Published in final edited form as:

J Pediatr. 2013 August ; 163(2): 320–326. doi:10.1016/j.jpeds.2013.03.077.

Disorders of Protein Misfolding: Alpha-1-antitrypsin Deficiency as Prototype

Gary A. Silverman, M.D., Ph.D., Stephen C. Pak, Ph.D., and David H. Perlmutter, M.D.

Departments of Pediatrics, Cell Biology and Physiology, University of Pittsburgh School of Medicine, Children's Hospital of Pittsburgh and Magee Women's Hospital of UPMC

When taken together, three conceptual paradigms have led to major advances in understanding the clinical manifestations of protein misfolding and recently have led to novel therapeutic strategies. First, disorders caused by misfolded proteins are now classified according to the mechanism by which they cause clinical effects, either loss-of-function or toxic gain-of-function. Loss-of-function is the result of mutations that specifically alter folding such that the function of the protein is impaired or such that the protein does not reach the cellular destination where its function is required or both. Cystic fibrosis is the prototype disease caused by a loss-of-function mechanism in that all of the disease manifestations arise from lack of chloride transport. The CFTR Δ F508 variant does not reach the apical surface of epithelial cells predominantly because of misfolding in the endoplasmic reticulum (ER) and rapid degradation by the proteasome. The small amount of CFTR Δ F508 that does reach the cell surface is unstable and this probably also contributes to loss of chloride transport activity at epithelia. Toxic gain-of-function mechanisms are attributable to the pathologic activity of the mutant protein itself or to the effect of its mislocalization or both. This type of mechanism is implicated when the mutant protein produces a toxic effect in a cell line or live animal model. Huntington's disease and early-onset forms of Alzheimer's disease are prototypes of the gain-of-function mechanism as protein misfolding leads to degeneration of neurons. Diseases with childhood onset also fit into the paradigm, including conditions as diverse as respiratory failure in the newborn (1) and early-onset diabetes (2), among many others.

A second paradigm has come from the recognition that cells possess multiple mechanisms by which they can respond to the consequences of protein misfolding, whether involving proteotoxicity and/or protein mislocalization. These mechanisms are now referred to as the proteostasis regulatory network (3). The proteostasis network is constituted by systems that are designed to counteract proteotoxicity including chaperones to prevent mis-folding and disposal pathways, such as the ubiquitin-dependent proteasomal pathway and the autophagic response, to degrade mis-folded intermediates. The proteostasis machinery also includes signaling pathways such as the unfolded protein response and the heat shock response that alter the cellular transcriptome to counteract proteotoxicity by a broad series of mechanisms at multiple intracellular sites. Third, we have come to realize that disease caused by misfolded proteins reflects the net effect of the alteration in the protein together with the

© 2013 Mosby, Inc. All rights reserved.

D.P. is PI of "Carbamazepine for severe liver disease due to alpha-1-antitrypsin deficiency" (ClinicalTrials.gov: NCT 01379469).

The other authors declare no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

response of the cellular proteostasis network. For example, in gain-of-function diseases clinical manifestations occur only when proteotoxic effects overwhelm the proteostasis network. This means that polymorphic variants in the proteostasis network may constitute genetic modifiers of the disease phenotype. Moreover, it means that drugs which enhance endogenous proteostasis mechanisms could theoretically prevent or delay the progression of clinical disease that is caused by proteotoxicity.

We will use α 1-antitrypsin deficiency (ATD) as a prototype of diseases caused by misfolded proteins and review recent findings about its pathobiology and the development of novel pharmacological strategies. The classical form of this deficiency is a relatively unique member of the protein misfolding diseases in that it causes disease in one target organ, chronic obstructive pulmonary disease (COPD), by loss-of-function and causes diseases in another target organ, hepatic fibrosis and carcinoma, by gain-of-function mechanism(s).

Alpha-1-antitrypsin deficiency causes target organ injury by loss- and gain-of-function mechanisms

The classical form of ATD is characterized by a point mutation that leads to misfolding of mutant alpha-1-antitrypsin Z (ATZ). ATZ accumulates in the endoplasmic reticulum (ER) of cells in which it is synthesized with reduced secretion such that serum levels are only 10–15% of normal. Because liver is the predominant site of AT synthesis, accumulation of mutant ATZ within the ER of liver cells leads to proteotoxic consequences, including hepatic fibrosis/cirrhosis and carcinogenesis, by gain-of-function (4). In contrast, lung disease is predominantly caused by a loss-of-function mechanism. Because the major function of alpha-1-antitrypsin (AT) is inhibition of neutrophil proteases, including neutrophil elastase, cathepsin G and proteinase 3, these enzymes are unchecked and degrade the extracellular matrix of the lung to cause chronic obstructive lung disease (5).

In common with many other diseases of misfolded proteins, there is wide variability in the clinical phenotypes of ATD, implicating genetic and environmental modifiers as key determinants of disease prevalence and severity. Cigarette smoking markedly accelerates the rate and severity of COPD in ATD. It is believed that active oxygen intermediates that are released by mononuclear phagocytes in response to cigarette smoking functionally inactivate the small amount of AT that circulates in the blood, body fluids and lungs in ATD. Even when this dominant environmental modifier is taken into consideration, there is evidence for variability in the lung phenotype of COPD that is thought to be attributable to genetic modifiers (5). There is also marked variability in the liver disease phenotype. Studies of a unique cohort of ATD individuals, identified by a nationwide newborn screening program in Sweden, have shown that only a subpopulation, 8–10% of the cohort, develop liver disease over the first 40 years of life (6). There is also a group of ATD individuals that develop progressive liver disease later in life. Adults with liver disease due to ATD represent 85–90% of the group that requires liver transplantation in the US (UNOS data, 2012).

We have made a number of hypothetical predictions about the modifiers that determine heterogeneity in the hepatic phenotype of ATD (reviewed in 4). First, we suspect that several specific complements of modifiers determine several distinct types of childhood-onset liver disease in ATD: a small subset of children with ATD develop liver disease in the first year of life (“infantile” type); another group develop liver disease at 1 to 8 years of age (“childhood” type); portal hypertension may also first develop in adolescence (“adolescent” type). Other sets of modifiers determine adult-onset liver disease and these sets probably overlap with the modifiers that cause other types of age-dependent degenerative diseases that involve proteotoxic mechanisms. Second, we have theorized that these modifiers constitute the proteostasis regulatory network of the host and act by influencing the fate of

mutant ATZ once it accumulates in the ER. Third, we have hypothesized that this proteostasis regulatory network predominantly works at 2 distinct levels, either by subtly altering intracellular degradative mechanisms or by modifying the signaling pathways that are designed to protect the cell from the consequences of the accumulation of aggregated protein in the ER (Figure). In terms of intracellular degradative mechanisms we know that at least 2 systems are involved, the proteasomal and autophagic systems (reviewed in 4). Recent studies have shown that a Golgi-to-lysosome pathway mediated by sortilin also plays a role (7) and most likely there are other, as yet to be fully characterized, mechanisms that influence intracellular degradation of mutant ATZ. In terms of the putative signaling mechanisms we know that accumulation of ATZ in the ER of cell line and mouse models activates a very distinct set of genes and signaling pathways including most prominently autophagy and NF κ B but does not include the unfolded protein response (UPR) (8–10). In recent studies, we have found that the insulin signaling pathway also has a very powerful effect on ATZ accumulation and its proteotoxicity (11). Other genes and signaling pathways that are activated in models of ATD, such as activation of ER- and mitochondrial-caspases and activation of TGF β signaling (9,10,12), are believed to play a role in the type of hepatic damage that develops. The overall concept is that genetic variations in any of these mechanisms would lead to either infantile, childhood, adolescent or adult-type liver disease or protect the ATD individual from any clinically significant liver disease.

Autophagy appears to be particularly important in protecting cells and tissues from the proteotoxic effect of mutant ATZ accumulation in ATD. Autophagy is an ancient, conserved process induced by stress states, such as starvation, involving the degradation of intracellular contents in lysosomes to generate new amino acids for survival. The process starts with formation in the cytoplasm of a double-membrane vesicle that envelopes cytosol and parts of subcellular organelles or entire organelles. This vesicle, termed autophagosome, eventually fuses with the lysosome, resulting in degradation of its contents and recycling of the amino acids that are generated. In cell line and mouse models of ATD autophagy is activated by accumulation of ATZ in the ER and the autophagic response is responsible for degrading ATZ, particularly the insoluble polymerized/aggregated ATZ (8,13). Recently, we found that an FDA-approved drug which enhances autophagic degradation of ATZ, carbamazepine (CBZ), reduces hepatic ATZ load and hepatic fibrosis in a mouse model of ATD (14). This drug is now being tested in a clinical trial for severe liver disease due to ATD. Because autophagy is specialized for disposal of aggregation-prone proteins, drugs which capitalize on this endogenous proteostasis mechanism may be applicable to other age-dependent diseases involving aggregation-prone proteins and proteotoxicity. Indeed the decline in autophagy with age has been implicated in the pathogenesis of Alzheimer's disease, cancer, cardiovascular disorders, inflammatory diseases and glucose intolerance/metabolic syndrome (15).

The theory that modifiers of the hepatic phenotype would target components of the proteostasis regulatory network was validated in a general way by early studies in which skin fibroblast cell lines from ATD patients with and without liver disease were engineered for expression of mutant ATZ. These studies showed that there is a lag in intracellular degradation of ATZ specifically in ATD patients with severe liver disease (16). However, there is still limited genetic proof of this theory. A single nucleotide polymorphism (SNP) in the downstream flanking region of ER mannosidase I has been implicated in early-onset liver disease in ATD (17). Because ER mannosidase I plays a role in the retrograde translocation/ubiquitin-dependent proteasomal degradation pathway that is generally referred to as ER-associated degradation, a polymorphism that affects its function would be consistent with our theory that components of the proteostasis network, in this case a degradation pathway, are potential modifiers of the hepatic phenotype in ATD. However, this SNP was not validated in another population (18) and so further epidemiological studies

are needed to determine if it is a true modifier. A SNP in the upstream flanking region of the AT gene itself has also been implicated in liver disease susceptibility (19). The most logical way that the upstream SNP could impact the hepatic phenotype would be by affecting an increase in transcription or translation but the published data did not substantiate that notion. SNPs in several genes have been implicated in severity of lung disease in ATD, including variants of endothelial nitric oxide synthase, glutathione s-transferase p1, TNF α , IL-10, cholinergic nicotine receptor $\alpha 3$ and iron regulatory binding protein 2 (20), but it is early in their validation and so they are still considered ‘potential’ genetic modifiers of the lung phenotype.

There is still relatively limited information about the final steps by which proteotoxicity impairs liver cell function and leads to the dominant hepatic pathological effects, fibrosis and carcinogenesis. Alterations in mitochondrial structure and function together with activation of ER-specific and mitochondrial caspases have been observed in model systems as well as in the liver of patients (12) and therein suggest that mitochondrial dysfunction is part of the cytotoxicity. Chronic activation of TGF β signaling has been demonstrated as a specific response to hepatic accumulation of ATZ (10) and this is likely to play a role in the fibrosis/cirrhosis that is characteristic of this liver disease. A theory for oncogenesis which involves a trans-effect of liver cells that have marked intracellular accumulation of mutant ATZ (so-called “globule-containing hepatocytes”) causing chronic hyper-proliferation of liver cells that have less ATZ accumulation (“globule-devoid hepatocytes”) has been proposed (21).

Use of *Caenorhabditis elegans* to model ATD

Variation in age-of-onset and severity of illness, even for simple Mendelian disorders, has prompted the vigorous search for genetic modifiers associated with human disease. Identification of these modifiers will not only broaden our understanding of disease pathogenesis, but also in themselves serve as potential therapeutic targets. For rare diseases like ATD, human population-based studies are difficult to conduct, so investigators have turned to cell lines and animal systems to help elucidate the role of different genes on disease phenotypes. For ATD, comparisons between genetically modified cell lines or transgenic mice expressing either the wild-type (ATM) or ATZ proteins have been instrumental in defining several genes that modify the ATD phenotype (4). Although these model systems proved to be invaluable for conducting proteomic or global expression studies using microarrays or RNA sequencing, they are still somewhat unwieldy for conducting genome-wide modifier screens by systemic knock-downs or knock-outs using RNAi or chemical/insertional mutagenesis, respectively. For this reason, we determined whether a simpler multicellular organism more amenable to genome-wide analysis could be used to phenocopy the cellular toxicity associated with the ATD gain-of-function phenotype. We selected *C. elegans* because many of the basic cellular physiological processes (e.g., protein synthesis, secretion and degradation) between nematodes and humans are well-conserved, and this organism can be easily manipulated by transgenic approaches (22). Moreover, whole-genome RNAi screens in *C. elegans* can be conducted by simply feeding this organism bacteria (their normal food source) expressing different double-stranded RNAs derived from a library of plasmids containing *C. elegans* DNA (23).

Because *C. elegans* is transparent, we generated several transgenic lines with intestinal expression of ATZ or ATM fused to the green fluorescent protein (GFP). Intestinal cells were selected for expression because they assume most of the biosynthetic and detoxification functions of hepatocytes, which are missing in this organism. Using simple epifluorescence microscopy, we could follow the fate of the human wild-type and mutant proteins in real time throughout the lifespan of the animals. Animals expressing ATM

secreted the protein, whereas animals expressing ATZ showed a marked accumulation of misfolded, aggregated protein within the ER (Fig. 2). Moreover, the disposition of ATZ is governed by the proteostasis pathways (e.g., endoplasmic-reticulum associated degradation and autophagy) operating in higher vertebrates (4). Animals expressing ATZ also had a shorter lifespan, smaller brood sizes and delayed development compared with ATM-expressing or wild-type animals. Taken together, these studies showed that *C. elegans* phenocopies many of the molecular, cellular and clinical features of ATZ expression in mouse models of ATD and in patients.

These “humanized” ATZ-expressing *C. elegans* strains were used to develop automated assay systems that permit high-throughput drug screening for novel compounds or FDA-approved drugs that alter the disposition of ATZ (24). A phenotype-based screen of the Library of Pharmacologically Active Compounds (LOPAC) has already provided several new hits that are being examined for efficacy in mouse models. In addition, we have developed a similar semi-automated assay system to assess the effects of RNAi knockdown of ~18,000 *C. elegans* genes on ATZ accumulation (11). Using these data, we have identified several genes that have a major effect on ATZ accumulation. Many of these genes are homologous to human genes that have already served as druggable targets and are listed in several databases.

Treatment

The treatment options currently available to patients with liver disease due to ATD include supportive management of symptoms owing to liver dysfunction and prevention of complications and liver transplantation when the hepatic dysfunction becomes progressive (25). This is why several new strategies for prevention and/or amelioration of this liver disease have generated considerable excitement.

One of these promising new strategies is based on the concept that endogenous proteostasis mechanisms can be targeted to pharmacologically counteract the proteotoxicity of intracellular ATZ accumulation. We recently used this concept, targeting autophagy as a particularly important proteostasis mechanism in ATD because autophagy plays a major role in intracellular disposal of ATZ and because accumulation of ATZ in the ER specifically activates autophagy. From a list of compounds that are purported to enhance autophagy we selected carbamazepine (CBZ) because it has been used extensively in humans with a relatively wide margin of safety. We found that CBZ mediated increased intracellular degradation of ATZ in cell line models of ATD (14). To our surprise, the effect of CBZ was mediated by effects on several different degradative mechanisms in addition to stimulating the autophagic degradative system. Most importantly, oral administration of CBZ to the PiZ mouse model led to reduction in the hepatic ATZ load and decreased hepatic fibrosis after treatment intervals as short as 2 weeks. Because it is FDA-approved and has an extensive safety profile, CBZ could be moved into a phase II/III clinical trial immediately and is currently being tested in a double-blind randomized protocol for severe liver disease due to ATD.

As mentioned above, automated high-content screening of drug libraries using the *C. elegans* model has provided further validation for the autophagy enhancer class of drugs and has suggested some additional concepts for drug development. An initial screen of the LOPAC drug library identified 5 hit compounds that mediated dose-dependent reductions in whole organismal ATZ load at magnitudes greater than 4-fold (24). Interestingly, 4 of these 5 hit compounds have the property of enhancing autophagy. These 4 compounds are all in active clinical use and so, like CBZ, they can immediately be tested in clinical trials, ‘re-purposing’ them for ATD. Another very interesting aspect of these findings is that 2 of the

compounds are from the phenothiazine family, which is structurally related to the tricyclic antidepressants that are closely related to CBZ. The phenothiazines have also been shown to enhance autophagic degradation of the aggregation-prone protein huntingtin that causes Huntington's disease (26,27). Thus, this type of screening platform provides a wonderful new model for drug discovery for ATD and 2 new strategies for chemical and computational-based drug discovery using the autophagy enhancer drug paradigm and the phenothiazine structure.

Several studies have shown that a class of compounds called chemical chaperones can reverse the cellular mislocalization or misfolding of mutant plasma membrane, lysosomal, nuclear, and cytoplasmic proteins (including CFTRF508, prion proteins, mutant aquaporin molecules in nephrogenic diabetes insipidus, and mutant galactosidase A in Fabry disease) (28). We have found that 2 of these compounds, glycerol and 4-phenylbutyric acid (PBA), mediate a marked increase in secretion of ATZ in a model cell line system (29). Moreover, oral administration of PBA was well tolerated by PiZ mice (transgenic for the human ATZ gene) and consistently mediated an increase in blood levels of human AT, reaching 20 to 50% of the levels present in PiM mice and normal humans. Because PBA had been used safely for years in children with urea cycle disorders, this drug was considered a candidate for chemo-prophylaxis of both liver and lung disease in ATD. Although, a pilot study in 10 patients with liver disease due to ATD did not show an increase in serum levels of AT after 14 days of treatment with PBA (30), it is not entirely clear that this was a sufficient duration of treatment or that the ability of patients with chronic liver disease to tolerate the large dose of PBA required for effects in humans limited the outcome. Because PBA selectively affects secretion of ATZ and mediates its effect *in vivo*, and further because this kind of effect has not been observed for any other pharmacological agent, it will be important to further investigate PBA if more favorable formulations become available.

Recently a drug with chemical properties very similar to PBA, suberoylanilide hydroxamic acid (SAHA), was shown to increase secretion of ATZ in cell line models of ATD and this effect was probably mediated by inhibition of histone deacetylase HDAC7 (31). However this drug was not tested *in vivo*. Furthermore, unlike PBA, SAHA mediates a substantial increase in synthesis of ATZ through a transcriptional activation mechanism. It was not entirely clear whether the increase in ATZ in extracellular fluid could be largely attributable to the increase in synthesis. Even if the results are consistent with increases in synthesis as well as an increase in translocation through the secretory pathway the effect of this drug may be associated with increased accumulation of ATZ in the ER and therein possibly increased proteotoxicity.

Several groups have utilized a strategy for drug development that begins with designing peptides to prevent polymerization of the mutant ATZ molecule with the theory that this will influence its potential for secretion. A peptide that targets a lateral hydrophobic cavity in the ATZ was found to prevent polymerization but, in a cell model, it increased intracellular degradation rather than increased secretion (32). Another peptide which targets the reactive center loop appeared to increase the rate of secretion of mutant ATZ in a cell line but it was not clear if it also led to increased intracellular accumulation (33).

The treatment options currently available for emphysema due to ATD are also relatively limited. Avoidance of cigarette smoking is one very important principle for all patients with ATD. Cigarette smoking markedly accelerates the destructive lung disease associated with ATD, reduces the quality of life, and significantly shortens longevity (34). Some patients with ATD and emphysema are currently receiving replacement therapy with purified or recombinant plasma AT either by intravenous or intratracheal aerosol administration (35). This therapy is associated with an improvement in serum concentrations of AT and in

neutrophil elastase inhibitory capacity in bronchoalveolar lavage fluid, without significant side effects but there is limited evidence of clinical efficacy (36,37). A number of patients with severe emphysema from ATD undergo lung transplantation with five-year survival rates in the range of 60% (38).

Replacement of AT by somatic gene therapy has also been discussed in the literature (39). Before clinical trials involving gene therapy are conducted, it would be helpful to know that replacement therapy with purified AT, as it is currently applied, is effective in ameliorating emphysema in this deficiency. This strategy would be useful only in ameliorating emphysema because liver disease is not caused by a loss-of-function mechanism. However, two recent studies have suggested that gene therapy could be used to knockdown expression of the mutant ATZ gene (Figure 3) using vectors that also encode the wild type AT and therein would reconstitute the host (40,41). In one case, adeno-associated virus encoding short hairpin RNA to knockdown endogenous AT gene expression together with a codon-optimized wild type AT transgene cassette was used (40). In the other report, an adeno-associated virus encoding microRNA to silence endogenous AT gene expression together with a microRNA-resistant wild type AT gene was delivered (41). These approaches both led to high levels of human AT in the serum of a transgenic mouse model of ATD and significant reduction in hepatic ATZ accumulation but the reduction was not sufficient to alleviate liver fibrosis. Thus, the impression is that silencing would need to be more potent and widespread within the liver to prevent proteotoxicity.

Because transplanted hepatocytes can repopulate the diseased liver, cell transplantation therapy for ATD has also been discussed. Indeed, Ding et al demonstrated that wild-type donor hepatocytes can almost completely re-populate the liver of the PiZ mouse model of ATD (42). Use of this type of ‘cellular’ therapy could be an alternative approach to replacement therapy to prevent COPD and perhaps also to prevent/treat liver disease because the transplanted hepatocytes have a selective proliferative advantage over endogenous hepatocytes eventually completely eliminating the latter.

A combination of gene targeting and cell-based therapy may also be a potential strategy for COPD and perhaps liver disease in ATD. Yusa et al recently reported exciting results in which the mutation in the AT gene was corrected in human induced pluripotent stem cells (iPS cells) from a patient homozygous for ATZ by a combination of zinc finger nucleases and transposon technology and then the iPS cell line was shown to engraft into the liver of a transgenic mouse model (43).

Taken together, these advances in strategies for treatment of ATD parallel novel therapies being tested for many other diseases caused by misfolded proteins (44). We believe that further advances are likely in the near future, particularly from continued efforts to discern the unifying concepts that come from comparing different types of protein misfolding disorders to each other.

Acknowledgments

Thanks to Cliff Luke for the confocal image.

Funded by the National Institutes of Health (P01DK096990, R01DK084512, R01DK076918, R01DK079806), Children’s Hospital of Pittsburgh, and Magee Womens Hospital of UPMC.

References

1. Whittsett JA, Wert SE, Weaver TE. Alveolar surfactant homeostasis and the pathogenesis of pulmonary disease. *Annu Rev Med.* 2010; 61:105–119. [PubMed: 19824815]

2. Liu M, Hodish I, Haataja L, Lara-Lomus R, Rajpal G, Wright J, Arvan P. Proinsulin misfolding and diabetes: mutant INS gene-induced diabetes of youth. *Trends Endocrinol Metab.* 2010; 21:652–659. [PubMed: 20724178]
3. Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. *Science.* 2008; 319:916–919. [PubMed: 18276881]
4. Perlmutter DH. Alpha-1-antitrypsin deficiency: importance of proteasomal and autophagic degradative pathways in disposal of liver disease-associated protein aggregates. *Annu Rev Med.* 2011; 62:333–345. [PubMed: 20707674]
5. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. *N Engl J Med.* 2009; 360:2749–2757. [PubMed: 19553648]
6. Piitulainen E, Carlson JA, Ohlsson K, Sveger T. Alpha-1-antitrypsin deficiency in 26-year-old subjects: lung, liver and protease/protease inhibitor studies. *Chest.* 2005; 128:2076–2081. [PubMed: 16236857]
7. Gelling CL, Dawes IW, Perlmutter DH, Fisher EA, Brodsky JL. The endosomal protein-sorting receptor sortilin has a role in trafficking α -1 antitrypsin. *Genetics.* 2012; 192:889–903. [PubMed: 22923381]
8. Kamimoto T, Shoji S, Hidvegi T, Mizushima N, Umebayashi K, Perlmutter DH, Yoshimori T. Intracellular inclusions containing mutant alpha-1-antitrypsin Z are propagated in the absence of autophagic activity. *J Biol Chem.* 2006; 281:4467–4476. [PubMed: 16365039]
9. Hidvegi T, Schmidt BZ, Hale P, Perlmutter DH. Accumulation of mutant α 1-antitrypsin Z in the ER activates caspases-4 and -12, NF κ B and BAP31 but not the unfolded protein response. *J Biol Chem.* 2005; 280:39002–39015. [PubMed: 16183649]
10. Hidvegi T, Mircics K, Hale P, Ewing M, Beckett C, Perlmutter DH. Regulator of G signaling 16 is a marker for the distinct ER stress state associated with aggregated mutant α 1-antitrypsin Z in the classical form of α 1-antitrypsin deficiency. *J Biol Chem.* 2007; 282:27769–27780. [PubMed: 17635928]
11. Long OS, Luke CJ, Gosai SJ, Benson JA, Miedel MT, Vetica AC, King DE, Hidvegi T, Ewing M, Perlmutter DH, Silverman GA, Pak SC. A *C.elegans* model of proteotoxicity due to α 1-antitrypsin deficiency. (in review).
12. Teckman JH, An JK, Blumenkamp K, Schmidt B, Perlmutter DH. Mitochondrial autophagy and injury in the liver in alpha-1-antitrypsin deficiency. *Am J Physiol.* 2004; 286:G851–G862.
13. Kruse K, Brodsky JL, McCracken AA. Characterization of an ERAD gene as VPS30/ATG6 reveals two alternative and functionally distinct protein quality control pathways: one for soluble Z variant of human alpha-1-proteinase inhibitor (A1PiZ) and another for aggregates of A1PiZ. *Mol Biol Cell.* 2006; 17:203–212. [PubMed: 16267277]
14. Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, Maurice N, Mukherjee A, Goldbach C, Watkins S, Michalopoulos G, Perlmutter DH. An autophagy-enhancing drug promotes degradation of mutant α 1-antitrypsin Z and reduces hepatic fibrosis. *Science.* 2010; 329:229–232. [PubMed: 20522742]
15. He C, Bassik MC, Moresi V, Sun K, Wei Y, Zou Z, An Z, Loh J, Fisher J, Sun Q, Korsmeyer S, Packer M, May HI, Hill JA, Virgin HW, Gilpin C, Xiao G, Bassel-Duby R, Scherer P, Levine B. Exercise-induced Bcl2-regulated autophagy is required for muscle glucose homeostasis. *Nature.* 2012; 481:511–514. [PubMed: 22258505]
16. Wu Y, Whitman I, Molmenti E, Moore K, Hippenmeyer P, Perlmutter DH. A lag in intracellular degradation of mutant alpha-1-antitrypsin correlates with liver disease phenotype in homozygous PiZZ alpha-1-antitrypsin deficiency. *Proc Natl Acad Sci USA.* 1994; 91:9014–9018. [PubMed: 8090762]
17. Pan S, Huang L, McPherson J, Muzny D, Rouhani F, Brantly M, Gibbs R, Sifers RN. Single nucleotide polymorphism-mediated translational suppression of endoplasmic reticulum mannosidase I modifies the onset of end-stage liver disease in alpha-1-antitrypsin deficiency. *Hepatology.* 2009; 50:275–281. [PubMed: 19444872]
18. Chappell S, Guetta-Baranes T, Hadzic N, Stockley R, Kalsheker N. Polymorphism in the endoplasmic reticulum mannosidase I gene is not associated with liver disease in individuals

homozygous for the Z variant of the alpha1-antitrypsin protease inhibitor (PiZZ individuals). *Hepatology*. 2009; 50:1315. [PubMed: 19739260]

19. Chappell S, Hadzic N, Stockley R, Guetta-Baranes T, Morgan K, Kalsheker N. A polymorphism of the alpha-1-antitrypsin gene represents a risk factor for liver disease. *Hepatology*. 2008; 47:127–132. [PubMed: 17972336]
20. Kim WJ, Wood AM, Barker AF, Brantly ML, Campbell EJ, Eden E, McElvaney G, Rennard SI, Sandhaus RA, Stocks JM, Stoller JK, Strange C, Turino G, Silverman EK, Stockley RA, DeMeo DL. Association of IREB2 and CHRNA3 polymorphisms with airflow obstruction in severe alpha-1 antitrypsin deficiency. *Resp Res*. 2012; 13:16–25.
21. Rudnick DA, Perlmutter DH. Alpha-1-antitrypsin deficiency: A new paradigm for hepatocellular carcinoma in genetic liver diseases. *Hepatology*. 2005; 42:514–521. [PubMed: 16044402]
22. Silverman GA, Luke CJ, Bhatia SR, Long OS, Vetica AC, Perlmutter DH, et al. Modeling molecular and cellular aspects of human disease using the nematode *Caenorhabditis elegans*. *Pediatr Res*. 2009; 65:10–8. [PubMed: 18852689]
23. Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biol*. 2001; 2:2.1–2.10.
24. Gosai SJ, Kwak JH, Luke CJ, Long OS, King DE, Kovatch KJ, Johnston PA, Shun TY, Lazo JS, Perlmutter DH, Silverman GA, Pak SC. Automated high-content live animal drug screening using *C. elegans* expressing the aggregation prone serpin α 1-antitrypsin Z. *PLoS One*. 2010; 5:e15460. [PubMed: 21103396]
25. Perlmutter, DH. Alpha-1-antitrypsin deficiency. In: Schiff, ER.; Sorrell, MF.; Maddrey, WC., editors. *Schiff's Diseases of the Liver*. 11. Oxford: Wiley-Blackwell; 2012. p. 845-867.
26. Zhang L, et al. Small molecule regulators of autophagy identified by an image-based high-throughput screen. *Proc Natl Acad Sci USA*. 2007; 104:19023–28. [PubMed: 18024584]
27. Tsetkov AS, Miller J, Arrasate M, Wong JS, Pleiss MA, Finkbeiner S. A small-molecule scaffold induces autophagy in primary neurons and protects against toxicity in a Huntington disease model. *Proc Natl Acad Sci USA*. 2010; 107:16982–7. [PubMed: 20833817]
28. Perlmutter DH. Chemical chaperones: a pharmacological strategy for disorders of protein folding and trafficking. *Pediatr Res*. 2002; 52:832–6. [PubMed: 12438657]
29. Burrows JAJ, Willis LK, Perlmutter DH. Chemical chaperones mediate increased secretion of mutant α 1-antitrypsin (α 1-AT) Z. A potential pharmacological strategy for prevention of liver injury and emphysema in α 1-AT deficiency. *Proc Natl Acad Sci U S A*. 2000; 97:1796–801. [PubMed: 10677536]
30. Teckman JH. Lack of effect of oral 4-phenylbutyrate on serum alpha-1-antitrypsin in patients with alpha-1-antitrypsin deficiency: a preliminary study. *J Pediatr Gastroenterol Nutr*. 2004; 39:34–37. [PubMed: 15187777]
31. Bouche-careilh M, Hutt DM, Szajner P, Flotte TR, Balch WE. Histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA)-mediated correction of α 1-antitrypsin deficiency. *J Biol Chem*. 2012; 287:38265–38278. [PubMed: 22995909]
32. Mallya M, et al. Small molecules block the polymerization of Z alpha-1-antitrypsin and increase the clearance of intracellular aggregates. *J Med Chem*. 2007; 50:5357–63. [PubMed: 17918823]
33. Alam S, Wang J, Janciauskiene S, Mahadeva R. Preventing and reversing the cellular consequences of Z alpha-1-antitrypsin accumulation by targeting s4A. *J Hepatol*. 2012; 57:116–24. [PubMed: 22425623]
34. Janus ED, Phillips NT, Carrell RW. Smoking, lung function and alpha-1-antitrypsin deficiency. *Lancet*. 1985; i:152–4. [PubMed: 2857224]
35. Crystal RG. Alpha-1-antitrypsin deficiency, emphysema and liver disease: genetic basis and strategies for therapy. *J Clin Invest*. 1990; 95:1343–52. [PubMed: 2185272]
36. Stockley RA, Parr DG, Piitulainen E, Stolk J, Stoel BC, Dirksen A. Therapeutic efficacy of alpha-1 antitrypsin augmentation therapy on the loss of lung tissue: an integrated analysis of 2 randomised clinical trials using computed tomography densitometry. *Resp Res*. 2010; 11:136.

37. Gotzsche PC, Johansen HK. Intravenous alpha-1 antitrypsin augmentation therapy for treating patients with alpha-1-antitrypsin deficiency and lung disease. *Cochrane Database of Systematic Reviews*. 2010; 7:CD007851. [PubMed: 20614465]
38. Lynch JP, Saggar R, Weigt SS, Ross DJ, Belperio JA. Overview of lung transplantation and criteria for selection of candidates. *Semin Respir Crit Care Med*. 2006; 27:441–469. [PubMed: 17072794]
39. Flotte TR. Adeno-associated virus-based gene therapy for inherited disorders. *Pediatr Res*. 2005; 58:1143–1147. [PubMed: 16306183]
40. Li C, Xiao P, Gray SJ, Weinberg MS, Samulski RJ. Combination therapy utilizing shRNA knockdown and an optimized resistant transgene for rescue of diseases caused by misfolded proteins. *Proc Natl Acad Sci USA*. 2011; 108:14258–63. [PubMed: 21844342]
41. Mueller C, et al. Sustained miRNA-mediated knockdown of mutant AAT with simultaneous augmentation of wild type AAT has minimal effect on global liver miRNA levels. *Mol Ther*. 2012; 20:1131–8. [PubMed: 22395529]
42. Ding J, et al. Spontaneous hepatic repopulation in transgenic mice expressing mutant human α 1-antitrypsin by wild-type donor hepatocytes. *J Clin Invest*. 2011; 121:1930–4. [PubMed: 21505264]
43. Yusa K, et al. Targeted gene correction of α 1-antitrypsin deficiency in induced pluripotent stem cells. *Nature*. 2011; 478:391–4. [PubMed: 21993621]
44. Lindquist SL, Kelly JW. Chemical and biological approaches for adapting proteostasis to ameliorate protein misfolding and aggregation diseases: progress and prognosis. *Cold Spring Harb Perspect Biol*. 2011; 3:307–340.

Figure 1. Hypothetical paradigm for mechanisms of protection and susceptibility to liver disease in ATD. A putative liver cell from the protected host is shown on the left and compared to the susceptible host on the right. Mutant ATZ accumulates in the ER in each case but in cells of the susceptible host there is a subtle block in either ER degradation pathways (lower left) or cellular protective responses (lower right), leading to more accumulation/proteotoxicity.

Figure 2.

A *C. elegans* animal expressing a transgene driving expression of ATZ fused to GFP. (Top) Large aggregates of retained ATZ (green) within intestinal cells appear in this single plane confocal image. The wild-type protein is rapidly secreted into the pseudocoelomic space and only visible at very high integrations (not shown). The red region is a pharyngeal marker to mark the head region. (Bottom) DIC image of the same animal showing normal anatomy except for large vacuolated regions in the intestinal cells corresponding to the positions of the ATZ aggregates in the image above. Scale bar is 100 microns.

Figure 3.

Cellular targets of novel therapies for ATD. A liver cell is depicted with the putative targets of potential therapeutic strategies including gene silencing strategies that would inhibit synthesis of ATZ, autophagy enhancer drugs that increase degradation of ATZ and drugs like PBA and SAHA that could possibly increase secretion of ATZ.