Alpha-1-Antitrypsin: The Lung, New Therapies and Early Identification of Alpha-1-Antitrypsin Deficient Individuals

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Objectives

Learn Alpha-1-antitrypsin Lung Disease and genetics
Learn about research for new therapies
Learn how to identify individuals with Alpha-1-antitrypsin Deficiency
Learn how you can help bring new therapies to the Alpha1 Community
Alpha-1-Antitrypsin Deficiency

- AAT level 5.75 µM PI*Z (>95%)
- PI*Z Glu to Lys at 342
- Common genetic disease, 75-100,000 individuals in USA (1:2500-4000)
- Clinical Presentation
  - Asthma
  - Bronchiectasis
  - Pneumonia
  - COPD
  - Cirrhosis
- Rapid decline in lung function
- Premature disability and death
\( \alpha_1 \)-Antitrypsin Deficiency

The Problem

- Most common inherited risk factor for COPD (1:10-PI*Z, PI SZ or PI MZ)
- Individuals who smoke cigarettes die 20 years before non-smokers
- Passive smoking is a major risk factor particularly in childhood
- Early diagnosis and treatment is associated with health benefits
\( \alpha_1 \)-Antitrypsin

- 52 kDa glycoprotein
- Acute phase reactant “anti-inflammatory”
- Secreted in large amount from hepatocytes, but also express in many other tissues
- At least 100 different alleles
- 34 alleles associated with deficiency or dysfunction
- Mutations may affect the amount secreted and/or its function
- The frequency of the Z allele suggest a selective advantage
Protease-Antiprotease Balance

- Is lung damage solely the result of a burden of neutrophils and a lack of sufficient AAT?
AAT is More than an Antiprotease

- 52 kDa glycoprotein
- Acute phase reactant “anti-inflammatory”
- Secreted in large amount from hepatocytes, but also express in macrophages & bronchial epithelium
- Broad spectrum anti-protease
- Inhibits $\alpha$-defensin cytotoxicity and pro-inflammatory properties
- Anti-oxidant with 9 methionines
Lung Injury in AAT Deficient Individuals

Initiation
- Smoking
- Infections
- Polymers of Z AAT
- Cellular Response to UPR

Propagation and Maintenance
- Pro-Inflammatory Cells (PMN, Mast Cells, Lymphocytes & AM)
- Recruitment-Expansion-Activation
- Fibrosis
- Pro-Inflammatory Molecules (LTB4 & IL8)

Airway Alveolus

Effects of Injury
- Oxidants
- Proteases
- α-Defensins

α1AT
Protease Inhibitor (PI) Typing
Relationship Between PI Type and Serum AAT Level

![Diagram showing the relationship between PI type and serum AAT level. The x-axis represents AAT PI Type (MM, MS, MZ, SZ, ZZ, QO) and the y-axis represents Serum AAT Level (µM). The graph indicates varying levels of AAT depending on the PI type.]
Typical Pedigree
Lung damage is the result of both a burden of neutrophils and a lack of sufficient AAT. It is likely that genetic and other biological factors are also important.
Treatment of Lung Disease

Deficiency

Reduce the burden of neutrophil products

Neutrophil Elastase Burden

Anti-neutrophil protection

Augment the lung concentration

AT
Treatment of Lung Disease in Deficiency

Reduce the burden of neutrophil products

- Smoking Cessation
- Prevention
  - Vaccines
  - Frequent Hand Washing
- Early Treatment of Exacerbations
- Inhaled Steriods

Intravenous Augmentation
Aerosolized Augmentation
Gene Therapy
Anti-polyer drugs
Anti-Elastase drugs
Aerosolized Hyluronic Acid
Aerosolized rAAT
  - Transgenic
  - Yeast
  - Plant

Augment the lung concentration

AT
Barriers to the Development of New and Better Therapies for Alpha

- Limited Number of Study Subjects
- Diverse Geographical Distribution of Subjects
- Perception by Industry that the Market is Small
- Adequate Outcome Variables to Prove Treatment Efficacy
- Limited Access to AAT Patient Tissue
- Limited Number of Alpha’s willing to Participate in Research Studies
Resources that Accelerate the Development of New Therapies

- Alpha1 Foundation-Advocacy
- AAT Detection Programs
- Research Registry
- DNA and Tissue Bank
- Researchers
- Industry Partners
- The Alpha1 Community
- Research Funding
Hope Through the Development of New Therapies

- Pathway to Successful Therapies
- Studies in Progress or Just Completed
- Studies Just Around the Corner
- Studies in Around More than One Corner
Pathway to Successful Therapies

- Pre-Clinical Studies
- Phase I
- Phase II
- Phase III
- Phase IV
Completed Studies

- PPL Intravenous Study (Phase I)
- PPL-Bayer Aerosolized Transgenic AAT (phase II)
- Aventis-Behring IV Study (Phase II/III)
- Alpha Therapeutics IV (Phase II/III)
- EACTLY Trial
- Talecris MP IV AAT Study
Current Studies (Continued)

- ARC Phase I (IV AAT)
- Arriva Phase Ia/b Aerosolized rAAT (Yeast)
- AAV-AAT gene therapy trial at UF
- Kamada API Phase I Aerosol Study
Where is the AAT in the Lung?
The Promise of Aerosolized AAT
Why Does Dr. Brantly Like to “Bronch” Me?
Elements Necessary for Final Development of Aerosolized AAT

- High Efficiency Aerosol Delivery Devices
- Highly Purified AAT
- 52 kDa

Aerosolized AAT

Robust Outcome Variables
Aerosolization of AAT to Lower Respiratory Tract

α1-antitrypsin

Powder Particles
1 - 2 µm

Powder Packaging
(Unit dose blisters)

PVC foil laminates

~ 6 mg α1-antitrypsin / blister
Gene Replacement Therapy

- **Routes of administration**
  - Muscle*
  - Lung
  - Pulmonary Vessel
  - Liver
- **Data in Animal Studies**
- **UF Study now on 2nd Study**
Why Gene Therapy?

- Direct extension of protein replacement strategy
- Potential for single dose treatment
- Stable plasma levels
  - Avoid fluctuations between peaks and troughs
Gene Therapy Tools

Vehicle which does not cause disease and is a carrier or “vector” to deliver genes to cells of patients as a therapy.
Adeno-associated Virus (AAV)-2

- **Adeno-Associated Virus**
- Parvovirus
- 4.7-kb ss-DNA genome
- 20-nm icosahedral non-enveloped capsid
- Requires helper virus to replicate
- Non-pathogenic

Wt-AAV2
AAV Life Cycle

- **Viral Attachment**
- **Proviral Integration into Chromosome 19**
- **AAV DNA Replication**
- **Packaging of Progeny Virions**
Recombinant AAV2-Alpha1-Antitrypsin Vector

- Packaging limit: 4.7 kb
- ITRs retained
- CMV enhancer/chicken beta actin promoter
- Efficient for gene transfer:
  - Terminally differentiated: neurons, myofibers
- Bronchial epithelium
- Hepatocytes
Therapeutic Protein Secretion from Viral Vectors Delivered to Muscle

1. Create vector
2. IM Delivery
3. Systemic Effect
Studies Ongoing or Starting in the Next 6 Months

- EXACTLY Study Prolastin IV-
- Phase IV Aralast Study (Baxter) Australia
- Phase IV Zemiara (ZLB-Behring) USA-Europe
- AAV1 Muscle Gene Therapy (UF) USA
- Kamada-Pari Aerosol Trial Phase I/II-2007
- Phase III IV AAT (Talecris) Nearing Completion
- Phase II (NIH) Aerosolized AAT-2008
- Phase II/III Kamada IV Study 2007
Studies Ongoing or Starting in the Next 6 Months (continued)

- ATRA Study in AAT Deficiency-(UK)
- Azithromycin Study?
- QUANTUM Study
- Natural History of Liver Disease Study (soon)
- PFT Lab Based Screening Study
- UF Molecular Basis of AAT Deficiency Study
Studies in Around a More than One Corner

- Ribozyme Therapy
- Gene Correction Therapy
- Hyaluronic Acid Protective Therapy
- Stem Cell Replacement Therapy
- Small Molecule Chaperones
Making the Promise of New Therapies a Reality for Alphas

- Identification of New Alphas
  - Screening and Detection
  - Research Registry
- DNA and Tissue Bank
- Scientists interested in Alpha related studies
- Research Funds
- Pharmaceutical Company Commitment
- Alphas Willing to Participate in Clinical Studies
First

- It’s a Inherited Disorder--- Counseling and Family Screening
Approach Targeted Screening in the State of Florida

- Establish a consensus with the community that the benefit of screening exceeds the risk
- Establish a high Through-put Central AAT Deficiency Screening Lab
- Establish Professional and Lay Educational Materials
- Develop Easy to Use collection System
- Program Marketing
- Establish Tertiary Referral System
  - Lung-Liver transplant
  - Genetic Counseling
  - Specialty Clinic

How do We Flip the Berg

~95,000

5,000
Professional and Lay Educational Materials
DBS Collection System
Individual with Obstruction

DBS Genotyping for S & Z Alleles

Negative for S or Z alleles (98% are MM)

Stop or PI type from plasma or serum

Positive for S or Z alleles

DBS AAT Level

Identification of Genotype ZZ, SZ, SS, MS* & MZ*

Plasma Sample Second Confirmation by PI Typing

*MS & MZ with low AAT Levels are likely Null or Rare Low Level AAT Alleles and Plasma Sample is requested for PI typing & SNP scan by High Resolution DNA Melt.
2001-2007 YTD Screening Programs by Genotype (n>30,000)

Screening Program Totals by Genotype

- MM: 71%
- MS: 7%
- SS: 0%
- MZ: 13%
- SZ: 1%
- ZZ: 2%
- Rejected: 4%
- Other: 2%

Jan 2001 - May 2007
WHO-ATS-ERS Recommendations

- All individuals with COPD should be tested once with PI/genotyping plus an $\alpha_1$-antitrypsin level
- All individuals with asthma and a non-reversible component
- Family members of individuals with $\alpha_1$-antitrypsin deficiency
- Individuals with “cryptogenic” cirrhosis
Why Bother to Identify AAT Deficient Individuals?

- Belief that early diagnosis and preventive care translate into health benefits
- Initiation of AAT specific therapies
- Identification of at risk individuals and targeted allocation of resources to prevent the development of disease
  - Aggressive smoking cessation program
  - Job counseling
- Increase the number of individuals available for clinical trials
  - Speeds development of therapies for AAT community
  - Larger market attracts more pharmaceutical players to develop drugs for this rare disease.
Summary: COPD Management

Diagnose

Reduce risk

Reduce symptoms

Reduce complications

Spirometry

AAT Testing!!!

Smoking cessation

Pharmacotherapy

Pulmonary rehabilitation

Imunize

Treat exacerbations

Consider oxygen

Education
State of Florida AAT Testing and National Detection Programs

- Dr. Jorge Zamudio 1-888-825-7421 ext. 246
- www.alphaone.ufl.edu
DNA and Tissue Bank Update

- 6th Year
- >1660 individuals Registered
- >650 Deficient Study Subjects
- Novel Alleles by High Resolution DNA Melt-DNA Sequencing- More than 10 new AAT Gene in the last 18 months
- Request for Tissue began last year
- Primary Publication-2007
Characterization of AAT Genotype

- PI Typing by Isoelectric Focusing
- AAT Level by Nephelometry
- Genotype by TaqMan (S&Z alleles)
- Novel Alleles by High Resolution DNA Melt-DNA Sequencing - More than 10 near AAT Gene in the last 18 months
Subject Enrollment

# enrolled

Year 2001 | Year 2002 | Year 2003 | Year 2004 | Year 2005 | Year 2006
---|---|---|---|---|---
# enrolled
Enrollment Demographics by Genotype (n=1669)

- ZZ: 39%
- MM: 22%
- MZ: 20%
- SZ: 3%
- ZZ: 39%
- Null: 5%
- Other: 0%
- Liver Tx: 1%
- Pending: 7%
ZZ Subject Demographics by Age
(n=561)
ZZ Subject FEV1% (n=232)

FEV1 % Distribution

Normal Lung Function
n=54
Non Augmented ZZ Subject Plasma AAT Concentration (n=144)
ZZ Subject Characteristics: Lung and Liver Disease

- Lung Disease:
  - Emphysema
  - Chronic Bronchitis
  - COPD
  - Asthma

- Liver Disease:
  - Cirrhosis
  - Hepatitis
  - Jaundice

n = 574 for Lung Disease
n = 571 for Liver Disease
n = 571 for Both
Family Clusters

- Families with 2 members: 174
- Families with 3 members: 37
- Families with 4 members: 14
- Families with >4 members: 13
ZZ Subject Augmentation Status
(n=576)

- Augmentation: 73.6%
- No Augmentation: 26.4%
Summary

- The Alpha 1 Foundation established a DNA & Tissue Bank to accelerate research on AAT Deficiency
- Genomic DNA is available on ~600 PI*ZZ subjects
  - The phenotype of these subjects is characterized by FEV1, liver function and a >350 element questionnaire
- Access to this valuable research resource is available by contacting the PI at mbrantly@alphaone.ufl.edu