Explorations of Mutations of Alpha-1-Antitrypsin

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Introduction

Alpha-1-Antitrypsin (A1AT) is a protein consisting of 394 amino acids that functions as a protease inhibitor specifically for neutrophil elastase, an enzyme which breaks down elastin in the lungs. Mutations in the Alpha-1-Antitrypsin gene can ultimately result in various liver and lung diseases from deficient or ineffective protein. These mutations can be divided into four main categories: normal, deficient, null, and dysfunctional. Normal mutations result in normal serum levels of Alpha-1-Antitrypsin and function normally. Deficient mutations result in Alpha-1-Antitrypsin levels that are less than 35 percent of normal. Null mutations result in no detectable Alpha-1-Antitrypsin present in the serum. With a dysfunctional mutation, the protein is present but functions abnormally.

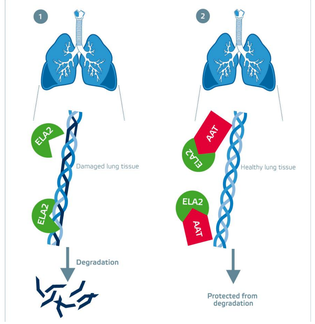

Figure 1

Figure 2

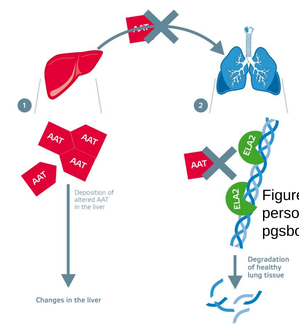

Figure 3

Figure 1,2,3 Source: bio-logis personal genomics services, pgsbox.com

Figure 1: Degradation by elastase due to protein imbalance
Figure 2: Individual with normal, functional Alpha-1-Antitrypsin
Figure 3: Example of mutation causing both liver and lung diseases

Research Objectives

Objective: Conduct literature searches to (1) gather information on normal and abnormal amino acids and corresponding codon sequences in the A1AT gene, (2) understand effects on the body, and (3) determine the susceptibility of disease for the most prevalent mutations that cause a deficiency in A1AT.

Data Collection

This literature search was conducted using:

- SciFinder—Online database that allows access to relevant scientific journal articles when given specific research topics, authors, journals, etc.
- Online Mendelian Inheritance in Man (OMIM)—Online database that provides detailed information about genetic disorders.
- Zotero—A reference management software used to collect and organize the journal articles used.

Results and Discussion

Deficient Mutations

Mutation Name	Normal Amino Acid/Codon	Abnormal Amino Acid/Codon	Residue Location	Effects	Risk for Liver Diseases	Risk for Lung Diseases
Z	glutamic acid-glu-E GAG	lysine-lys-K AAG	342	Alteration in secondary structure, accumulation of mis-folded Z-AAT in ER of hepatocytes (liver cell)/AAT-producing cells, reduced neutrophil elastase inhibitory capacity	X	X
S	glutamic acid-glu-E GAA	valine-val-V GTA	264	Does not accumulate in AAT-producing cells, AAT protein degraded intracellularly before secretion, occurs on M1(Val213) haplotype background	---	X (Emphysema)
I	arginine-arg-R CGC	cysteine-cys-C TGC	39	Compound heterozygotes with Z or null allele have risk of emphysema	---	X (Emphysema)
M-Mineral Springs	glycine-gly-G GGG	glutamic acid-glu-E GAG	67	Intracellular aggregation of mutant protein, protein that reached circulation had reduced ability to inhibit neutrophil elastase	---	X (Emphysema)
M-procida	leucine-leu-L CTG	proline-pro-P CCG	41	Somewhat reduced catalytic activity, low concentration in plasma due to instability and resulting intracellular degradation before secretion	---	X (Emphysema)
M-heerlen	proline-pro-P CCC	leucine-leu-L CTC	369	Occurs in Exon 5	---	---
M-duarte	aspartic acid-asp-D GAT	valine-val-V	256	Same deficiency-producting change as that in P (Lowell), differs from polymorphic nucleotides at other positions in the gene	No information found	
M-malton	phenylalanine-Phe-F	Deletion	51 or 52	Abnormal intracellular accumulation of newly synthesized AAT protein, showed imflammation, mild fibrosis, and intrahepatocyte accumulation of the protein in the liver, impaired secretion	X	X (Emphysema)

Null Mutations

Mutation Name	Normal Amino Acid/Codon	Abnormal Amino Acid/Codon	Residue Location	Effects	Risk for Liver Diseases	Risk for Lung Diseases
Null-Bellingham	lysine-lys-K AAG	Stop Codon TAG	217	Single base substitution in exon 3	---	X (Emphysema)
Null-Granite Falls	tyrosine-tyr-Y TAC	Stop Codon TAG	160	Deletion of third nucleotide in amino acid tyr	---	X (Emphysema)
Null-Mattawa	leucine-leu-L TTA	phenylalanine-phe-F, generating premature stop codon at location 376	353	For monocytes: mRNA translated at normal rate producing truncated version of antitrypsin protein	---	X (Emphysema)
Null-Hong Kong	leucine-leu-L CTC	Dinucleotide deltetion of TC resulting in stop codon (TAA) at location 334	318	---	---	X (Emphysema)

Dysfunctional Mutations

Mutation Name	Normal Amino Acid/Codon	Abnormal Amino Acid/Codon	Residue Location	Effects	Risk for Liver Diseases	Risk for Lung Diseases
Alpha1AT-Pittsburgh	methionine-met-M ATG	arginine-arg-R	358	Only known dysfunctional variant, single aa substitution at active inhibitory site, inhibitor of thrombin and poor inhibitor of neutrophil elastase, results in a bleeding disorder	---	---

Normal Variants

Variant Name	Normal Amino Acid/Codon (in comparison to M1 and M2)	Abnormal Amino Acid/Codon (in comparison to M1 and M2)	Residue Location
M1 [Ala 213]	---	---	---
M1 [Val 213]	---	---	---
M2	arginine-arg-R CGT	histidine-his-H CAT	101
	glutamic acid-glu-E GAA	aspartic acid-asp-D GAC	376
M3	glutamic acid-glu-E GAA	aspartic acid-asp-D GAC	376
F	arginine-arg-R CGT	cysteine-cys-C TGT	223
Note: At least 42 other normal variants			

Figure 4: Number of mutations from the literature search that result in susceptibility to various liver diseases, lung diseases, or both. This shows that lung disease, specifically emphysema, is more prevalent than liver disease in the mutations that were researched.

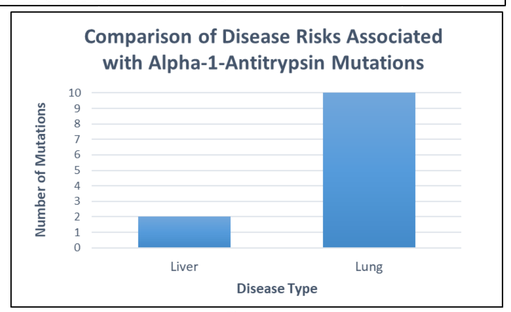

Figure 4

Conclusions/Future Work

Conclusions: These literature searches will allow for the creation of a library of mutated forms of Alpha-1-Antitrypsin in the laboratory. Understanding and having access to these mutated versions will allow for studies of the inhibitory capacity of various Alpha-1-Antitrypsin forms.

Future Work:

- Using restriction enzyme HindIII in an effort to cut Null-Hong Kong gene from a plasmid stored in the lab
- Checking the plasmid for the correct gene sequence that codes for the truncated form
- Exploring viability of alpha complementation experiments to confirm that the mutated form is inserted into the plasmid

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