Chronic Obstructive Pulmonary Diseases:
Journal of the COPD Foundation®

Original Research

Novel *SERPINA1* Alleles Identified Through a Large Alpha-1 Antitrypsin Deficiency Screening Program and Review of Known Variants

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Abstract

The *SERPINA1* gene encodes the serine protease inhibitor alpha-1 antitrypsin (AAT) and is located on chromosome 14q31-32.3 in a cluster of homologous genes likely formed by exon duplication. AAT has a variety of anti-inflammatory properties. Its clinical relevance is best illustrated by the genetic disease alpha-1 antitrypsin deficiency (AATD) which is associated with an increased risk for chronic obstructive pulmonary disease (COPD) and cirrhosis. While 2 single nucleotide polymorphisms (SNPs), S and Z, are responsible for more than 95% of all individuals with AATD, there are a number of rare variants associated with deficiency and dysfunction, as well as those associated with normal levels and function. Our laboratory has identified a number of novel AAT alleles that we report in this manuscript. We screened more than 500,000 individuals for AATD alleles through our testing program over the past 20 years. The characterization of these alleles was accomplished by DNA sequencing, measurement of AAT plasma levels and isoelectric focusing at pH 4-5. We report 22 novel AAT alleles discovered through our screening programs, such as $Z_{\text{little rock}}$ and $QO_{\text{chillicothe}}$, and review the current literature of known AAT genetic variants.

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Abbreviations:

AAT=alpha-1 antitrypsin; **AATD**=alpha-1 antitrypsin deficiency; **COPD**=chronic obstructive pulmonary disease; **IEF**=isoelectric focusing; **PCR**=polymerase chain reaction; **SNPs**=single nucleotide polymorphisms

Funding Support:

This work was supported by the Alpha-1 Foundation.

Citation:

Wiesemann GS, Oshins RA, Flagg TO, Brantly ML. Novel *SERPINA1* alleles identified through a large alpha-1 antitrypsin deficiency screening program and review of known variants. ***Chronic Obstr Pulm Dis.*** 2023;10(1):7-21. doi: https://doi.org/10.15326/jcopdf.2022.0321

Publication Dates:

Date of Acceptance: October 19, 2022
Published Online Date: October 24, 2022

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Keywords:

alpha-1 antitrypsin; novel alleles; allele characterization; genomics; PolyPhen-2; COPD

Introduction

The alpha-1 antitrypsin (AAT) gene (*SERPINA1*) is located on chromosome 14q31-32.3. This serine protease inhibitor is predominantly produced by hepatocytes,[1] but also expressed by macrophages,[2] neutrophils,[3] monocytes,[4] and epithelial cells.[5] AAT is synthesized as a single polypeptide chain that undergoes co/post-translational modification which includes a 24 amino acid N-terminal clip and the addition of 3 N-linked glycosylated oligosaccharides to produce the di-, tri-, and tetra-antennary structure.[6]

AAT is an acute phase reactant protein known for its anti-inflammatory properties. This is demonstrated by increases in AAT levels within hours after inflammation or

infection begins. Allelic variations in this genetic disease can lead to deficiency/dysfunction of the AAT protein. The protein may misfold and accumulate in the endoplasmic reticulum of hepatocytes leading to increased susceptibility for development of cirrhosis.[7] Since levels of circulating AAT are decreased, less of this molecule can reach the lungs and inhibit neutrophil elastase. The balance of protease to anti-protease is shifted towards lung destruction in deficiency. This is classically seen with the development of chronic obstructive pulmonary disease (COPD) at earlier ages even with minimal or no tobacco history in affected individuals.[8]

AAT's clinical relevance is best demonstrated by the genetic disease alpha-1 antitrypsin deficiency (AATD) which predisposes individuals to developing COPD and cirrhosis. In the homozygous state, it is associated with development of emphysema at an early age[8] and with an increased incidence of hepatitis, usually progressing to cirrhosis.[7] There are 2 major alleles, S and Z, which encompass greater than 95% of all known AAT mutations. The S mutation results from the substitution of valine for glutamic acid at amino acid position 264 in exon III (g.9628 A>T). The Z mutation results from the substitution of lysine for glutamic acid at amino acid position 342 in exon V (g.11940 G>A). However, hundreds of mutations exist, including variants associated with normal circulating plasma levels and those associated with deficiency and/or dysfunction.

Our laboratory has identified several novel mutations through our nationwide testing program where we screened more than 500,000 individuals. Individuals with abnormal or ambiguous screening results were invited to join the Alpha-1 Foundation DNA and Tissue Bank and enrolled at their own discretion. The Alpha-1 Foundation DNA and Tissue Bank was established in 2002 and contains approximately 2400 DNA and plasma samples from AATD patients and their families. These individuals were initially screened based on medical indications from liver, lung, or family history that pointed towards a possible diagnosis of AATD. Characterization of novel alleles was accomplished using DNA sequencing, measuring AAT levels in plasma, and isoelectric focusing (IEF) at pH 4–5. Pathogenic variants were determined using PolyPhen-2, a program which estimates the probability that an amino acid change significantly affects protein structure. In this report we characterized 22 alleles discovered at the University of Florida AAT Genetics Laboratory and provide a comprehensive review of known AAT allelic variants.

Material and Methods

Approach to Detection of Abnormal SERPINA1 Alleles

The majority of samples were screened for abnormal alleles using dried blood spot cards containing whole blood collected in three 12 mm circles on 903 paper. Punches from the 903 Whatman filter paper containing whole blood were used to determine AAT levels by nephelometry. DNA was extracted from the blood spots and genotyped by TaqMan polymerase chain reaction (PCR) using primers directed to the Z and S alleles. When a sample had a low AAT level inconsistent with the genotype of MZ, SZ, and ZZ, a letter was sent to either the patient or the patient's physician (depending on the screening program) inviting the individual to join the Alpha-1 Foundation DNA and Tissue Bank and submit a clinical questionnaire and a whole blood sample.

Samples

Genomic DNA was isolated from whole blood samples obtained from participants in the Alpha-1 Foundation DNA and Tissue Bank at the University of Florida, protocol UF-IRB-201500842, after giving written consent. Candidate samples were prescreened and selected for DNA melting, DNA sequencing, or both based on 3 criteria, which, when considered together, suggested the existence of a rare or novel allele. In most cases TaqMan allelic discrimination had to indicate the existence of a non-S or non-Z allele, the AAT protein level had to be lower than 10μM by nephelometry, and IEF had to present an unusual protein migration signature. For melt experiments, DNA concentration was adjusted to 10μg/mL. An M1M1 (rs 6647) sample from the Alpha-1 Foundation DNA and Tissue Bank was used for a control.

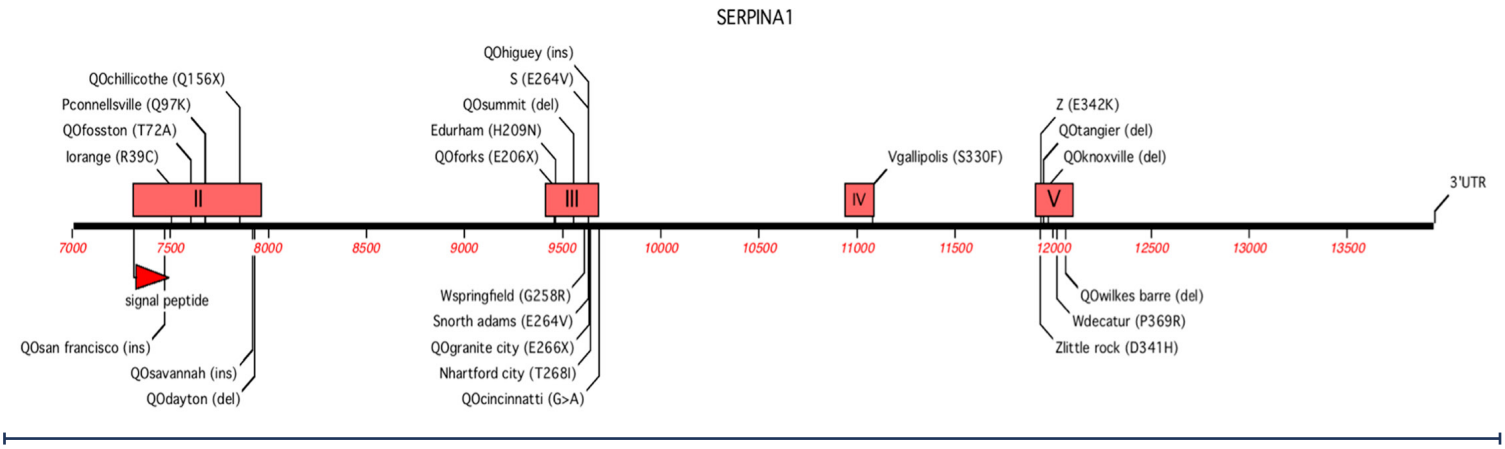
Primers

Two different sets of primers were designed for the pre-melt PCR amplifications and pre-sequencing PCR amplifications due to the inability of the melt experiment to accurately detect mutations with high fidelity in amplicons greater than 400 base pairs. Exons II and V were subdivided into 3 and 2 sections, respectively, for the pre-melt amplification (Figure 1). Primers were designed to begin approximately 20 base pairs upstream of intron-exon junctions so splice site mutations, as well as intra-exon mutations, could be identified. We did not routinely screen the promotor regions of the AAT gene for 2 reasons: (1) in our previous sequencing studies of AATD participants we have identified promotor single nucleotide polymorphisms (SNPs) but were unable to attribute any reductions in plasma AAT to the SNPs and, (2) all participants we screened using this approach had SNPs that explained the decreases in plasma AAT, e.g., nonsense mutations, frameshifts, stop codon, and splicing mutations.

Pre-melt PCR

The pre-melt amplification solution included 1μL of genomic DNA and 9μL of a PCR master mix that included a

Figure 1. Genomic Map of Novel Alleles of the Alpha-1 Antitrypsin Gene



SERPINA1 Exons II, III, IV, and V

Klentaq enzyme and LCGreen Plus (Idaho Technology, Inc., Salt Lake City, Utah). Reaction mixtures were pipetted into opaque black and white 96-well plates with a 20µL mineral oil overlay, sealed with optical adhesive tape and amplified. The PCR had an initial denaturation step of 95°C for 2 minutes, 35 cycles of denaturation at 95°C for 15 seconds, reannealing at 65°C for 20 seconds, and elongation at 72°C for 15 seconds, followed by a 95°C hold for 30 seconds and a 26°C hold for 30 seconds.

Melt Acquisition

High resolution melt scanning was performed on an Idaho Tech Lightscanner. After completion of PCR amplification, the instrument's heating block was warmed to a holding temperature of 70°C, at which point the 96-well tray was inserted. Samples were melted within a temperature range of 74°C to 94°C with fluorescence levels measured over the interval.

Melt Analysis

Results were analyzed using the light scanner software package, which presented each melt event as a curve plotted as fluorescence versus temperature. All melt curves corresponding to a single amplicon section were grouped together and normalized by declaring 100% and 0% fluorescence levels at regions before and after the denaturation event. The temperature shift was set to 5% fluorescence. Using the M1M1 control sample as the baseline, the software generated -dF/dT derivative plots that gave steep parabolic curves for samples containing heteroduplexes.

Sanger Sequencing

Exons determined to contain mutations were amplified using in-house sequencing primers. The PCR included an initial denaturation step of 94°C for 1 minute, 40 cycles of denaturation at 94°C for 10 seconds, reannealing at 56°C for 30 seconds, and elongation at 72°C for 1 minute. Sequencing was performed by the UF ICBR sequencing core using an Applied Biosystems Model 3130 Genetic Analyzer or by GeneWiz (South Plainfield, New Jersey). Returned sequences were aligned against National Center for Biotechnology Information-Gene consensus sequences using the MacVector ClustalW/Multiple Sequence Alignment (Apex, North Carolina).

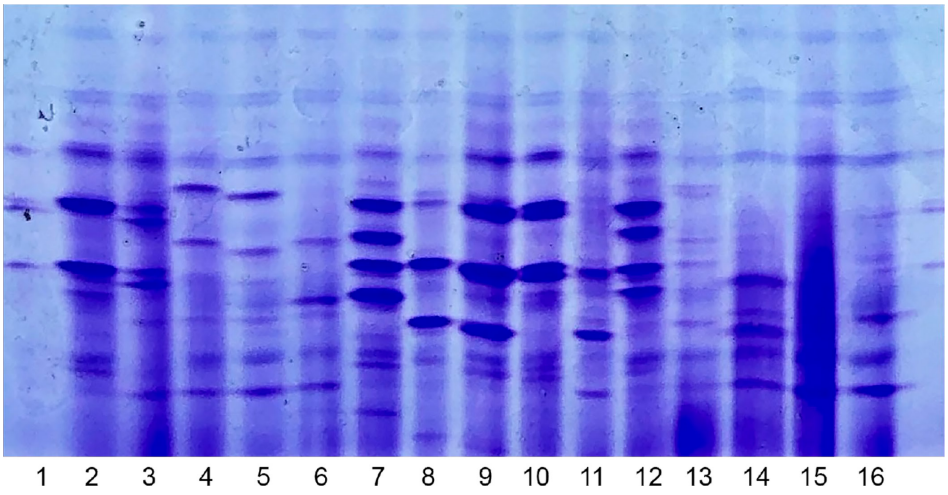
PolyPhen-2

PolyPhen-2 is a program designed to predict the impact of amino acid substitutions on the structure and function of human proteins.[9] Position of the variant within the protein, along with the specific amino acid substitution was inputted to determine a score from 0–1 to indicate if the overall protein would be deficient, dysfunctional, normal, or null. A score of 0.8 or greater is considered probably damaging.

Naming

Novel alleles were named according to the birthplace of the individual with the novel variant. Designation of M, Z, S, QO, etc., were implemented based on the pattern of the protein on the IEF gel. Novel alleles were named according to the Human Genome Variation Society and described at the DNA level with the format "position substituted" "reference nucleotide" > "new nucleotide." The reference transcript does not include the 24 residues of the signal peptide. Much of the variation in AAT variants is based on amino acid substitutions that alter the electrophoretic migration in an IEF gel at pH 4–5. The most common alleles are the M alleles, M1–3, where the differences are based on a combination of SNPs encoding differentially charged amino acids that can be identified by IEF (see example in Figure 2). We used M1(val213) as the base allele to compare all variants.

Figure 2. Novel Alleles Discovered at University of Florida, Isoelectrophoresis Gel



Left to right: 1-M_4E_{durham}, 2-M_1M_1, 3-$ZN_{hartford\ city}$, 4-ZI, 5-ZI_{orange}, 6-ZP_{lowell}, 7-$M_1P_{connellsville}$, 8-SS, 9-$M_3W_{springfield}$, 10-M_1M_2, 11-$ZW_{decatur}$, 12-$M_1S_{north\ adams}$, 13-SZ, 14-$ZV_{gallipolis}$, 15-$ZZ_{little\ rock}$, 16-ZZ

Approach to Literature Review

Construction of tables of known variants of AAT was accomplished by using the search term "alpha-1 antitrypsin" in the PubMed database. The search was accomplished in early 2020 and there were approximately 14,000 articles with the search word in the title and/or abstract. All 14,000 article abstracts and titles available on PubMed were screened for terms indicating a report of novel AAT alleles. Following the identification of these articles the authors reviewed the articles for accuracy and availability of sufficient data to support the variant.

Results

From the Alpha-1 Foundation DNA and Tissue Bank, we identified 22 novel alleles (Figure 1 and Table 1). The majority of these novel alleles were discovered in asymptomatic individuals who underwent genetic testing due to a family history of AATD. Three individuals with novel mutations who presented with pulmonary symptoms at a younger age are discussed in greater detail below.

I$_{orange}$

The proband was a 33-year-old female with a history of asthma. She was a non-smoker with a family history of COPD. During initial screening, she was determined to be carrying a Z allele and subsequent DNA sequencing revealed a heterozygous mutation at codon 39 (Arg CGC > Cys TGC) on an M2 (Arg101 CGT > His CAT) background which causes it to run cathodal to the known I mutation by IEF.

QO$_{san\ francisco}$

This patient was a 41-year-old female with a history of hepatitis, asthma, and emphysema. She was on supplemental oxygen and receiving AAT augmentation therapy at the time of sample collection, though she reported a very low plasma level of AAT prior to initiation of augmentation therapy. DNA sequencing appeared to be homozygous for a null allele in exon II (Pro28 CCC, insertion of T, shift to stop TGA 32) though there is a possibility she has a complete deletion of her second AAT allele. The patient had 2 children who were tested at the time, a 16-year-old with a plasma AAT level of 12.8μM and a 7-year-old with a level of 15.4μM, both are heterozygous for this novel null allele.

QO$_{knoxville}$

The proband was a 57-year-old male with an extensive family history of lung disease, including emphysema, COPD, chronic bronchitis, and asthma. In addition to a Z allele, he was determined to have a novel null allele (a frame shift deletion in exon V resulting in a stop codon at 373). Family testing facilitated by the Alpha-1 Foundation DNA and Tissue Bank showed his son, a 28-year-old with asthma was also heterozygous for this null allele. More family members were screened and a 34-year-old niece of the proband with no active lung disease was found to have the $SQO_{knoxville}$ genotype.

Other Novel Alleles

Because the genetic screening program focused predominately on individuals identified as AAT deficient, the majority of alleles identified represent disease-associated mutations and fall into 2 major categories of AATD: null

Table 1. Novel Alleles from the University of Florida Alpha-1 Antitrypsin Genetics Laboratory

Allele	Base	Exon	Mutation	Description	Genomic Location	PolyPhen-2 Score
Null						
QO chillicothe	M1(Val213)	II	Q156[a]	Gln 156 CAG to stop TAG		
QO dayton	M1(Val213)	II		Thr 180 ACA/Val 181 GTT to AG delete, shift to stop TAA 190	7927/7928 del AG	
QO fosston	M2	II	T72A	Thr 72 ACT to Ala GCT	7601 A>G	
QO san francisco	M1(Ala213)	II		Pro 28 CCC to insert T, shift to stop TGA 32	7469 ins T	
QO savannah	M1(Val213)	II		8 base pair insertion at amino acid 177	aa177 is 7916:7918	
QO granite city	M1(Ala213)	III	E266[a]	Glu 266 GAA to stop TAA	9633 G>T	
QO higuey	M1(Ala213)	III		Asn 265 AAT to insert A, shift to stop TGA 266	9630/9631 ins A	
QO summit	M1(Val213)	III		Val 239 GTG to G delete, shift to stop TGA 241	9554 del G	
QO wilkes-barre	M1(Val213)	III/V		Leu 383 CTC to T delete, shift to stop TGA 389	12064 del T	
QO knoxville	M1(Ala213)	V		Glu 354 GAG/Ala 355 GCC to G delete, shift to stop TAA 373	11978/11979 del G	
QO tangier	M1(Val213)	V		Glu 346 GAA to G delete, shift to stop TAG 353	11954 del G	
QO cincinnati	M1(Val213)			G>A substitution at position +1 of intron III		
QO forks			E206[a]	Glu 206 GAG to stop TAG		
Deficient						
I orange	M2	II	R39C	Arg 39 CGC to Cys TGC	7502 C>T	1.000
E durham	M4	III	H209N	His 209 CAC to Asn AAC	9462 C>A	0.958
S north adams	M4	III	E264V	Glu 264 GAA to Val GTA	9628 A>T	1.000
W springfield	M3	III	G258R	Gly 258 GGG to Arg AGG	9609 G>A	0.990
V gallipolis	M1(Val213)	IV	S330F	Ser 330 TCC to Phe TTC	11802 C>T	1.000
W decatur	M2	V	P369R	Pro 369 CCC to Arg CGC	12022 C>G	1.000
Z little rock	S	V	D341H	Asp 341 GAC to His CAC	11937 G>C	0.867
Normal						
P connellsville	M1(Val213)	II	Q97K	Gln 97 CAG to Lys AAG	7676 C>A	0.197
N hartford city	M1(Val213)	III	T268I	Thr 268 AAC to Ile ATC	9640 C>T	0.407

[a]Denotes premature stop codon

(n=13) and deficient (n=7). Only 2 normal alleles were identified, $P_{connelllsville}$ and $N_{hartford\ city}$ (Table 1, Figures 1 and 2). Two of the deficient alleles, I_{orange} and $S_{north\ adams}$, show altered IEF migration patterns of the I and S alleles, respectively, and only differ from them with respect to their base alleles which in these novel cases appear on the M_2 and M_4 backgrounds rather than on the more common M_1 background. Electrophoretic differences such as these have previously been reported in the P family of alleles.

Novel deficiency alleles were called "disease associated" based on a PolyPhen-2 score above 0.8 and a clinical history of respiratory and/or liver disease. The molecular mechanisms of abnormal secretion of AAT typically were associated with amino acid substitutions that cause a significant charge alteration, such as a neutral amino acid to a charged amino acid or vice versa. Novel null alleles were most commonly caused by single base deletions and subsequent sequence frameshifts, leading to a premature stop codon. One null allele, $QO_{cincinnati}$, was the result of a base change in a splice junction in intron III (Figure 1). Two other null mutations were the result of a base substitution that created a stop codon (Figure 1 and Table 1).

We have grouped known variants into similar categories as the reported novel variants of normal, deficient/dysfunctional, and null variants (Table 2)[10-31] (Table 3)[32-59] (Table 4).[60-84] Alleles listed without a name were discovered via sequencing alone and were not given a name based on their pattern of IEF. We included a table that lists variants that have been identified by IEF and did not have DNA sequencing (Table 5).[85-106]

Discussion

Our laboratory has been screening individuals for AAT variants for several years using a series of improving and more efficient technologies to simplify the accurate identification of new alleles. In the process, we have identified a number of alleles that provide insight into the molecular basis of AATD-based key relationships between structure and function. While rare deficiency alleles do not play a significant role in the vast majority of AATD individuals, they may play an important role in guiding novel therapies involving chaperones, gene editing, and gene silencing to modulate the consequences of misfolded AAT.

While more than 95% of all disease-affected AATD individuals have severe deficiency due to the presence of the Z allele, novel mutations in the AAT gene provide insight into the key structural elements of the AAT protein. Modeling of

Table 2. Normal Alleles (Review)

Allele	Base	Exon	Mutation	Description	Genomic Location	RS Number
E johannesburg[10]	M1(Val213)	II	H15N	His 15 CAC to Asn AAC	7430 C>A	138070585
M1 bruxelles[11]	M1	II	H15L	His 15 CAC to Leu CTC	116 A>T	
M2[12]	M3	II	R101H, E376D	Arg 101 CGT to His CAT, Glu 376 GAA to Asp GAC/T	7690 G>A	709932
M3 riedenburg[13]	M3	II	L118L	Leu 118 CTG to Leu TTG	7739 C>T	20546
M4[14]	M1(Val213)	II	R101H	Arg 101 CGT to His CAT	7689 G>A	
M5 berlin[13,1]	M1(Val213)	II	P88T	Pro 88 CCG to Thr ACC	7649 C>A, 7651 G>C	
M5 karlsruhe[13]	M1(Val213)	II	A34T	Ala 34 GCC to Thr ACC	7487 G>A	149319176
M rouen[15]	M1/M2	II	R39H	Arg 39 CGC to His CAC	188 G>A	764726147
O thonon-les-bains[11]	M1	II	D159N	Asp 159 GAT to Asn AAT	547 G>A	759578830
P yonago[16]	M1(Val213)	II	D19A	Asp 19 GAT to Ala GCT	7443 A>C	
S roubaix[11]	M1	II	S47R	Ser 47 AGC to Arg CGC	211 A>C	11575873
Trento[17]	M1(Ala213)	II	E75V	Glu 75 GAA to Val GTA	296 A>T	
V[18]	M1(Val213)	II	G148N	Gly 149 GAC to Asn AAC	7832 G>A	112030253
V munich[19]	M1(Val213)	II	D2A	Asp 2 GAT to Ala GCT	7392 A>C	199422212
W saint-avre[11]	M1	II	E122K	Glu 122 GAG to Lys AAG	436 G>A	537285845
[20]		II	E89D	Glu 89 GAG to Asp GAC/GAT		
E cincinnati[21]	M1(Ala213)	III	K274D	Lys 274 AAG to Asp AAT	9659 G>T	
M1(Ala213)[22]		III		Ala 213 GCG	9475	6647
M1(Val213)[22]	M1(Ala213)	III	A213V	Ala 213 GCG to Val GTG	9475 C>T	6647
M1 lille[11]	M1	III	H269Q	His 269 CAC to Gln CAA	879 C>A	141095970
P st albans[23]	M1(Val213)	III/V	D341N, D256D	Asp 341 GAC to Asn AAC, Asp 256 GAT to Asp GAC	11937 G>A, 9605 T>C	43370956/28929471
P st louis[24]	M2	III	M221T	Met 221 ATG to Thr ACG	9499 T>C	
Puerto Real[20]		III	T249A	Thr 249 ACC to Ala GCC	817 A>G	
X[25]	M1(Val213)	III	E204K	Glu 204 GAG to Lys AAG	9448 G>A	199422208
X curis[11]	M1	III	N247D	Asn 247 AAT to Asp GAT	811 A>G	755851961
M1 lyon[11]	M1	IV	A284S	Ala 284 GCC to Ser TCC	922 G>T	141620200
E taurisano[11]	M2	V	K368E	Lys 368 AAA to Glu GAA	12018 A>G	
G saint-sorlin[11]	M2	V	K394X	Lys 394 AAA to stop UAA	1252 A>T	
L offenbach[13]	M1(Val213)	V	P362T	Pro 362 CCC to Thr ACC	12000 C>A	12233
M3[27]	M1(Val213)	V	E376D	Glu 376 GAA to Asp GAC	12044 A>C	1303
M5 gunma[28]	M3	V	P362S	Pro 362 CCC to Ser TCC	12000 C>T	
P donauwoerth[13]	M1(Val213)	V	D341N	Asp 341 GAC to Asn AAC	11937 G>A	28929471
São Tomé[29]	M3	V	P362H	Pro 362 CCC to His CAC	1050C>A	569384943
W bethesda[30]	M1(Ala213)	V	A336T	Ala 336 GCT to Thr ACT	11922 G>A	1802959
X christchurch[31]		V	E363K	Glu 363 GAG to Lys AAG	12003 G>A	121912712

X=stop codon

$Z_{little\ rock}$ (Figure 3) and $QO_{Chillicothe}$ (Figure 4) demonstrate how amino acid changes may create different interactions that alter the structural stability of the protein. These observations have allowed structural biologists to identify key mechanisms of misfolding including the serpin shutter disruption, importance of the C-terminus in structure, and our understanding of the mechanisms of polymerization of variants.[107,108]

Centers throughout the world devoted to screening for known and novel AAT alleles need to continue their work. While the ease of sequencing DNA has made major leaps, specialized protein analysis and retaining key clinical information of alleles remain very important resources for the AATD community and requires specialists. The Alpha-1 Foundation has been one of the most generous funders of these specialized *detection* centers. There is much to do before there is a cure for AATD, a condition that affects nearly half a million individuals world-wide. A major step towards developing a cure for AATD includes screening for deficient individuals with informative structural changes and using this information to better understand the structural basis of AATD. As has been said by more than one geneticist, nature through its rich variation has done all the interesting experiments, we just need to determine what we can learn from them.

Acknowledgements

Author contributions: All authors contributed to the conception and design of the study, acquisition of data, data analysis and/or interpretation, writing the article, and revisions prior to submission

Table 3. Dysfunctional/Deficient (Review)

Allele	Base	Exon	Mutation	Description	Genomic Location	RS Number
Cadiz[32]		II	E151K	Glu 151 GAA to Lys AAA	523 G>A	149770048
I[33]	M1(Val213)	II	R39C	Arg 39 CGC to Cys TGC	7502 C>T	28931570
L frankfurt[13]	M2	II/III	Q156E, P255T	Gln 156 CAG to Glu GAG, Pro 255 CCT to Thr ACT	7853 C>G, 9600 C>A	
M riedenburg[13]	M3	II	L118L	Leu 118 CTG to Leu TTG	7739 C>T	20546
M1 saint-rambert[11]	M1	II	G95V	Gly 95 GGC to Val GTC	356 G>T	
M2 obernburg[13]	M1(Ala213)	II	G148W	Gly 148 GGG to Trp TGG	7829 G>T	112030253
M6 bonn[13]	M1(Ala213)	II	S45F	Ser 45 TCC to Phe TTC	7521 C>T	199687431
M6 passau[13]	M1(Val213)	II	A60T	Ala 60 GCC to Thr ACC	7565 G>A	111850950
M malton[34]	M2	II	F51/52del	Phe 51/52 TTC to delete	7538	775982338
M mineral springs[35]	M1(Ala213)	II	G67E	Gly 67 GGG to Glu GAG	7587 G>A	28931568
M nichinan[36]	M1(Val213)	II	F51/52del, G148R	Phe51/52 TTC to delete, Gly 148 GGG to Arg AGG	7538, 7829 G>A	112030253
M palermo[13]	M1(Val213)	II	F51/52del	Phe 51/52 TTC to delete	7538	
M procida[37]	M1(Val213)	II	L41P	Leu 41 CTG to Pro CCG	7509 T>C	28931569
P gaia[38]	M1(Val213)	II	E162G	Glu 162 GAG to Gly GGG	7872 A>G	
Queens[23]	M1(Val213)	II	K154N	Lys 154 AAG to Asn AAC/AAT	7849 G>C or T	
S donosti[32]		II	S14F	Ser 14 TCC to Phe TTC	113 C>T	745463238
S iiyama[39]	M1(Val213)	II	S53F	Ser 53 TCC to Phe TTC	7545 C>T	55819880
Sevilla[32]		II	A58D	Ala 58 GCT to Asp GAT	245 C>A	
Tijarafe[32]		II	I50N	Ile 50 ATC to Asn AAC	221 T>A	
W vernaison[11]	M1	II	L126R	Leu 126 CTA to Arg CGA	449 T>G	
Z bristol[40]	M1(Val213)	II	T85M	Thr 85 ACG to Met ATG	7641 C>T	199422213
Z wrexham[41]		II		Ser TCG to Leu TTG at codon 19 in signal peptide		140814100
[20]		II	Y138C	Tyr 138 TAC to Cys TGC		
[20]		II	S14F	Ser 14 TCC to Phe TTC		
[42]	M3	II	I50N	Ile 50 ATC to Asn AAC		
F[18]	M1(Val213)	III	R223C	Arg 223 CGT to Cys TGT	9504 C>T	28929470
M pisa[26]	M1(Val213)	III	K259I	Lys 259 AAA to Ile ATA	9613 A>T	
N nagato[43]	M2	III	L276P	Leu 276 CTG to Pro CCG	9664 T>C	
Novel variant[44]		III	F208L	Phe 208 TTC to Leu TTG	9461 C>G	
P brescia[45]		III	G225R	Gly 225 GGC to Arg CGC	9510 G>C	
P duarte[46]	M4	III	D256V	Asp 256 GAT to Val GTT	9604 A>T	121912714
P lowell[23]	M1(Val213)	III	D256V	Asp 256 GAT to Val GTT	9604 A>T	121912714
P loyettes[11]	M1	III	M221T	Met 221 ATG to Thr ACG	734 T>C	766260108
P solaize[11]	M2	III	M221I	Met 221 ATG to Ile ATA	735 G>A	
S[47]	M1(Val213)	III	E264V	Glu 264 GAA to Val GTA	9628 A>T	17580
T[48]	M2	III	E264V	Glu 264 GAA to Val GTA	9628 A>T	17580
Tarragona[32]		III	F227C	Phe 227 TTT to Cys TGT	752 T>G	759837735
Y barcelona[49]		III/V	D256V, P391H	Asp 256 GAT to Val GTT, Pro 391 CCC to His CAC	9604 A>T, 12088 C>A	
M1 brest[11]	M1	IV	Y297C	Tyr 297 TAT to Cys TGT	962 A>G	774775536
P salt lake[50]		IV	G320R	Gly 320 GGG to Arg AGG	11051 G>A	
S munich[13]	M1(Val213)	IV	S330F	Ser 330 TCC to Phe TTC	11082 C>T	201788603
Valencia[32]	M1(Val213)	IV	K328E	Lys 328 AAG to Glu GAG	1054 A>G	
Baghdad[51]		V	A336P	Ala 336 GCT to Pro CCT	11922 G>C	
E tokyo[32]	M1(Val213)	V	K335E	Lys 335 AAG to Glu GAG	11919 A>G	
Kings[33]	M1(Val213)	V	H334D	His 334 CAT to Asp GAT	11916 C>G	
M1 cremeaux[11]	M1	V	H334Q	His 334 CAT to Gln CAA	1074 T>A	
M heerlen[54]	M1(Ala213)	V	P369L	Pro 369 CCC to Leu CTC	12022 C>T	199422209
M vall d'hebron[55]	M1(Ala213)	V	P369S	Pro 369 CCC to Ser TCC	12021 C>T	
M wurzburg[56]	M1(Val213)	V	P369S	Pro 369 CCC to Ser TCC	12021 C>T	61761869
P pittsburgh[57]		V	M358R	Met 258 ATG to Arg AGG	11989 T>G	121912713
Y orzinuori[26]	M1(Val213)	V	P391H	Pro 391 CCC to His CAC	12088 C>A	
Z[58]	M1(Ala213)	V	E342K	Glu 342 GAG to Lys AAG	11940 G>A	28929474
Z ausburg[59]	M2	V	E342K	Glu 342 GAG to Lys AAG	11940 G>A	28929474

Table 4. Null Alleles (Review)

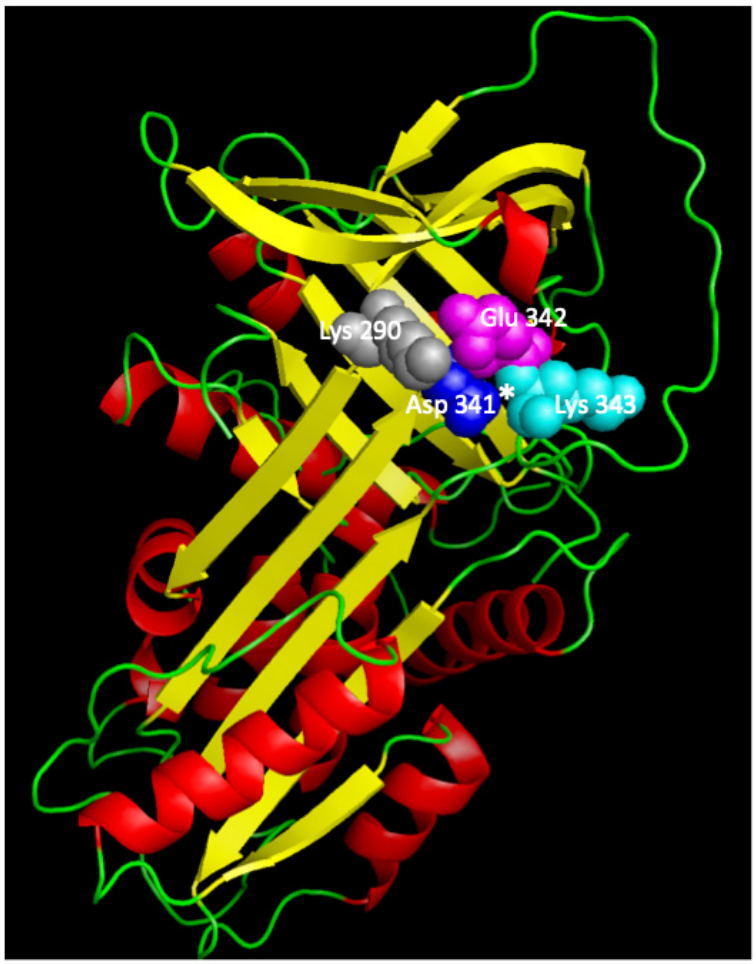
Allele	Base	Exon	Mutation	Description	Genomic Location	RS Number
M varallo[60]		II		8 base pair deletion leads to stop codon TGA 70/71		
QO amersfoort/ bredevoort[61,62]	M1(Ala213)	II	Y160X	Tyr 160 TAC to stop TAG	7867 C>G	199422210
QO casablanca[11]	M2	II		His 73 CAC Met fs*7	288_297del	
QO cork[63]		II		Thr 180 ACA, del CA to stop 190 TAA	7926:7927 del CA	
QO granite falls[64]	M1(Ala213)	II		Tyr 160 TAC to C delete, shift to stop TAG 160	7867 del C	267606950
QO isola di procida[37]		II-V		10kb deletion of exons II - V		
QO kowloon[65]	M1(Val213)	II	Y38X	Tyr 38 TAC to stop TAA	7501 C>A	
QO lille[11]	Z	II		His 73 CAC Met fs*7	288_297del	
QO lisbon[13]	M1(Val213)	II		Thr 68 ACC to Ile ATC	7590 C>T	
QO ludwigshafen[66]	M2	II	I92N	Ile 92 ATC to Asn AAC	7662 T>A	28931572
QO newport/devon[41]	M1(Val213)	II	G115S	Gly 115 GGC to Ser AGC	7730 G>A	11558261
QO riedenburg[67]		II-V		deletion of exons II-V		
QO saint-etienne[11]	M4	II	K163X	Lys 163 AAG to stop ATG	559 A>T	
QO savannah[68]	M1(Val213)	II		8 base pair insertion at amino acid 177	aa177 is 7916:7918	
QO soest[62]	M1(Ala213)	II		Thr 102 ACC, del A to stop 112 TGA	7691 del A	
QO bellingham[69]	M1(Val213)	III	K217X	Lys 217 AAG to stop TAG	9486 A>T	199422211
QO brescia[63]		III	E257X	Glu 257 GAG to stop TAG	9606 G>T	
QO cairo[70]	M1(Ala213)	III	K259X	Lys 259 AAA to stop TAA	9612 A>T	1802963
QO cardiff[71]		III	D256V	Asp 256 GAT to Val GTT	9604 A>T	121912714
QO gaia[72]	M1(Ala213)	III	L263P	Leu 263 CTG to Pro CCG	9625 T>C	
QO milano[73]	M1(Val213)	III		Thr 259 AAA, del 17bp to stop UGA	9752-9768	
QO perugia[63]		III		Val 239 GTG, del G to stop 241 TGA	9552 del G	
QO trastevere[74]	M1(Val213)	III	W194X	Trp 194 TGG to stop TGA	9419 G>A	
QO oliveira do douro[38]	M3	III-IV		Arg 281 AGA to AG delete, shift to stop TGA 297	9679:9680 del GA	
QO cosenza[63]		IV	Q305X	Gln 305 CAA to stop TAA	11006 C>T	
QO hong kong[75]	M2	IV		Leu 318 CTC to TC delete, shift to stop TAA 334	11046_11047 del TC	1057519610
QO new hope[65]	M1(Ala213)	IV-V	G320E, E342K	Gly 320 GGG to Glu GAG, Glu342 GAG to Lys AAG	11052 G>A, 11940 G>A	
QO pordenone[63]	M1(Val213)	IV	L327X	Leu 327 CTG, del T to stop 338 TGA	11073 del T	
QO torino[63]		IV	Y297X	Tyr 297 TAT to stop TAA	10984 T>A	
[76]		IV-V		Arg CGT 223 to Cys TGT, Pro 362 CCC to insert C, shift to stop TGA 376		
QO bolton[77]	M1(Val213)	V		Pro 362 CCC to C delete, shift to stop TAA 373	12000 del C	
QO clayton[78]	M1(Val213)	V		Pro 362 CCC to insert C, shift to stop TGA 376	12000 ins C	
QO dublin[63]	M1(Val213)	V	V337X	Phe 370 TTT, del T to stop 373 TAA	12024 del T	
QO lampedusa[63]	M2	V	V2337X	Val 337 GTG, del G to stop 338 TGA	11925 del G	
QO montluel[11]	M1	V	V389X	Val 389 GTG to stop	1237_1239del	760849035
QO ourem[79]	M3	V		Leu 353 TTA to insert T, shift to stop TGA 376	11973 ins T	
QO saarbruecken[13]	M1(Ala213)	V		Pro 362 CCC to insert C, shift to stop TGA 376	12000 ins C	
QO mattawa[80]	M1(Val213)	V		Leu 353 TTA to insert T, shift to stop TGA 376	11973 ins T	28929473
QO faro[38]	M1(Val213)	Intron 1C		C. -5+2 dupT		
QO madrid[81]	M3	Intron 1C		C. -5+2 dupT		
QO achicourt[11]	S	intron 3			917+1 G>A	750779440
QO saint-avold[11]	M1	intron 3			918-1 G>A	
QO amiens[11]	M1	intron 4			1065+1 G>A	781591420
QO bonny blue[65]	M1(Val213)			G deletion at position #1 of intron II splice acceptor		
QO boston[82]				Unknown		
QO porto[83]				G>A substitution at position +1 of intron 1C		1243161
QO vila real[38]	M3			Met 374 ATG to ATGA delete, shift to stop TGA 292	12036 A>G	
QO west[84]	M1(Val213)			G>T at position #1 of intron II splice donor		

X=stop codon

Table 5. Alleles Identified by Isoelectric Focusing (Review)

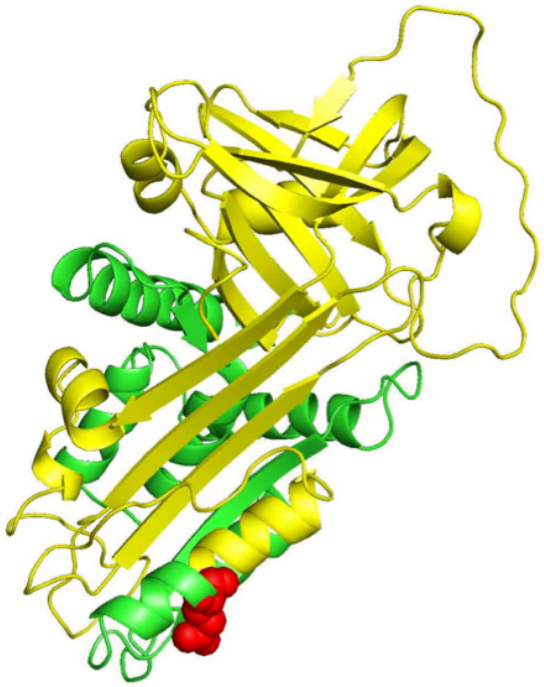
Allele			
B[85]	L beijing[98]	N Adelaide[86]	P weishi[100]
B saskatoon[87]	M chapel hill (M baldwin)[97,101]	N grossoeuvre[88]	P yasugi[43]
C[89]	M cobalt[77]	N hampton[33]	QO boston[82]
D[89]	M duarte[102]	N letrait[90]	N hampton[33]
E[91]	M hailin[100]	N yerville[88]	R[94]
E franklin[92]	M huariou[100]	P[93]	W salerno[103]
E lemberg[94]	M lamb[97]	P budapest[94]	X alban[92]
E matsue[43]	M salla[61]	P castoria[92]	X fengcheng[100]
G[91]	M toyoura[104]	P clifton[95]	Y brighton[103]
G cler[96]	M whitstable[105]	P kyoto[52,97]	Y hagi[43]
J houyao[96]	M5 germany[59]	P oki[16]	Y toronto[94]
L[99]	N[106]	P onomichi[43]	Z pratt[21]

Figure 3. Tertiary Protein Structure of Variant Allele $Z_{little\ rock}$



Variant D341H (D341*) in blue surrounded by key amino acids likely important in maintaining reactive loop stability. G342 purple, K343 aqua and K290 grey. This variant is structurally very interesting since the protein is electrophoretically like PI*Z but does not have the PI*Z mutation and suggests that the base of the reactive site loop is stabilized by more than just the Lys 290 and Glu 342 salt bridge.

Figure 4. Tertiary Protein Structure of Variant Allele $QO_{chillicothe}$



Q156 termination site highlighted in red, yellow denotes predicted missing structure. $QO_{chillicothe}$ is an example of the most commonly seen mechanism for null variants, i.e., a premature stop codon.

The authors are grateful for the help of referring physicians and the generosity of enrollees participating in the Alpha-1 Foundation DNA and Tissue Bank

Data sharing: No participant data has or will be shared.

Declaration of Interests

All listed authors will not receive any compensation for the work of the submitted manuscript, have any financial relationships outside the submitted work, or have any other relationships or activities that readers could perceive to have influenced the manuscript. There are no intellectual properties or copyrights that are broadly relevant to the work.

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