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Review

Leveraging Population Genomics for Individualized Correction of the Hallmarks of Alpha-1 Antitrypsin Deficiency

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Abstract

Deep medicine is rapidly moving towards a high-definition approach for therapeutic management of the patient as an individual given the rapid progress of genome sequencing technologies and machine learning algorithms. While considered a monogenic disease, alpha-1 antitrypsin (AAT) deficiency (AATD) patients present with complex and variable phenotypes we refer to as the "hallmarks of AATD" that involve distinct molecular mechanisms in the liver, plasma and lung tissues, likely due to both coding and non-coding variation as well as genetic and environmental modifiers in different individuals. Herein, we briefly review the current therapeutic strategies for the management of AATD. To embrace genetic diversity in the management of AATD, we provide an overview of the disease phenotypes of AATD patients harboring different AAT variants. Linking genotypic diversity to phenotypic diversity illustrates the potential for sequence-specific regions of AAT protein fold design to play very different roles during nascent synthesis in the liver and/or function in post-liver plasma and lung environments. We illustrate how to manage diversity with recently developed machine learning (ML) approaches that bridge sequence-to-function-to-structure knowledge gaps based on the principle of spatial covariance (SCV). SCV relationships provide a deep understanding of the genotype to phenotype transformation initiated by AAT variation in the population to address the role of genetic and environmental modifiers in the individual. Embracing the complexity of AATD in the population is critical for risk management and therapeutic intervention to generate a high definition medicine approach for the patient.

Abbreviations: alpha-1 antitrypsin, **AAT**; alpha-1 antitrypsin deficiency, **AATD**; machine learning, **ML**; spatial covariance, **SCV**; cystic fibrosis, **CF**; Neimann-Pick C1, **NCP1**; chronic obstructive pulmonary disease, **COPD**; endoplasmic reticulum, **ER**; neutrophil elastase, **NE**; genome-wide association study, **GWAS**; carbamazepine, **CBZ**; trimethylamine N-oxide, **TAMO**; 4-phenylbutric acid, **PBA**; histone deacetylase inhibitor, **HDACi**; suberoylanilide hydroxamic acid, **SAHA**; wild-type, **WT**; adenovirus, **AVV**; retrovirus, **RTV**; adeno-associated virus, **AAV**; small interfering RNA, **siRNA**; human Z-AAT, **hZ-ATT**; human-induced pluripotent stems cells, **hIPSCs**; human mesenchymal stem cells, **hMSCs**; allele frequency, **AF**; variation spatial profiling, **VSP**; Gaussian process regression, **GPR**; cystic fibrosis transmembrane conductance regulator, **CFTR**

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Introduction

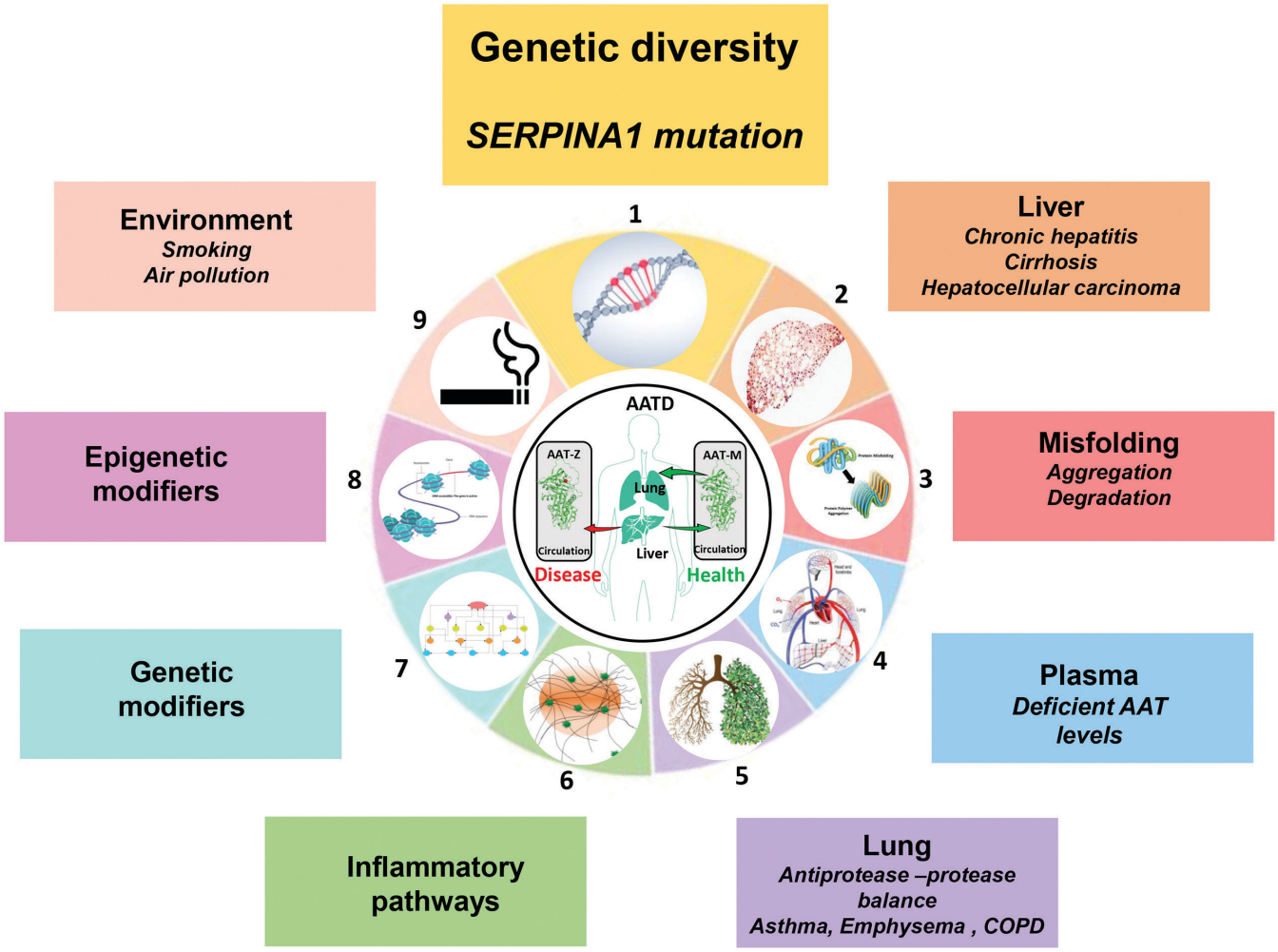
Recent advances in genome sequencing technology is transforming our understanding and application of genomics to monogenic rare disease, for example, developing molecular therapeutics that target specific genetic variants in cystic fibrosis (CF) and Niemann-Pick C1 (NPC1),[1-5] as well as to complex polygenic diseases[6,7] such as risk assessment for chronic obstructive pulmonary disease (COPD).[8] In addition to genomics, the development of techniques to monitor other personal omics, for example, epigenomics,[5,9,10] proteomics,[11,12] metabolomics,[13] microbiomics,[14] environmental exposomics[15] and human activity tracking,[16] as well as rapid progress of machine learning (ML) analytical tools,[1,17,18] is already advancing deep medicine[19] at a high-definition[20] and high-performance level.[21] Here, the patient is viewed as an individual in a population rather than being treated as an average of the population as occurs in conventional medicine.

Alpha-1 antitrypsin deficiency (AATD) is a monogenic familiar disease that is driven by basic and clinical hallmarks (Figure 1), each of which is uniquely influenced by genetic diversity in the population. Each of these hallmarks illustrate the complexity of disease associated with each patient in terms of genetics, tissue pathology (liver, plasma, lung), time of onset, progression and the environment. AATD is caused by genetic variants (alleles) in the *SERPINA1* gene which has been identified in all ethnic groups worldwide with a frequency of 1 in every 2500 whites of European descent.[22] Thought of as a monogenetic disease, AATD patients present highly variable phenotypes at different time frames with unique mechanisms that involve both gain and loss of function in different tissues (Figure 1). Alpha-1 antitrypsin (AAT) is synthesized in the endoplasmic reticulum (ER) and secreted from the liver through the exocytic pathway in large quantities on a daily basis to maintain a plasma concentration at 1-2 g/L.[23,24] A well-established biological function of AAT is its antiprotease activity within the lung that prevents tissue degradation by neutrophil elastase (NE) and is currently the most established genome-wide association study (GWAS) modifier leading to COPD.[25] More recently, AAT has been shown to have anti-inflammatory and immunomodulatory functions independent of the antiprotease activity that may contribute to AATD phenotypes.[26-29]

Approximately 95% of severe AATD cases are caused by the Z allele[22,30] where the glutamic acid residue is mutated to lysine residue at position 366 in the AAT polypeptide (sequence numbering including the signal polypeptide sequence). The Z variant leads to misfolding and polymerization of AAT in the ER of hepatocytes which can trigger hallmarks of liver disease phenotypes including chronic hepatitis, cirrhosis and hepatocellular carcinoma[31] (Figure 1). Liver disease phenotypes are very heterogeneous in presentation with only about 10%-15% of infants with homozygous Z variant (PiZZ) developing clinically relevant liver disease,[32-34] although all homozygotes have some degree of accumulation of misfolded Z protein in the liver[35] upon aging and progression of the disease (Figure 1). Epigenetic mechanisms including DNA methylation[10] as well as polymorphisms in ER mannosidase 1 gene (*MAN1B1*)[36] impacting glycosylation patterns, contribute to AATD liver phenotypes in subgroup cohorts[37] (Figure 1). There is also evidence that inherited traits influencing intracellular proteolysis mechanisms play a role in liver disease susceptibility. Due to the accumulation and degradation of Z-AAT in the ER of hepatocytes, a key hallmark of the disease is that only 10% to 15% of Z-AAT is secreted into the circulation (Figure 1). The loss of AAT function disrupts protease-antiprotease balance in the serum and lung, triggering emphysema and COPD[34] (Figure 1). Environmental factors, including cigarette smoke[38] and air pollution[39,40] contribute to the lung disease phenotype (Figure 1). Moreover, genetic modifiers including, for example, variants in matrix metalloproteinase 1 (*MMP1*),[41] tumor necrosis factor,[42] interleukin 10 (*IL10*),[43] iron regulatory binding protein 2 (*IREB2*) and cholinergic nicotine receptor alpha3 (*CHRNA3*)[44] impact the lung disease phenotypes in AATD (Figure 1). The heterogeneity and complexity of AATD presentation in the clinic indicates the importance of developing a high-definition, deep medicine[20] approach for each individual patient based on their genome sequence (Figure 1).

Herein, we briefly review the current therapeutic approaches for AATD. Not surprisingly, all efforts to date nearly exclusively focus on restoration of function of the dominant Z-variant polymerization. Thus, current approaches illustrate potential avenues for intervention for a single variant but fall short of the real problem– diversity in the population which

Figure 1. Hallmarks of Alpha-1 Antitrypsin Deficiency



AATD is a familial disease that occurs in response to genetic polymorphism in the *SERPINA1* gene. Clockwise in the figure, the first and most critical hallmark is genetic diversity in the population that triggers dysfunction of the liver (the second hallmark of disease) where ATT misfolding (the third hallmark of disease) produces aberrant forms of AAT depending on the AAT variant. Misfolding challenges the proteostasis program which is critical for the proper folding of AAT and thousands of other proteins during nascent synthesis in the ER. In some cases, a variant (such as the rare PiZZ variant state) leads to accumulation and polymerization of AAT in the liver due to loss of proteostasis balance which (approximately 15% of the PiZZ population) can lead to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. These results illustrate the potential impact of accumulated ER aggregate at the site of synthesis (ER) of nearly 1/3 of genome-encoded proteins. The loss of secretion of AAT into the circulation system (or the secretion of an inactive form) leads to reduced plasma levels of AAT activity (the fourth hallmark of disease). Low levels of AAT in plasma lead to reduced AAT to the lung (the fifth hallmark of disease) that results in loss of anti-elastase activity against NE and related proteases resulting in increased inflammation (the sixth hallmark of disease). The disruption of the antiprotease-protease balance in the lungs leads to onset and progression of asthma, emphysema and COPD that varies considerably between patients, reflecting the genetic variant of AAT driving disease in the individual relative to that population, and largely unknown genetic impacts of modifier genes including *MMP1*, *TNF*, *IL10*, *IREB2* and *CHRNA3* (the seventh hallmark of disease). The overall onset of disease is further reflected in epigenetic mechanisms (the eighth hallmark of disease) affecting *SERPINA1* gene expression and function. All of the first 8 hallmarks are subject to the impact of environmental factors from birth to death (the ninth hallmark). For example, smoking and air pollutions are highly prognostic for early deterioration of the lung given the environment challenged folding in the ER and activity of AAT in the lungs which is sensitive to oxidative modification leading to a more rapid progression and severity of disease. These results illustrate the global importance of proteostasis balance in management of the disease state in the individual.

AATD=alpha-1 antitrypsin deficiency; AAT=alpha-1 antitrypsin; ER=endoplasmic reticulum; NE=neutrophil elastase;

makes it patient-unique. To address this problem, we highlight genotypic diversity of AAT variants and the impact of variation on the hallmarks of AATD (Figure 1) driving clinical presentation. Finally, we discuss the importance of understanding the genotype to phenotype transformation from the perspective of a new paradigm based on the concept of spatial covariance (SCV) in biology to individualize treatment of each patient from a *genotype first* perspective. In essence, we embrace the concept of "leave no patient behind," a current goal of the Cystic Fibrosis Foundation,[2] as the operational paradigm for AATD treatment.

Current Generic Therapeutic Approaches for Z-Variant Alpha-1 Antitrypsin Deficiency

The vast majority of AATD therapeutic efforts target the dominant Z-variant allele. Here, the patient is treated as an average of the disease etiology of the Z-variant population based on either a reduced level of AAT in plasma and/or progression to endpoint liver/lung damage.

Enzyme Replacement

The current standard of care is based on the principle of enzyme replacement. Here, a quantitative increase in the serum pool of full-length AAT is mediated by intravenous infusion of plasma derived AAT (60mg/kg/wk), elevating transiently serum AAT to 50% of normal.[45] Treatment with 120 mg/kg/wk in a recent clinical trial has been shown to approximate a low-normal serum level of AAT with an associated increased antiprotease activity, decreased elastin degradation, and reduced airway inflammation.[46] A problem with this approach is that it fails to prevent the inherent accumulation and toxic effects of the expressed Z-variant in the patient.

Chemical and Biochemical Approaches

A number of small-molecule chemical and biochemical approaches are under development for AATD therapy with the goal of fixing the protein misfolding problem in the ER. These efforts largely focus on the aberrant polymerization events that leads to liver pathology and markedly reduces protein secretion into the plasma leading to reduced function in the lung.

One small-molecule chemical approach that addresses Z-variant aggregation in the liver makes use of the Food and Drug Administration (FDA)-approved drug carbamazepine (CBZ) that enhances autophagic pathways thereby reducing Z-AAT polymer intracellular accumulation in multiple cell and mouse models of the disease.[47] CBZ is currently in phase 2 clinical trials for individuals with severe liver disease (NCT01379469).[30] Furthermore, the bile compound nor-ursodeoxycholic acid has been shown to clear > 70% of intrahepatic Z-AAT and reduced hepatocellular death through autophagy mechanism, suggesting a novel therapeutic approach for the treatment of liver disease in AATD.[48,49]

An alternative approach to reduce aggregation involves the FDA-approved drug rapamycin, an mTOR inhibitor, that has been demonstrated to reduce the accumulation of hepatic polymerized Z-AAT and hepatocellular injury in a Z-AAT transgenic mice model.[50] Moreover, the generic proteostasis[51-53] drug trimethylamine N-oxide (TAMO) has been shown to protect Z-AAT protein from heat-induced polymerization in vitro, although it has no effect on Z-AAT protein secretion or protein re-folding in cell models.[54] In contrast, beside inhibiting Z-AAT polymerization and enhancing polymerized Z-AAT degradation, 4-phenylbutyric acid (PBA), as a proteostasis effector of unknown function, has been shown to promote Z-AAT protein secretion and function in both cell model and mouse models.[55] However, in 2 small human phase 2 trials in AATD patients, PBA had either no significant change on serum AAT levels, or led to a small increased serum AAT level in 11 PiZZ AATD patients.[45]

While the above compounds are thought to directly impact proteostasis pathways, histone deacetylase inhibitors (HDACi), including suberoylanilide hydroxamic acid (SAHA), have been shown to increase Z-AAT protein secretion from epithelial cell lines to 50% of wild-type (WT) levels.[56] The exact mechanism remains unknown but could reflect epigenetic mechanisms (histone acetylation) leading to changes in the gene expression profile impacting protein folding programs and AAT stability in the ER, or post-translational mechanisms reflecting acetylation/deacetylation of AAT or membrane trafficking pathways facilitating AAT delivery in the cell.[56]

An alternative chemical strategy is to prevent

AAT polymerization using small molecules[57,58] and peptides.[59,60] AAT polymerization blocking molecules have been developed through virtual ligand screening against the hydrophobic cavity which is formed by s2A β-sheet, helix D and helix E in the AAT structure.[57,58] A number of reagents have been identified as potential binders that block Z-AAT polymerization in vitro and in the cell leading to improvement of liver pathology, although delivery of these reagents to intracellular compartments where the problem is initiated remains a major challenge.[30,58,61]

In contrast to small-molecule chemical approaches impacting folding and stability of the Z variant, a biochemical approach involving the AAT-specific monoclonal antibody 4B12, which interacts with AAT protein by binding a conformation sensitive epitope involving residues Glu32, Glu39, His 43 and Leu 306, has been shown to block AAT polymerization in vitro, providing a potential approach to reduce the aggregation prone phenotype of Z-AAT.[62] A single chain-variable-fragment intrabody has been shown to rescue Z-AAT function by both blocking intracellular Z-AAT polymerization and increasing functional Z-AAT protein secretion in cell and animal models, although the physiological mechanism remains a mystery given that intracellular Z-AAT pools in the ER are unlikely to be targeted by exogenous delivery of these reagents.[63] However, these results provide evidence that monoclonal antibodies could be used as a therapeutic approach in treatment of AATD, possibly targeting Z-AAT that is secreted into plasma and in lung tissue to prevent further polymerization.[64-66]

While all small-molecule chemical- or biochemical-based approaches are preliminary, each of these results raise the hope for small-molecule efforts for Z-variant AATD therapy by targeting mechanistically principles of fold design responsible for stability, monomer-polymer ratio and/or restoration of activity in the liver.[45]

Genetic Therapy for Alpha-1 Antitrypsin Deficiency

An alternative strategy to the chemical- or biological-based interventions described above, are genetic therapy approaches. Here, the goal is to alter the genotype of the liver (or lung) to replace or convert the variant to a WT genotype in order to directly prevent aggregation disease in the liver and/or provide the lung with endogenously produced AAT to prevent destruction of the lung by NE and related protease activities, thereby reducing the impact of disease hallmarks (Figure 1).

In the past decade, a number of delivery vectors have been developed to administer WT AAT to the liver including adenovirus (AVV), retrovirus (RTV) and adeno-associated virus (AAV) vectors.[24,67] AVV gene transfer has been used to express human AAT protein in rat lung respiratory epithelium and hepatocytes.[68,69] RTV has been used for expression of human WT-AAT mice fibroblasts and dog hepatocytes.[70,71] Currently, AAV vectors are the most promising tool for gene delivery given their low toxicity, targeting specificity (liver, muscle, lung), and efficient long-term expression.[72] Multiple serotypes have been used in preclinical AATD studies, whereas AAV1, AAV2 and AAVrh.10 have been used in clinical studies.[73] To date, AAV1 is considered the vector of choice given sustained expression of AAT, although in phase 2 clinical trials only 2% of the needed therapeutic level was sustained over 5 years, suggesting considerable ground for improvement.[74] A major drawback of these efforts is the inability to remove the toxic impact of the Z-variant allele in the patient.[67]

To relieve the burden of accumulated variant protein-triggering liver damage, another strategy is to reduce the level of misfolded protein in the ER. Small interfering RNA (siRNA) and microRNA have been developed to efficiently silent hepatocyte human Z-AAT (hZ-AAT) protein expression and serum Z-AAT protein level in hZ-AAT mice through delivery by recombinant adeno-associated virus delivery vectors.[75] Moreover, antisense oligonucleotides targeting hAAT reduced Z variant plasma levels and reduced intracellular AAT protein accumulation in hZ-AAT transgene expression in mouse models. With long-term treatment, liver disease was reversed and a decrease in liver fibrosis was observed.[76] A similar result was observed in human PiZZ transgene expressing mice treated by AAV8 delivering AAT specific shRNA. shRNA decreased 90% of the AAT mutant protein.[77] Furthermore, siRNA targeting liver production of Z-AAT has been used in human clinical trials by 2 delivery vectors: ALN-AAT (NCT02503683) and ARC-AAT (NCT02363946) by Alnylam Pharmaceuticals and Arrowhead Research Corporation, respectively.[78] Phase 1/2 clinical

trials using ALN-AAT have been conducted using homozygous Z-AAT allele patients since 2015. Clinical data shows a nearly 88.9% reduction of circulating Z-AAT.[77] In ARC-AAT clinical trials, the vector was tolerated and had sustained reduction of AAT (up to 90%) in a phase 1 trial.[30] These studies demonstrate that RNA interference represents a potential therapeutic approach for AATD liver disease but is limited by failing to fix the primary problem–the need for a sufficient level of functional AAT delivery to the plasma to protect against lung disease.

An alternative strategy to disease is to apply gene editing approaches such as CRISPR/Cas9 and related rapidly evolving technologies.[79] As a powerful tool for genome modification, gene editing systems have now been used to correct genome variants in multiple rare disease model systems.[80-83] Editing vectors transfected into Z-AAT transgenic PiZZ mice provides a proof-of-principle for the approach where hZ-AAT variant expression was reduced, circulating hATT protein levels were increased, and liver fibrosis and protein aggregation reduced.[84-86]

Cell Therapy for Alpha-1 Antitrypsin Deficiency

While liver transplantation is the end-point strategy for treatment of liver disease, it has severe limitations given the need for donor tissue as well as the need for comprehensive immune suppression to prevent rejection. As an alternative approach to transplantation, AAT protein secretion has been observed by transplantation of hepatocytes from LacZ-transgenic ROSA26 mice into human Z-AAT transgenic mice. These results raised the possibility of hepatocyte transplantation as a therapeutic method for treatment of AATD in humans.[87] One possibility is that liver cells differentiated from stem cells from a normal individual found to express normal level of WT-AAT protein[88,89] could be used as a transplantation approach, although issues associated with immune rejection and toxic Z-variant load remain. Alternatively, human-induced pluripotent stem cells (hIPSCs) from the PiZZ patients could be differentiated into hepatocyte-like cells which express Z-AAT.[90,91] hIPSC-Z-AAT expressing cells show all the hallmarks of disease-disrupted mitochondrial structure, oncogenic protein AKR1B10 expression, upregulated inflammatory genes and induction of the unfolded protein response pathways.[92] hIPSC Z-AAT can be corrected by gene editing using a combination of zinc finger nucleases and piggyBac with restored function and AAT secretion. When edited hIPSCs were transplanted into a mouse model of liver injury (Alb-uPA+/+; Rag2-/-; Il2rg-/-) they were distributed throughout the liver leading to AAT secretion.[93] Moreover, edited human mesenchymal stem cells generated from patient stem cells have recently been shown to improve liver pathology as reflected in reduced inflammatory response and decreased fibrosis and apoptotic death compared to hZ-AAT-expressing hepatocytes in a mouse model.[94]

Tackling Genotypic Diversity in Alpha-1 Antitrypsin Deficiency One Patient at a Time

While the Z-variant is the current focus of most therapeutic efforts, the complexity of AATD management is considerably amplified by the large number of variants now detected in the world-wide population through genotyping and genome sequencing efforts.[95-99] In the genome aggregation database (gnomAD, v2.1.1),[96] which includes the genome sequence information for 141,456 individuals, 601 variants in the *SERPINA1* gene are reported. Among these reported variants, 277 are missense variants introducing amino acid residue changes in the polypeptide sequence with 116 being synonymous variants; 88 are in the intron region; 61 are in the untranslated region; 31 are splicing variants and the remaining 28 are deletion, truncation or frameshift. The top-3 most frequent variants are E400D (allele frequency [AF] = 27%), V237A (AF=22%) and R125H (AF=15.6%) which are historically referred to as M alleles (M3, M1 and M4 respectively) to indicate their WT-like activity. The S (AF=2.3%) and Z alleles (AF=1.1%) are fourth and fifth in allele frequency as missense variants, respectively.[95] The S allele (E288V) leads to a milder deficiency of AAT in plasma and the heterozygous PiSZ has a lower risk of lung disease than PiZZ individuals.[100] However, the clinical impact of the majority of rare variants is poorly understood or completely unknown. Thus, many alleles may be contributing in unknown ways to other features of disease etiology impacting the hallmarks of disease (Figure 1).

Besides the more common alleles listed above, we examined the distribution of 40 AAT missense

variants found in the AATD population contributing to liver and/or lung phenotypes on the AAT structure, to illustrate the potential impact of genetic diversity AAT protein fold design (Table 1[101-147]; Figure 2, upper panel). Here, patients carrying the variants illustrated in red have been reported to show both liver and lung phenotypes. Patients carrying orange variants have been reported with only the liver-associated phenotype while patients with yellow variants have been reported with only the lung-associated phenotype, whereas variant M382R, which is indicated in light blue, has a unique bleeding phenotype likely impacting the proteases in the coagulation cascade.[145,148-150] Variants found in the region of the reaction center loop adjacent to β-sheet A or in β-sheet A (Figure 2A, left panel, oval 1), such as E366K (Z), H358D and V357M, all have liver-associated phenotypes consistent with the known loop-sheet mechanisms leading to the polymerization of AAT in the liver that are predicted to impact AAT stability.[61,151,152] Furthermore, deletion of F76 (F76del) and S77F at the back of β-sheet A (Figure 2A, left panel, oval 1) also shows liver disease phenotypes, consistent with the interpretation that they may disrupt the stability of β-sheet A leading to an increase of the polymerization propensity of AAT.[146,153,154] Variants in β-sheets B and C (Figure 2A, left panel, oval 2) have more diverse phenotypes, for example, most of them are lung phenotype-associated variants, though some of variants also show liver-associated phenotypes. These variants are close to the "gate" region which has been shown to allosterically mediate loop-sheet polymerization.[147,152,155-158] Surprisingly, the variants localized to the bottom of the molecule (Figure 2A, left panel, oval 3) are exclusively associated with lung disease phenotype, indicating they do not cause aggregation in the liver but could affect secretion. Moreover, mapping these variants on AAT in complex with NE (Figure 2B) reveals that they are near the binding interface to NE, indicating these variants may even exclusively impact anti-protease (NE or other proteases) activity in the plasma or lung. It is possible that the many other variants have uncharacterized functions, for example, they may also contribute to ER-associated degradation promoting AAT deficiency, contribute to stability and/or translocation of AAT from the plasma to the lung, or modulate inflammation-related sensing in the lung.

Applying Deep Medicine to Individualizing Treatment in Alpha-1 Antitrypsin Deficiency

We now appreciate that the genome encodes key, evolutionary conserved information that reflects protein fold design principles that are largely unknown, yet define the functional requirements driving natural selection in response to a changing environment.[1]

To appreciate how the genome encodes these protein fold design principles, the Balch laboratory has developed variation spatial profiling (VSP) based on the principle of SCV[1] (Figure 3A). VSP is a Gaussian process regression (GPR)-based machine learning (GPR-ML)1 approach that re-describes central dogma in the context of SCV matrices that link sequence-to-function-to-structure.[1] Here, SCV relationships are defined by a sparse collection of variants found in the extant human population for any protein (such as the 40 variants found in AAT) (Figure 2). We refer to these known variants as *trusted* or *fiduciary* reporters, as they record the evolving genomic rules driving protein fold design. Using these variants to train the hidden layers using GPR-ML (Figure 3A), we generate sequence-to-function descriptions of the entire protein fold, that we refer to as "phenotype landscapes"[1] (Figure 3A and 3B). Phenotype landscapes can be generated by using the sparse collection of variants from any protein to build SCV relationships based on their sequence position in the genome relative to functional features. For example, we have pioneered application of SCV using the rare disease protein cystic fibrosis transmembrane conductance regulator (CFTR). CFTR, like AAT, traffics through the secretory pathway. At the cell surface, instead of being secreted like AAT, it functions as a Cl- channel function (Figure 3A and 3B)[158] When defective in disease in response to over 600 variants in the population,[3,159] patients present with markedly diverse clinical phenotypes, even for the same prominent F508del allele,[2,3,160] These known relationships are then used to predict the function of all uncharacterized residues in the protein sequence design (Figure 3A and 3B) that is displayed as a phenotype landscape and which can be mapped to protein structure at atomic resolution (detailed methods are explained in Wang et al[1]) (Figures 3A and 3C). Phenotype landscapes are very robust in design, allowing the construction of phenotype landscapes that measure and predict the protein sequence response

Table 1. Alpha-1 Antitrypsin Missense Variants with Described Patient Phenotypes

Variant	Allele Name	Clin Var	Lung Syndrome	Liver Syndrome	Other Syndrome	Reference
S6L	$Z_{Wrexham}$	Pathogenic	Pneumonia; Emphysema; Asthma	No evidence for liver disease		101
S38F	SDonosti	NA	Mild COPD; Idiopathic pulmonary fibrosis	NA		102
H39N	$E_{Johannesburg}$	Uncertain significance	Asthma	NA		103
R63C	I	Pathogenic/ Likely pathogenic	Bronchitis; Pneumonia	Liver cirrhosis; Liver enlargement		104-106
L65P	$M_{procida}$	Pathogenic, other	Emphysema; Dyspnea	No accumulation of AAT in hepatocytes		107,108
I74N	Tijarafe	NA	Emphysema	NA	Chronic inflammatory demyelinating polyneuropathy; Chronic diarrhea	102,109
F76del	$M_{malton\ (\Delta TTC)}$ or $M_{Palermo(\Delta TTC)}$	Pathogenic/ Likely pathogenic, other	Dyspnea; Emphysema; Pneumonia; COPD	Hepatic inflammation; Raised serum alanine aminotransferase levels and serum aspartate aminotransferase levels; Portal inflammation		106, 110-113
S77F	S_{iiyama}	Pathogenic, other	Overinflated lungs and bullous changes; Obstructive ventilatory impairment; Dyspnea	No apparent liver dysfunction, but PAS positive inclusion bodies in hepatocytes		146
A82D	Sevilla	NA	Severe COPD	NA		102
G91E	$M_{mineral\ springs}$	Pathogenic, other	Dyspnea; Severe emphysema	No evidence of liver disease		114
T92I	$Q0_{lisbon}$	NA	Bronchial asthma	NA		115,116
E99V		likely benign	Emphysema; Obstructive defects	NA		117
N107K	$Z_{la\ palma}$	NA	Bronchial asthma or bronchial hyperreactivity	No found evidence for liver disease		118
T109M	$Z_{bristol}$	Uncertain significance	Breathlessness	Perinatal deaths from fulminant liver disease of living offspring		119,120
I116N	$Q0_{ludwigshafen}$	Pathogenic, other	Lungs affected	Liver affected		121-124

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G139S	$Q0_{newport}$ or Q0Devon	Pathogenic, other	Asthma; Bronchitis; Pneumonia	Neonatal hepatitis; Raised serum AST and ALT		101, 124, 125
E175K	Cadiz	NA	Asthma	NA		102
E186G	$Q0_{Gaia}$		Dyspnea and Cough	NA		126
E228K	X	Likely benign	NA	Liver in a fibrotic stage		109, 127, 128
F232K		NA	Dyspnea on exertion; Emphysema; Asthma	NA		109, 118
V234E	$M1_{pierre\text{-}bénite}$	Uncertain significance	Emphysema; Atelectasis; COPD	NA		109, 129
R247C	F	pathogenic	Dyspnea; COPD	No history of liver disease		106,128, 130,131
G249R	$P_{brescia}$	Likely pathogenic	NA	Raised serum levels of transaminases; Mild liver steatosis		132
F251C	Tarragona	NA	Severe COPD	NA		102
T273A	Puerto Real	NA	Mild COPD	NA		102
D280V	P_{lowell} or $Q0_{cardiff}$	Likely pathogenic, other	Bronchitis with wheezing; Dyspnea; Emphysema	AST and ALT raised; Palpable liver; no evidence for developed as liver disease yet		106, 133, 134
K283I	M_{pisa}	Uncertain significance	Dyspnea; Emphysema	NA		106, 147
L287P	$Q0_{gaia}$	NA	Diffuse emphysema	NA		135
E288V	S	Pathogenic, other	COPD	No evidence		106,128, 136,137
G344R	$P_{salt\ lake}$	NA	Emphysema	NA		128, 133, 138
S354F	Smunich	Uncertain significance	Family spontaneous pneumothorax; Respiratory distress syndrome	NA		128, 139
V357M		NA	NA	Chronic liver disease; Fibrosis and inflamination in liver		109, 140
H358D	King's	Likely pathogenic	NA	Prolonged neonatal jaundice; Portal inflammation; Minimal portal fibrosis; Periportal steatosis; PAS granule		141
E366K	Z	Pathogenic, other	COPD	Liver cirrhosis		106, 142
M382R	Pittsburgh	Pathogenic	No evidence of lung disease	No evidence of liver disease	Bleeding	145
K392E	$E_{Taurisano}$	NA	Panlobular emphysema	Mild hyper-transaminasemia		126,147

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P393L	$M_{heerlen}$	Pathogenic/ Likely pathogenic, other	severe COPD	No evidence of liver disease		108, 116, 126
P393S	$M_{wurzburg}$	Pathogenic	NA	Intrahepatic accumulation of AAT; Portal fibrosis		116, 126, 143
M409T		Pathogenic	COPD	NA		144
P415H	$Y_{orzinuovi}$ or $Y_{Barcelona}$	NA	NA	Liver inflammatory, mild steatosis; Persistent increased transamine levels		147

COPD=chronic obstructive pulmonary disease; NA=not applicable; AAT=alpha-1 antitrypsin; PAS=periodic acid-Schiff stain; AST=aspartate aminotransferase enzyme; ALT=alanine aminotransferase enzyme

on a residue-by-residue basis to a broad spectrum of basic and clinical functional features, and in response to the changing environment that drives onset and progression of human disease.[1,161]

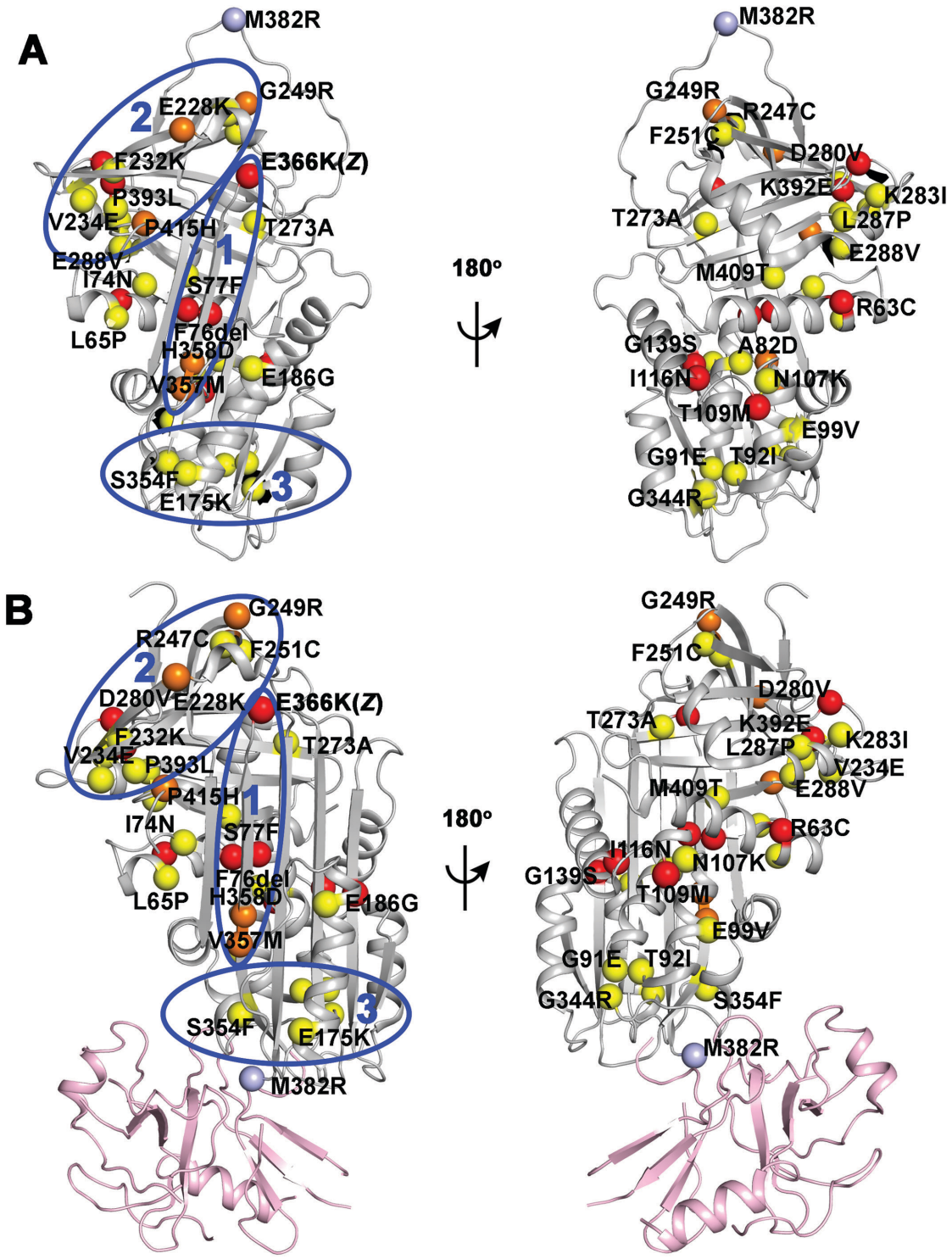
As an example of the utility of SCV relationships in understanding therapeutic development and with the goal of understanding the impact of genetic diversity on management of AAT in the clinic, we generated phenotype landscapes based on 63 CFTR variants[1] to reveal the function of all residues across the entire CFTR sequence, illustrating that some variants effect ER export, while others only impact chloride channel function at the cell surface (Figure 3B, left panel). Whereas the most common variant, F508del, prevents ER export, the rare CFTR variant G551D was the basis for the first FDA-approved personalized therapy for CF—the drug ivacaftor (Figure 3B and 3C).[159,162] G551D is a gating mutation that does not affect trafficking to the cell surface, as observed for some AAT variants that are efficiently secreted yet lack function (Figure 2). We have shown, using SCV relationships, that 63% of CFTR residues found in the disease population could be corrected by ivacaftor (Figure 3C).[1] More recently, in combination with other compounds targeting different features of the CFTR folding trajectory based on our current knowledge of the genotype impact on CFTR function,[1,3,159] a new triple combination of drugs improved the response of even the most severe F508del variant,[160,163-165] indicating that nearly all variants may be differentially accessible to correction in the clinical setting based on a complete understanding of SCV relationships contributing to disease. A second example are the rare variants found in the NPC1 protein (600 patients world-wide) responsible for NPC1 disease which maintains cholesterol homeostasis in all cell types.[166,167] Over 300 variants are now known to contribute to the differential onset and progression of cholesterol mismanagement leading to early onset neurodegenerative disease.[4] Application of GRP-ML to NPC1 disease, using around 50 variants triggering disease, reveals that both proteostasis[4] and epigenetic[5] approaches can be used to correct cholesterol deficiencies of many but not all variants, revealing the power of SCV relationships in addressing the individualized nature of human disease where each patient in essence is unique and therefore requires a comprehensive understanding of disease phenotype.

Managing Alpha-1 Antitrypsin from a Spatial Covariance Perspective

AAT is a metastable protein and, like the CFTR transmembrane channel that goes through rapid open and closed cycles for chloride conduction, is a remarkable example of a conformation that has evolved to be sufficiently stable to support its synthesis in the ER of the liver through the exocytic pathway and secretion to plasma,[151] while still maintaining its flexibility to perform in post-liver environments with its structural acrobatics that prevent the function of NE (or other proteases) in the lung in response to a large conformational change of molecule.[152,161,168] This dynamic conformational change is also, from a genetic diversity perspective, the Achilles heel of the disease.

Given the above, it is now apparent that different AAT variants will need to be understood in the context of their basic fold design principles from both basic

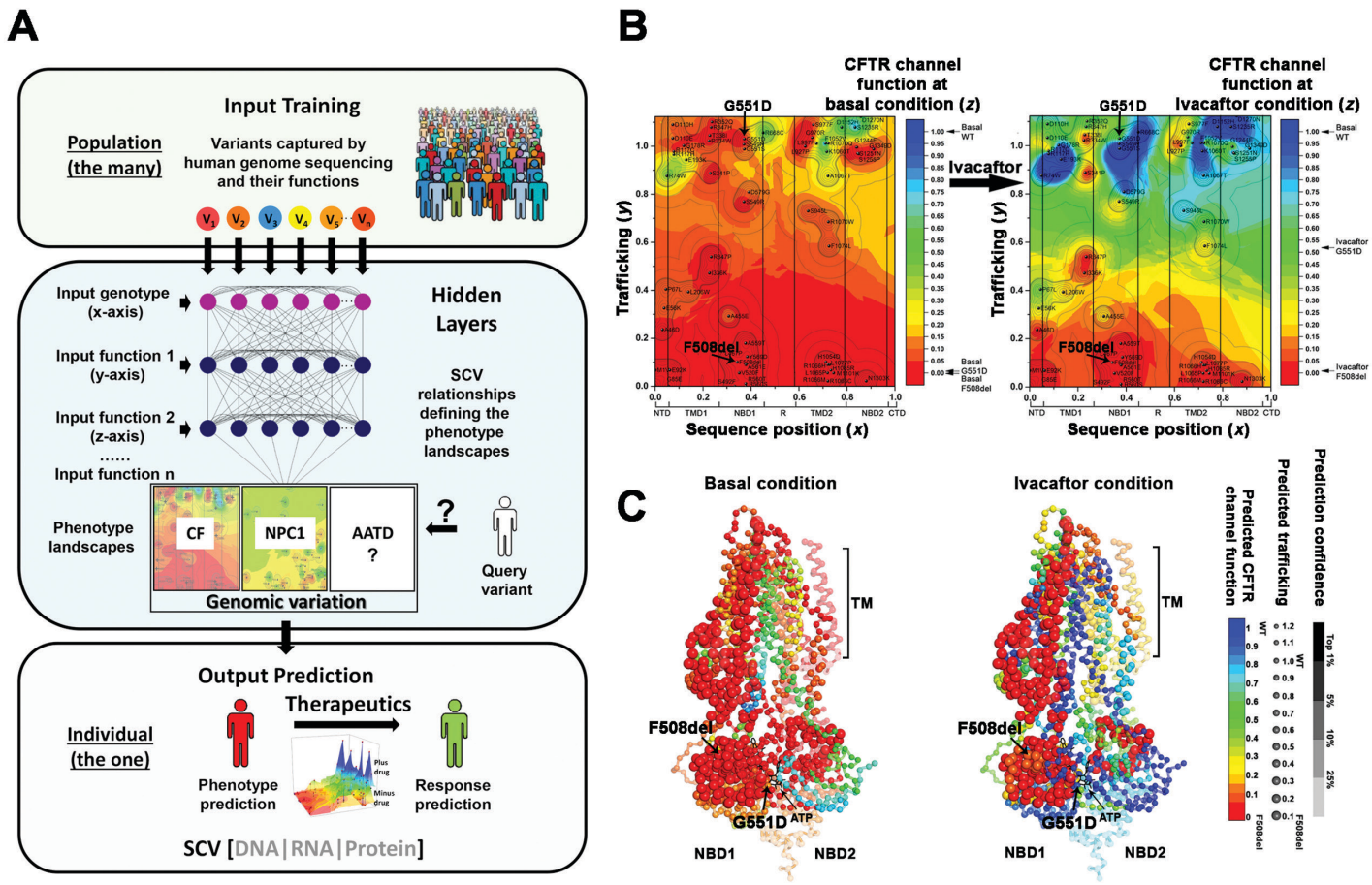
Figure 2. Mapping Phenotypic Diversity of Alpha-1 Antitrypsin Variants onto Alpha-1 Antitrypsin Structure



AAT variants in Table 1 are shown as balls in AAT structure (PDB:3n4E) (**A**) or the structure of the AAT and NE (PDB:1EZX) (**B**). A red ball illustrates that the variant is associated with both liver and lung disease phenotypes. An orange ball illustrates that the variant has only a liver-associated phenotype, while a yellow ball illustrates a variant with only a lung-associated phenotype. M382R is shown in light blue to indicate its unique bleeding phenotype.

AAT=alpha-1 antitrypsin; NE=neutrophil elastase

Figure 3. Applying Variation Spatial Profiling to Drive Individualized Medicine



(**A**) VSP[1] is a universal GPR-ML platform that utilizes a sparse collection of variants from human population (upper panel) as Input to quantitatively define through matrices (the hidden layers in the middle panel) representing sequence-to-function-to-structure relationships for every residue in a polypeptide sequence in terms of SCV-based phenotype landscapes (middle panel). As Output (lower panel), SCV relationships help us to perform risk management and understand the impact of therapeutics for each patient on a one-by-one (individual) basis. (**B**) An example of how knowledge of SCV relationships can be used to build phenotype landscapes reflecting the impact of the FDA-approved high-definition drug ivacaftor for the entire CFTR polypeptide sequence. The impact of a given variant on CFTR function can be assessed in the context of 3-dimensional coordinates, where the x-coordinate is sequence position of mutated residue, the y-coordinate represents the trafficking index to the cell membrane and the z-coordinate represents CFTR channel function (i.e., Cl- conductance). The z-coordinate is shown as color scale where red-to-yellow indicates low channel function of patient variants and green-to-blue indicates values approaching WT channel function. SCV uses information from only a sparse collection of variants to build a continuous landscape in the absence (left panel) and presence (right panel) using GRP-ML. Here, we illustrate the impact of ivacaftor on the basal state of the fold to show how every residue in the CFTR polypeptide sequence responds in the presence of ivacaftor. F508del and G551D are highlighted by arrows illustrating that F508del fails to respond to ivacaftor (as well as many other residues), whereas G551D and many other residues that traffic to the cell surface respond along a gradient from weak to robust. (**C**) The phenotype landscapes can be mapped on CFTR structure to generate a "functional structure" that illustrates the response of all residues at atomic resolution in the absence (left panel) and presence (right panel) of ivacaftor. Ball color represents predicted CFTR channel function, ball size represents predicted efficiency of export from the ER and ball transparency represents the confidence of the prediction (uncertainty) where over 95% of residues fall in the high confidence region for prediction. Figure taken from reference 1.

VSP=variation spatial profiling; GRP-ML=Gaussian process regression-machine learning; SCV= spatial covariance; FDA=Food and Drug Administration; CFTR=cystic fibrosis transmembrane conductance; ER=endoplasmic reticulum; WT=wild-type

and clinical sequence-to-function perspectives that can be captured by profiling SCV relationships.[4] For example, the plot of variants on the known structure of native and NE-bound AAT (Figure 2) already suggests that different variants and their projected SCV relationships defining the AAT fold will have functions that are likely independent of one another, not only given the physical spatial differences associated with their sequence position, but the contribution of a given variant for a particular known and/or unknown function in the complex environments of host (i.e., liver versus plasma versus lung). For example, the liver is involved in nascent synthesis and management of fold monomer solubility at high concentrations in the ER for efficient secretion that is differentially challenged by different variants, whereas the post-liver function in plasma and/or lung environments is critical for inhibitor activity driving disease presentation and progression in the clinic (Figure 2).

Thus, by selectively applying knowledge of genetic diversity found in the extant AATD population through building SCV relationships based on GPR-ML, it may become possible to tailor therapeutic development and management to specific alleles that are unique to an individual. Such a "genotype first" approach will allow us to specifically and differentially, mitigate the hallmarks of AATD in an individualized, deep medicine approach (Figure 1).

Summary and Perspective

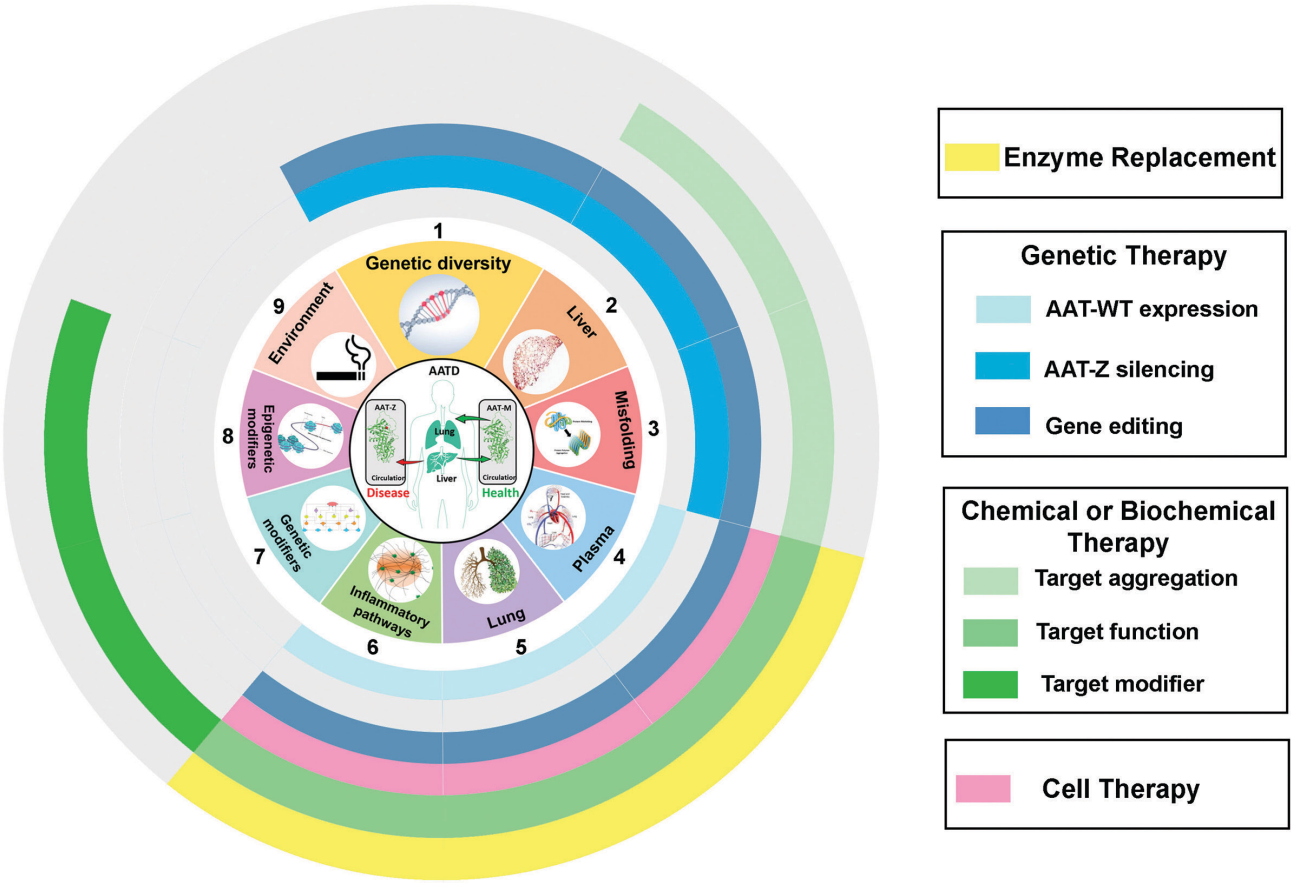
What is the best approach to manage AATD? Current approaches are illustrated in the context of hallmarks of AATD (Figure 1) and genetic diversity in the population with the genotype of the individual we posit being the ultimate gauge determining the basis for therapeutic management (Figure 4).[19-21]

Genetic approaches are currently at the forefront of personalized approaches (Figure 4). For example, silencing of variant expression through RNA interference technology is the farthest along in clinical trials for Z-variant liver disease with a strong likelihood of success in the near term.[78] The limitations of this focused strategy include the lack of a means to restore expression of WT ATT. In contrast, gene editing, now possible through multiple approaches including CRISPR-Cas9 improved probes, still remains at its infancy for human use, particularly in terms of targeting efficiency and off-target effects that could exacerbate disease and/or trigger other unexpected pathologies. A key limitation of both of these technologies is not only expense but deciding when to initiate treatment, particularly as a pre-emptive strike before tissue damage in either the liver or lung is beyond repair. As an alternative to handling genetic diversity, patient-specific stem and iPSC cell supplementation or replacement strategies provide a very different and potentially powerful approach, but will certainly require considerable advances in current technologies given our poor understanding of their stability in the patient and malignancy potential, the nature of the tissue niche to which they need to be targeted and maintained, and proper management of the level of expression. Moreover, this approach lacks the ability to remove the toxic threat of the AAT variant driving disease, a concern inherent in current enzyme replacement approaches as well as the genetic therapies discussed above.

Small-molecule chemical/biochemical approaches also hold great promise to manage genetic diversity in the population that is unique for the individual (Figure 4), particularly if screened for effectiveness in the context of genetic diversity of the AATD population.[1,4,5] However, they are generally limited by their associated dosing and safety issues in complex environments of the human, often reflecting an inability to target the drug to the site of interest. Ivacaftor treatment for G551D and the predicted 63% of CFTR residues that are likely to respond to the drug based on GRP-ML (Figure 3), as well as the more recent triple drug combination for management of CF,[2,160,165] are pioneering examples of a personalized genotype specific strategy that targets different variants through their impact on the protein fold to minimize off-target effects. The Balch laboratory has proposed[1,4,5] that by understanding how human evolution works to manage diversity leading to fitness in the population in response to function of the protein fold,[1,4,5] we will be able to "pre-train" a high throughput screening drug development program based on genetic diversity to "pre-tune" the therapeutic approach in advance of clinical efforts.

Given the above, the first challenge we posit will be to lay the groundwork for precision disease management in the context of genetic diversity in AATD by understanding the impact of given variants in the context of the operation of the full-length fold, as reported by detailed natural history and clinical perspectives

Figure 4. Impact of Therapeutics on Hallmarks of Alpha-1 Antitrypsin Deficiency Impacted by Genetic Diversity in the Alpha-1 Antitrypsin Deficiency Population



Given the diverse genotype and phenotypes driving AATD onset and progression in the population, treatment of AATD patients requires a deep medicine approach based on their unique genetic diversity that drives disease (**Figure 1**). Therapeutics fall into 4 main categories. Enzyme replacement, the current gold standard, has largely unknown effects on prevention of disease onset and progression, and would be expected to only effect Hallmarks 4-6. Genetic Therapy comprises 3 independent strategies. Over-expression of AAT-WT in the liver using, for example, AAV serotypes could impact levels of AAT in plasma (Hallmark 4) and thereby provide functional protein for improvement of lung function (Hallmarks 5-7). In contrast, silencing of hepatocyte AAT-Z expression (i.e., siRNA) could eliminate the toxic features of AAT misfolding (i.e., PiZZ polymerization) leading to protein aggregation in the liver and maladaptive stress responses impacting Hallmarks 1-3. It will likely have only minor effects on Hallmarks 4-6. If anything, such strategies may exacerbate the problem due to lack of even residual AAT variant function in the patient that has kept them healthy. Genome editing technologies (i.e., CRISPR/Cas9) provide a potential approach to either silence (like siRNA above) or, importantly, genetically engineer out pathogenic alleles to generate the WT allele, the latter, of course, impacting Hallmarks 1-6, although efforts are currently very early in development. Chemical and Biochemical approaches have potential for targeting different features of disease. Therapeutics that reduce aggregation load (i.e., compounds CBZ and Rapamycin) could lead to improved liver function (Hallmarks 2-3). TMAO and 4-PBA represent potential generic proteostasis modifiers altering Hallmarks 2 and 3), whereas the epigenetic modifiers including the HDACi SAHA may improve the overall plasticity of the folding in the ER and cellular/tissue environment to partially restore function as observed for NPC1 (see text), impacting Hallmarks 2-8. Finally, Cell Therapy is another therapeutic approach for AATD lung disease by generating AAT-WT protein through surrogate cell types. The latter technology is in infancy and faces many hurtles not withstanding stability and avoidance of metastatic disease. Using any of the above approaches, an improvement in AAT plasma levels could influence additional hallmarks indirectly including the impact of genetic modifiers (Hallmark 7), epigenetic state of the cell (Hallmark 8) and response to the environment (Hallmark 9). Peptide reagents and/or antibodies blocking AAT-Z polymerization in liver could also be useful, but their access to the nascent pool is problematic.

AATD=alpha-1 antitrypsin; WT=wild-type; AAV=adenovirus; siRNA=small interfering RNA; CBZ=carbamazepine; TAMO= trimethylamine N-oxide; 4-PBA=4-phenylbutric acid; HDACi= histone deacetylase inhibitor; SAHA=subeoylanilide hydroxamic acid; NDC1=Neimann-Pick C1

of the AATD patient population sequenced using whole-genome sequencing technologies (i.e., AllofUS).[169] Additional detailed biochemical/functional characterization of AAT variants are essential to understand the comprehensive genotype to phenotype landscape of variant AAT in response

to different tissue environments as we have shown for CFTR[1] and NPC1[4,5] from genetic, epigenetic and therapeutic perspectives. We anticipate a major component of AATD treatment in the future will be a *genotype first* approach revealed by whole-genome sequencing efforts that help us to understand not only an individual's AAT genotype but integrate that genotype with the many divergent basic and clinical features of disease reflected in the genetic diversity of the rest of the genome that make each one of us unique. These data will allow us to computationally formulate a common multi-dimensional framework to prioritize disease features that need to be corrected as well as their short- and long-term impact on the patient.

Put in another way, success will come from not only a deep understanding of the phenotype transformation driving disease in response to a patient's unique AAT genotype, but will likely be influenced by multiple non-AAT alleles that contribute to the modifier environment that is, like AAT specific alleles, also unique to each individual harboring disease. Characterizing this complex interplay of patient specific genomics with their unique phenomics (Figure 4) in the context of environmental factors such as smoking, air quality and life-style will contribute to a more globalized understanding of each patient as an individual for personalized treatment. Digitizing AATD in the context of multi-dimensional layers of -omics matrices as performed using GRP-ML and rapidly advancing deep learning technologies[18,20] we predict will bring a new era of personalized medicine to AATD patient health and disease management. These are not insurmountable goals given the rapid advances now beginning to drive the future of deep medicine,[19] one that embraces the need for active patient involvement in all aspects of therapeutic development, as the SCV strategy is about "this is me."[1]

Declaration of Interest

The authors have nothing to declare.

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