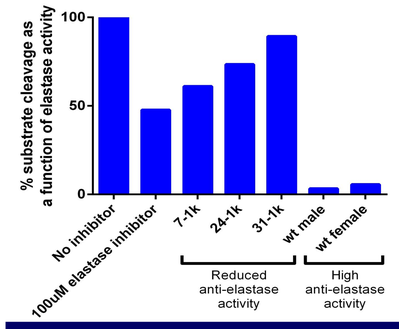


Figure 6: shows the results of screening for inhibitors of neutrophil elastase (NE). The relative percent inhibition of NE is determined on a colorimetric basis. When no inhibitor is added, the reaction between NE and the chromogenic substrate occurs unhindered, resulting in a detectable color change (column 1). At 1000x dilutions, the KO mice (7, 24, 31) exhibit some inhibitory activity of the reaction, but at a significantly reduced level as compared to the W/T control samples.

LPS Challenge

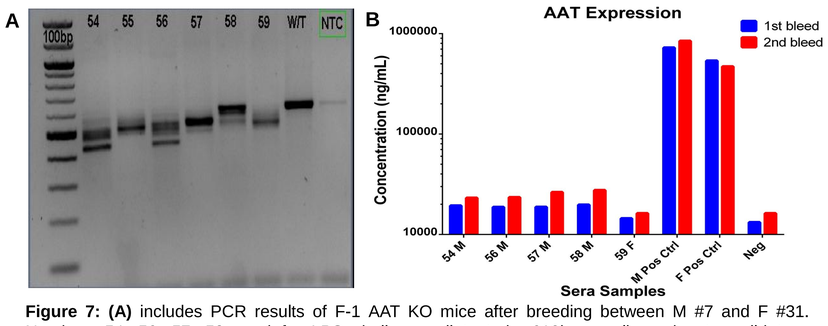


Figure 7: (A) includes PCR results of F-1 AAT KO mice after breeding between M #7 and F #31. Numbers 54, 56, 57, 58 used for LPS challenge pilot study. 610bp amplicon denotes wild type genotype. NTC: Non-template Control **(B)** Results from mouse specific AAT ELISA . Positive controls: 1 male and 1 female C57BL6 W/T. Negative control: human serum.

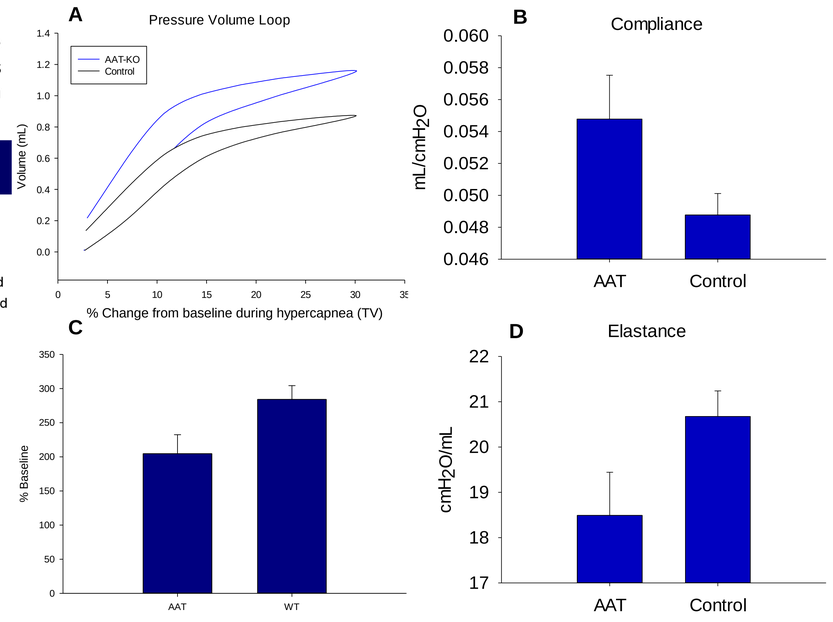


Figure 8: (A) Pressure-Volume loops for AAT-KO and W/T cohorts (Flexivent). **(B) & (D)** Compliance and Elastance measurements of pulmonary tissue from experimental groups. AAT-KO mice predicted to have increased compliance and decreased elastance due to uninterrupted elastase activity in the lungs (Flexivent). **(C)** AAT-KO and W/T percent changes from baseline of tidal volume with response to hypercapnic conditions 5 days post LPS administration (Plethysmography).

Conclusions

- From screening results, AAT knockout mice were successfully created using CRISPR/Cas9 genome editing technology (mice #: 7, 24, 31), which have been bred into F-2 generations from F-1 founder lines.
- After challenging knockout and wild type mice with LPS, the AAT KO group demonstrated decreased elastase and increased compliance as compared to the control counterparts.

Acknowledgments

R24 Grant from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to C.M.

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