

Conference Report

MZ carrier state in alpha-1 antitrypsin deficiency: Summary of the 16th Gordon L. Snider critical issues workshop

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BACKGROUND AND INTRODUCTION

Alpha-1 antitrypsin deficiency (sometimes called AATD but referred to as “Alpha-1” in this summary) is an autosomal recessive genetic condition that can result in serious lung disease in adults and/or liver disease at any age. Alpha-1 occurs when the blood lacks a protein called alpha-1 anti-

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rypsin, or AAT. AAT is mainly produced by the liver, and its primary function is to protect the lungs from inflammation caused by infection and inhaled irritants such as tobacco smoke. In most cases, a low level of AAT in the blood occurs because the AAT is abnormal and cannot be released from the liver at the normal rate. This leads to a buildup of the abnormal AAT in the liver, which can cause liver disease, and a decrease of AAT in the blood, which can cause lung disease.

People identified with Alpha-1 most commonly have two abnormal Z alleles (ZZ, or homozygous for that allele).¹ Current evidence suggests that at least 100,000 people in the United States have Alpha-1 and the ZZ genetic profile. However, multiple mutations can lead to an abnormal alpha-1 antitrypsin allele. The S allele is another common form of the abnormal gene, although less common than the Z allele. Thus, another deficient gene combination is SZ, although people with this gene combination are less likely to develop lung or liver problems than those with two Z genes. Normal alleles are called M. A person who does not have Alpha-1 will have two normal M alleles (MM, or homozygous for that allele).

Carriers of Alpha-1 have one copy of a normal allele (M) and one copy of an abnormal allele (Z, S, or another variant). Historically, it has been assumed that carriers—who are heterozygous for the genes—are symptom free or not predisposed to the clinical symptoms of Alpha-1. That is, having one normal allele (M) is protective. However, in recent years, the Alpha-1 patient community has raised concerns about the potential for lung and/or liver disease in the carrier state. In response, the Alpha-1 Foundation and its partners convened a 1-day workshop to summarize what is known about the carrier state and to identify future lines of research into disease mechanisms and clinical phenotypes in MZ heterozygotes. The ultimate goal is to understand the preventative and therapeutic implications for carriers who might be predisposed to symptoms of Alpha-1 or at risk for other disorders.

During the workshop, a series of presenters focused on what is known about carrier status in rare diseases and Alpha-1, and on results of observational and mechanistic studies in Alpha-1. The workshop concluded with the development of a series of research questions to pursue regarding the clinical implications of MZ status, and possible approaches for answering them and developing a clinical strategy to assist individuals who are Alpha-1 carriers.

CARRIER STATES IN RARE DISEASES

Carrier States of Mendelian Disorders

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The combination of large research cohorts linked to electronic health records (EHRs) has accelerated discovery in genomic medicine and has provided a new opportunity to study disease. Vanderbilt University has linked de-identified EHRs to a DNA repository, called BioVU, which contains nearly 250,000 samples¹. Some of the data and samples date back decades. This combination of rich data facilitates the study of the genomic basis of disease and drug response using real-world clinical data, although finding phenotypes in the EHR can be challenging. To address this challenge, Denny and colleagues combine billing data, laboratory data, medication exposures, and natural language processing to enable efficient study of genomic and pharmacogenomic phenotypes. The deployment of algorithms across multiple EHRs has been shown to accurately identify phenotypes, for example, in autoimmune hypothyroidism² and many other diseases³. The goal is to develop an accurate phenotype that can then be validated by looking at actual health records.

Scanning EHRs also enables the inverse experiment; that is, one can begin with a given genotype and discover all of the phenotypes with which it is associated by looking across EHRs, including

¹Some investigators refer to the Z, S, and M genes as PiZ, PiS, and PiM. For consistency, this summary uses the simpler terms of Z, S, and M.

exploration of rare genetic variants. This is called a phenome-wide association study (PheWAS)⁴. The hypothesis is that Mendelian genes influence complex diseases and traits and that these associations or correlations can be discovered if there are sufficient records to search. The growth in large populations with EHR data, such as the All of Us Research Program, will accelerate discovery for a wide range of diseases.

Recently, Denny and colleagues developed an approach that aggregates phenotypes based on patterns described by features seen in Mendelian diseases. They mapped the clinical features of 1,204 Mendelian diseases onto phenotypes captured from the EHR and summarized this evidence as phenotype risk scores (PheRS)⁵. The PheRS identifies Mendelian diseases by their component phenotypes and can identify known genetic diseases without using the disease label (e.g., finding patients with cystic fibrosis [CF] using its disease features such as bronchiectasis, infertility, pancreatic insufficiency, and pneumonias). It has also been used to identify novel, rare alleles associated with disease phenotypes, including demonstrating heterozygote risk in some Mendelian diseases classically thought of as recessive. Sequencing then confirms these variants as potentially pathogenic and reveals that heterozygotes have disease risk.

Denny and his colleagues analyzed homozygotes for Alpha-1S and Z alleles across population-based genotyped populations. As expected, scores were elevated for ZZ individuals. Individuals heterozygous for Z alleles also had mildly elevated scores, and individuals with SZ and SS genotypes tended toward higher scores than individuals without Z or S alleles, indicating that these individuals expressed some features of Alpha-1. Of interest, individuals with the MZ genotype and liver disease showed a higher incidence of Hepatitis C than would be expected, suggesting that infection with Hepatitis C might be the “second hit” needed to trigger disease. However, there is an apparent lack of lung disease in the MZ cohort.

During discussion, Dr. Denny said that his team will continue to retrospectively study the rates of liver versus lung disease among MZ cohorts across the EHR and will compare findings with other studies.

Genotype–Phenotype Correlations in Cystic Fibrosis

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Cystic Fibrosis (CF) is an autosomal recessive disease caused by single-gene mutations of the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene. It is the most common multisystem genetic disease in Caucasians, found in 1 in 2,500 to 3,500 live births in the United States. The gene defect produces an abnormal CFTR protein that results in defective ion transport. A series of factors increase rates of infection, mucus obstruction, inflammation, and bronchiectasis, resulting in end-stage lung disease. In the pancreas, the mucus prevents the release of digestive enzymes that allow the body to break down food and absorb vital nutrients^{1,2}.

While environment and CF modifier genes clearly play a role in disease phenotype and clinical outcome, the CFTR genotype plays a critical role³. One in 25 Caucasians are carriers for CFTR gene mutations, resulting in a carrier rate of 2% to 5%, with lower rates in African American and Asian communities. In some populations, relatively uncommon mutations are prevalent because of genetic founder effects⁴.

There are many causative CFTR mutations, with more than 1,900 recently described, of which 270 have been confirmed to be disease-causing^{3,5}. However, relatively few mutations account for the majority of CFTR alleles that are particularly common in the northern European descent population. The most prevalent mutation is F508del (c.1521_1523delCTT), which causes omission of phenylalanine at position 508, accounts for about 75% of all CFTR alleles, and generally causes severe disease⁶.

The functional basis of various CFTR mutations allows correlation of genotype to phenotype, although CFTR genetics alone explain only some (estimated to be around half of the variance) of the variable outcome among CF patients⁷. There are six mutation classes, with Classes I-III generally more severe¹. Depending on the nature of the genetic mutation and the resulting molecular defect, CFTR proteins can be minimally functional (no residual expression or function), or exhibit residual activity (partially retained expression and function, previously termed mild/variable mutations)⁸. Even small levels of residual function are enough to alter the CF phenotype⁹. For example, when two severe mutations (traditionally molecular Class I, II, or III, although clear exceptions exist) are present, pancreatic insufficiency usually results. The presence of two severe mutations also confers risk for severe phenotypic manifestations including progressive pulmonary disease, pancreatic insufficiency, meconium ileus, and hepatobiliary disease^{10,11}.

Moreover, phenotypic expression of CF is extremely heterogeneous. There is considerable age-related variability, and the severity of the disease in specific organs varies considerably within and among patients. In some affected organs, phenotypic variability is tightly linked to genotype. In others, modifier genes and extrinsic factors clearly influence disease heterogeneity^{12,13}.

The presence of one or more residual function mutations often confers sufficient CFTR activity to alter disease expression and severity. CF patients with these residual function mutations have intermediate sweat chloride elevation, which correlates with lower levels of pancreatic insufficiency, slower rates of respiratory decline, and later onset disease. Of note, sweat chloride tends to be highest in CF patients with pancreatic insufficiency¹¹. In addition, sweat chloride levels track with biomarker assays of CFTR function such as nasal potential difference (NPD)¹¹ or intestinal current measurements,^{14,15} which along with its steep relationship with CFTR function, explains why the sweat chloride test has been such an important diagnostic test that is also highly sensitive to changes in CFTR function¹⁶⁻¹⁸. Notably the genotype-phenotype correlations show overlap among the mutations, highlighting the complexity of CFTR mutations and their molecular consequences¹¹.

When two mild/variable mutations are present, atypical forms of CF arise (i.e., non-classic or CFTR-related disorders), such as congenital absence of the vas deferens, idiopathic pancreatitis, or late-onset respiratory disease without other characteristic features of the CF syndrome¹⁹⁻²¹.

At its most extreme, CF carriers do not exhibit overt manifestations of CF illness, although carrier status has been proposed to increase the propensity and/or severity of respiratory disorders such as asthma, allergic bronchopulmonary aspergillosis, chronic rhinosinusitis, and chronic bronchitis²²⁻²⁵. The variance in CF disease outcome has provided an important framework to predict the clinical efficacy of small molecules that restore CFTR function, and serves as an important model for the correction of other genetic disorders. For example, the CFTR potentiator ivacaftor highlights the need for precision drugs to target specific mutations. It acutely restores CFTR function in patients with the G551D CFTR mutation^{17,26,27} and illustrates the importance of mapping pharmacological CFTR responses onto a genotype-phenotype relationship, providing a conceptual framework for CFTR modulation and clinical response across mutations^{16,28}.

During the discussion, participants considered the linkages between CF carrier status and smoking, and how CFTR ion transport dysfunction has been implicated in chronic obstructive pulmonary disease (COPD) pathogenesis and associated with chronic bronchitis²⁹⁻³¹. The CFTR mutation heterozygosity appears to alter susceptibility to cigarette smoke-induced CFTR dysfunction²⁴. Consequently, COPD patients with chronic bronchitis may have a higher rate of CFTR mutations compared to the general population. Rowe and colleagues continue to look at the influence of environmental factors and the CFTR mutations associated with pancreatitis. It was noted that Alpha-1 lacks a tool such as the sweat chloride test in CF, which is stable throughout the lifespan and can be used for validation of other markers, mutations, and phenotypes.

OBSERVATIONAL AND MECHANISTIC STUDIES IN ALPHA-1 ANTITRYPSIN DEFICIENCY

Lung Function in MZ Patients: A Family-based Study

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The fact that Alpha-1 is an autosomal codominant disorder means that people with the MZ genotype have decreased levels of circulating and lung AAT¹. Several studies have sought to ascertain the risk of COPD in MZ heterozygotes, and initial results are conflicting. If MZ heterozygosity were a risk factor for COPD, one would expect to find both an increased odds ratio in the categorical studies and a reduction in lung function in the studies measuring continuous outcome of expiratory volume. Reduced mean forced expiratory volume (FEV1) is a defining feature of COPD.

Although case-control studies have shown an increased odds ratio for COPD in the MZ genotype compared to the normal (MM) population, this has not been confirmed in cross-sectional studies². Moreover, cross-sectional lung function studies showed no reduction in mean FEV1 in those with the MZ genotype. However, studies with larger samples sizes detected lower FEV1/forced vital capacity (FVC) ratios than would be expected. A case-control study found that MZ heterozygotes had more emphysema based on quantitative chest computed tomography data³. However, these early studies were limited by small sample size, selection bias, inconsistent use of spirometry in the diagnosis of COPD, and lack of control for age, sex, ethnicity, and cigarette smoking.

With this as background, McElvaney and colleagues undertook a family-based study to ascertain whether there is an increased risk of COPD in MZ heterozygotes⁴. They hypothesized that a subset of MZ siblings are at an increased risk for COPD due to additional genetic and environmental factors. Further, they hypothesized that lower FEV1 levels would be found in MZ siblings compared to their MM siblings in families of MZ COPD probands.

Index probands were identified from the Irish National Alpha-1 Targeted Detection Program. The index proband was the first person in the family diagnosed with MZ heterozygosity and Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II to IV, to enrich the sample with families with a predisposition to COPD. MM and MZ non-index subjects were recruited from 51 index probands who were confirmed to have COPD of GOLD stage II to IV. The primary outcome measures were quantitative variables of pre- and post-bronchodilator FEV1, FEV1 (% predicted), FEV1/FVC, forced expiratory flow mid-expiratory phase (FEF25-75) and FEF25-75% predicted, and a categorical outcome of COPD. Blood was collected and serum isolated, and assays were performed to measure AAT levels and AAT phenotyping or genotyping.

Screening for Alpha-1 in Ireland to date has yielded, of 16,000 tested, a rate of roughly 1 in 3 individuals with at least one abnormal AAT gene. In addition to 288 ZZ and 238 SZ individuals detected, more than 2,300 MZ individuals were identified. MZ heterozygotes were then recruited into a more intensive study evaluating baseline pulmonary function⁵.

The results demonstrated that MZ heterozygotes were at increased risk for impaired lung function and COPD and that this risk is strongly influenced by cigarette smoke exposure. The heterozygotes in this population who never smoked did not develop lung disease, demonstrating the importance of gene and environment interactions in the genesis of COPD in MZ individuals. MZ heterozygosity was associated with an adjusted odds ratio (OR) of 5.18 (95% confidence interval [CI], 1.27–21.15, $p=0.02$), which was significantly higher (OR of 10.65; 95% CI, 2.17–52.29; $p=0.004$) in individuals who ever smoked. The degree of lung decrement was also related to the smoking pack years. This study confirms the risk for COPD in MZ heterozygotes who ever smoke. Other factors may also play a role, including occupational exposures in MZ heterozygotes, and studies have identified a three-way

interaction among MZ genotype, smoking, and occupational exposure to vapors, gases, dusts, and fumes⁶. In addition, further evaluation is required to look at other genetic modifiers in the setting of MZ heterozygosity.

During discussion it was noted that, although ever smoking among MZ heterozygotes predisposes them to COPD, the triple hit of other exposures compounds the risk. However, MZ subjects exposed to occupational or other exposures (e.g., wood smoke) but who never smoked did not have the same level of risk, although it was higher than those with no exposures. Thus, smoking seems to be the key risk factor.

Participants also asked whether measures other than AAT might be informative, for example, other anti-inflammatory markers or the level of protease-antiprotease imbalance. There was discussion about the value of screening and when it might be most effective, for example, in newborns, adolescents, or adults. Those found to be MZ carriers can be counseled to avoid smoking themselves or around their MZ children. Family studies will continue to search for other modifier genes and potential biological scoring systems. Finally, the genotype-phenotype correlates that have been found in CF do not exist in Alpha-1, again highlighting the need to expand the Alpha-1 correlations and to develop a validating test (such as the sweat test in CF) for confirmation.

MZ Heterozygosity and Lung Structure/Function in Large Cohorts

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Controversy has endured concerning the association of MZ heterozygosity with risk for lung function impairment and COPD. Studies that vary by study design, sample size, and other factors have produced divergent conclusions. However, analyses of several large independent cohorts have provided strong support for the association of MZ heterozygosity with risk for COPD in multiple racial groups.

For example, Hersh and colleagues analyzed 16 studies from 1966 to 2003 and reported COPD as a categorical outcome¹. All studies employed isoelectric focusing, acid starch gel with crossed immunoelectrophoresis, or PCR-based genotyping. They found no mean difference between subjects with the MM and MZ genotypes when FEV1 was reported as a continuous outcome. Case-control studies were most strongly associated with COPD in comparison to population-based studies. They concluded that there was a consistent small increase in COPD risk in all subjects or a larger risk in a subset.

As another example, Sørheim and colleagues compared MZ heterozygotes and normal individuals from a case-control study in Norway and a multicenter family-based study from Europe and North America². Neither study found the MZ genotype to be associated with COPD or radiographic airway wall thickness.

As discussed by McElveney above, a family-based study in Ireland found the following: MZ heterozygotes had a 6.6% reduction in median post-bronchodilator FEV1% predicted; MZ heterozygotes had a 3% reduction in median post-bronchodilator FEV1/FVC; and MZ heterozygotes had a 9% reduction in median post-bronchodilator FEF25–75. Of note, family-based studies are less susceptible to population stratification and selection bias and may be enriched for environmental exposures³.

Finally, Foreman and colleagues assessed correlations of the MZ genotype with COPD in two racial groups, based on the COPDGene cohort. COPDGene is a cross-sectional, observational, multicenter cohort composed of smokers (current or former, > 10 pack years) from two racial groups⁴. The goal was to investigate the underlying genetic factors for the development of COPD and precisely clinically characterize and phenotype COPD subjects. MZ subjects were found to be less likely to be active smokers. However, MZ heterozygous individuals who smoke are at increased risk

for COPD and obstructive lung function impairment compared with Z-allele noncarriers, regardless of race.

In sum, the COPDGene and Irish family studies found the following:

- MZ heterozygosity is associated with COPD affection status in non-Hispanic white current or former smokers in the COPDGene Study.
- MZ heterozygosity is strongly and paradoxically associated with airflow obstruction in individuals of European and African descent in the COPDGene Study and in Irish ever smokers.
- MZ heterozygosity is associated with COPD-related phenotypes such as densitometric CT emphysema.

During discussion, it was noted that caution is needed in over attributing COPD to MZ genotype status. Multiple genes have been linked to COPD, and it would be informative to discern how they segregate to understand better the association and/or correlation between COPD and MZ status. As such, it would be useful to conduct genome-wide association studies on the entire COPDGene cohort.

Demographics and Patient Characteristics of the MZ Individuals from a U.S. National Detection Program

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University of Florida, 2GeneAidyx

Alpha-1 is caused by abnormalities in the AAT gene that may lead to low AAT serum levels or dysfunctional AAT, and an increased risk of respiratory and hepatic disease. The normal allele (M) is the most prevalent. Presence of the Z allele is associated with severe dysfunction and reduction in AAT levels. Patients with the MZ genotype may present with intermediate levels of AAT compared to MM genotype and maintain a risk for lung disease, particularly if they smoke or have persistent environmental or occupational exposures.

The World Health Organization and several clinical and scientific societies recommend that all individuals with COPD should be tested once with genotyping plus AAT level, as should all individuals with asthma and a non-reversible component, family members of individuals with Alpha-1, and individuals with “cryptogenic” cirrhosis.

Brantly and colleagues evaluated the demographics and patient characteristics of individuals identified with the MZ allele from the largest screening program for Alpha-1, the United States Targeted Detection Program for Alpha-1 Antitrypsin Deficiency, consisting of more than 580,000 samples from the United States and its territories.

To conduct the targeted screening, physicians submitted samples from patients identified as at risk for Alpha-1 and completed a questionnaire detailing demographic and clinical characteristics and reasons for ordering testing. Genotyping was performed, and AAT protein levels were determined by immunogenic assay for samples positive for a deficiency or dysfunctional alleles. The results database (July 2003 to February 2017) was analyzed for patients with the MZ alleles, and demographic and clinical characteristics were evaluated.

Samples from 469,409 patients were included in the analysis. The MZ state was detected in 26,403 (5.6%) of tested patients. The MM state was present in 443,006 patients (94.4%). Although clinical characteristics did not vary significantly between the two groups, other differences emerged. Mean (\pm SD) age at genetic diagnosis was 56.5 (15.7) years for MZ patients and 58.9 (14.5) years for MM patients (mean difference [95% CI], -2.47 [-2.67 , -2.28] years; $P < 0.0001$, 2-sample t -test). Thus, those with the MZ genotype are likely to be younger at genetic diagnosis than those with the MM genotype. Further, a greater proportion of MZ patients reported Alpha-1 and/or family history of Alpha-1, and lower AAT levels were measured, although not in the range considered deficient when

compared to the MM group. Because of this elevated risk it is advisable to screen those with lung disease for genotype status per the recommended guidelines and for clinical suspicion of disease.

Discussions asked whether there was a potential for bias in the sample because of over representation from the southern parts of the United States, and whether that suggests that people living in the South are more likely to be lung patients and therefore entered into the registry. It was noted that registry studies such as this one provide the opportunity to look at genotype and biochemical activity to detect patterns, associations, and correlations. Large, population-based datasets, such as that available through 23andMe, might provide a broader population sample that is more representative of the general population.

Heterozygous Carriage of the Alpha-1 Antitrypsin Z Variant Increases the Risk to Develop Liver Fibrosis

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Homozygous carriers of the Alpha-1 Z variant are strongly predisposed for developing liver cirrhosis¹. In contrast, the relevance of the heterozygous MZ genotype is less obvious².

Although the prevalence of the ZZ genotype varies worldwide, it appears in roughly 1 in 2,000 to 4,000 individuals. In contrast, the prevalence of the MZ genotype can be as high as 8% in Northern Europe³. The MZ genotype is associated with slightly reduced or even normal AAT levels. Whereas the Alpha-1 Z variant produces loss of function in the lung, it results in a gain of function in the liver due to an excess of misfolded AAT. This can result in chronic hepatitis, fibrosis, and cirrhosis^{1,2}. Notably, AAT is stress-inducible, that is, stress inducibility helps to compensate when there is a loss of function. But when there is a gain of function, inducibility may further amplify liver damage².

Currently, the strongest evidence of the disease-promoting effect of the MZ genotype comes from a large cohort of patients with CF⁴. CF is an inherited multi-organ disease caused by a mutation within the CFTR gene that results in viscous secretions. Although lung and pancreas disease are the most common CF manifestations, a clinically relevant liver disease develops in a minority of the patients⁴. The study by Bartlett et al. analyzed two separate international cohorts of CF patients consisting of 967 and 1,224 individuals. Out of them, 124 and 136 displayed a clinically relevant liver disease, and the presence of liver impairment was strongly associated with the carriage of Z allele (combined OR 5.04; 95% CI, 2.88–8.83; $p = 1.5 \times 10^{-8}$)⁴. Of note, the CFTR gene is expressed only in cholangiocytes, but not in hepatocytes, that are the primary targets of Alpha-1 Z-variant-mediated proteotoxicity. Accordingly, these data suggest that hepatocytes carrying the Alpha-1 Z-variant are more susceptible to the recurrent cholestatic injury caused by the viscous bile of CF patients.

In contrast to CF, only smaller studies analyzed the role of Z carriage in Alpha-1 as a modifier of other liver disorders. Among them, several focused on the impact of the Alpha-1 Z-variant on disease development in subjects with hereditary hemochromatosis caused by a mutation in the HFE gene. While HFE hemochromatosis leads to hepatocellular iron overload, only a small fraction of carriers develops an advanced liver pathology. In a small study, 3 of 15 patients with a HFE hemochromatosis were heterozygous for the Alpha-1 Z-variant, a frequency significantly higher than the occurrence of Z allele in the general population⁵. As a potential underlying mechanism, the Alpha-1 Z-variant was suggested to reduce the production of hepcidin, the central negative regulator of iron metabolism, and thereby contribute to development of hepatocellular iron overload⁶. However, a large Italian study did not observe an increased rate of Alpha-1 Z-variants in subjects with HFE hemochromatosis compared to controls, and the individuals with both genetic hits did not appear to have more severe liver disease⁷.

A large single-center study analyzed 641 patients who underwent liver transplantation due to end-stage liver disease and found that 8.2% of them carried a heterozygous Alpha-1 Z-variant, which was a significantly higher frequency than seen in the general population⁸. Another large study compared 1,847 liver biopsy cases with 1,030 autopsy cases and found a higher rate of heterozygous Z carriage in the former group, thereby suggesting that it predisposes to development of chronic liver disease⁹. The same group analyzed the amount of liver fibrosis in 30 matched alcoholic liver disease patients with and without heterozygous Z carriage. They demonstrated that MZ status promotes the alcohol-induced development of liver fibrosis¹⁰. In contrast, no obvious association between the presence of heterozygous Alpha-1 Z-variant and increased liver damage has been observed in a large cohort of patients with nonalcoholic fatty liver disease¹¹. Similarly, Alpha-1 Z-variant was not detected as a significant liver disease modifier in any genome-wide association study of individuals with alcoholic liver disease, nonalcoholic fatty liver disease, HFE hemochromatosis, or viral hepatitis.

Although many more examples are available, these data demonstrate that the role of heterozygous Z carriage on development of liver disease other than CF remains to be clearly proven. The likely reason for the described controversial results is that, compared to other well-established liver disease modifiers such as PNPLA3 and TM6SF2, the Alpha-1 Z-variant is much less common and therefore much larger sample cohorts are needed.

Potential Mechanisms of Liver and Lung Disease in the MZ Alpha-1 Antitrypsin Phenotype

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Alpha-1 most commonly results from the Z allele (Glu342Lys) and is found in a homozygous state (ZZ) in approximately 1 in 2,000 Caucasians of North European descent. Homozygous deficiency is clearly associated with neonatal hepatitis, cirrhosis, and hepatocellular carcinoma along with early onset panlobular emphysema^{1,2}. However, it is less clear whether the carrier genotype (MZ), which is found in 4% of individuals, is also associated with disease. MZ heterozygotes are overrepresented in individuals awaiting liver transplantation³, and they probably have a faster rate of decline in lung function if they smoke than non-carriers⁴⁻⁷.

The Z allele causes the protein to misfold and undergo a conformational transition to form ordered polymers that are retained within the endoplasmic reticulum of hepatocytes⁸⁻¹². These polymers form the characteristic periodic acid-Schiff (PAS) positive inclusions that are associated with liver disease^{13,14}. Lomas and colleagues have shown that heterozygotes for two polymerogenic alleles (the mild, slowly polymerizing I allele and the rapidly polymerizing Z allele) form heteropolymers in association with liver disease^{14,15}. It is unknown whether polymers that form in hepatocytes from MZ heterozygotes are MZ heteropolymers or form from the Z allele alone. Polymers, isolated from the livers of MZ heterozygotes and ZZ homozygotes, have the same characteristics. Thus, the underlying pathology is the same—the difference being the dose of the Z allele and hence the polymeric load. These polymers/inclusions are likely to be a cofactor that predisposes to liver disease in MZ heterozygotes in association with other factors such as alcohol, fatty liver, and viral hepatitis^{1,2}.

The lung disease associated with Alpha-1 results from the lack of an important protease inhibitor and over activity of neutrophil elastase^{16,17}. Lomas and colleagues showed that polymers are also present within the lung¹⁸. These polymers were pro-inflammatory *in vitro*, formed in transgenic mice that expressed human Z AAT following exposure to cigarette smoke, and were associated with excessive inflammation^{19,20}. Thus, the paradigm for the lung disease associated with Alpha-1 is a combination of deficiency of an important antiprotease, local inactivation of AAT by polymerization, and the drive toward further inflammation²¹.

It is now clear that every individual who is a homozygote for the Z allele has polymers of the protein in the peripheral circulation^{21,22}. Of note, these polymers disappear following liver transplantation. The polymers were also present in the plasma of MZ heterozygotes. This is likely to be important, as follow-up of a large pediatric cohort has shown that the level of circulating polymers from ZZ homozygotes correlates with portal hypertension and can predict children who will develop liver disease. Thus, they may serve as a biomarker for liver disease in Alpha-1.

In summary, a single copy of the Z allele of Alpha-1 is sufficient to form polymers within hepatocytes, within the circulation, and probably within the lung. These may act as cofactors in driving both the lung and liver disease associated within Alpha-1, and, as such, may represent a target for polymer-blocking therapy in these individuals.

During the discussion, participants discussed the value of the mouse model, which shows cigarette smoked-induced polymers *in vivo*. More study of this model could lead to a greater understanding of the mechanism of pathogenesis of emphysema in Alpha-1. Further research is needed on the combination of MZ deficiency as well as other genes and environmental factors.

RECOMMENDATIONS AND NEXT STEPS

Participants broke into two groups to discuss the implications of the day's discussions and to develop a series of questions and possible strategies for answering them. Discussions focused on the biology and genetics of MZ lung and liver disease and possible clinical trials designs

Biology and Genetics of MZ Liver and Lung Disease

Based on the state of the science, the following is known about the risk of lung and liver disease in individuals with the MZ genotype (items numbered for ease of discussion, not by priority):

1. Those who smoke are at greater risk for COPD than are smokers in the general population.
2. Other environmental risk factors, such as occupational and environmental exposures (e.g., air pollution) may also influence lung disease risk in smokers.
3. Those who do not smoke are not at significantly increased risk for COPD.
4. They are at increased risk for end-stage liver disease
5. Those with exposure to Hepatitis C or heavy alcohol use are likely at increased risk for end-stage liver disease.

The following key questions need to be answered to better understand prevention, prognosis, and treatment of individuals with the MZ genotype (items numbered for ease of discussion, not by priority):

Assessing risk and mechanisms of disease:

1. What is the magnitude of the lung and liver disease risk for this population?
2. Is the risk of lung and liver disease the same in all individuals with the MZ genotype, and is the severity of disease the same for all?
3. Is the clinical and pathological type of lung and liver disease that develops the same in the MZ as in the ZZ genotype? How does it compare to those with the MM genotype?
4. What environmental, genetic, and other factors influence lung disease risk?
5. What is the mechanism for increased risk of lung disease in those with the MZ genotype?
 - Is there a toxic gain of function?
 - Is the increased risk related to plasma AAT levels?
 - How much of it is related to protease-antiprotease imbalance?

6. What is the role of other “Omics” such as transcriptomics, metabolomics, proteomics, and epigenetics in understanding the risks and mechanisms of Alpha-1 lung and liver disease?
7. Are those with the MZ genotype at increased risk for other conditions, such as autoimmune diseases?
8. Are there genetic determinants of AAT levels (at baseline or with stress) other than Z variants, and, if so, do they influence lung disease risk?
9. How do the determinants of AAT levels relate to disease risk?
10. What is the impact of genetic modifiers within SERPINA1 and elsewhere in the genome on liver and lung disease?
11. Can other AAT protein functions beyond protease inhibition be studied in the laboratory that would give clues to mechanisms of disease in those with the MZ genotype?

Clinical issues:

12. Does COPD in those with the MZ genotype progress after smoking cessation?
13. Should we treat MZ heterozygotes with COPD differently than MM COPD patients?
14. Does silencing the Z protein in the liver have beneficial effects on lung disease?

Potential Research Strategies to Answer Key Questions

Overarching issues concern the need for well-defined goals and measurable endpoints in any study. For example, natural history designs could focus on lung disease risk, liver disease risk, or risks of other medical conditions in the MZ heterozygote population. General study design issues include

- selecting MZ heterozygotes based on their disease risk or for some other reason (i.e., an unbiased population);
- establishing necessary sample sizes for power (resolving conflicts between small focused studies and large, far-reaching studies);
- standardizing technology and terminology;
- establishing preferred measurements for the liver;
- choosing biomarkers (e.g., Desmosine, AAT levels, AAT function);
- setting parameters of study cohorts (e.g., family studies, MM control arms, comparator arms, and costs).

Specific research strategies could include the following:

- Use large population-based studies such as UK Biobank, TOPMed, BioVU, or COPDGene to assess risk for COPD and liver disease among those with the MZ genotype.
- Determine the relationships between plasma AAT levels (Z and total) and other SERPINA1 and other genetic variants.
- Develop cell-based assays to assess multiple functions of AAT.
- Look within well-phenotyped longitudinal studies such as COPDGene, ECLIPSE, and SPIROMICS for information about COPD progression in the MZ heterozygote population.
- Use multiple “Omics” approaches, including transcriptomics, proteomics, metabolomics, and epigenetics to understand variable risk for lung and liver disease in MZ heterozygotes.
- Employ cell-based and animal models to assess the impact of environmental factors such as viral infection and alcohol consumption on mechanisms of Z protein toxicity.
- Determine the impact of the Z protein on cell-based and animal models of lung disease.

- Analyze a dataset of lung pathology in MZ subjects; consider “Omics” follow-up with transcriptomics (the Lung Tissue Research Consortium could be a resource, but lung tissues are not inflated).
- Determine how to design a trial of MZ subjects for augmentation therapy.

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Heterozygous Carriage of the Alpha-1 Antitrypsin Z Variant Increases the Risk to Develop Liver Fibrosis

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Potential Mechanisms of Liver and Lung Disease in the MZ Alpha-1 Antitrypsin Phenotype

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