Alpha-1 antitrypsin deficiency: clarifying the role of the putative protective threshold

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Shareable abstract (@ERSpublications)
The protective threshold of 11 µM has historically been used to predict risk of COPD in AATD. The evidence now conclusively shows this to be inaccurate. 11 µM is a target for augmentation therapy, but should not be used as a determinant of risk of COPD. https://bit.ly/2TXXNus

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Abstract

Alpha-1 antitrypsin deficiency (AATD) is the only readily identifiable monogenic cause of COPD. To date the only condition-specific treatment for AATD-associated COPD is weekly administration of intravenous plasma-purified human alpha-1 antitrypsin (IV-AAT). Uncertainties regarding which AATD genotypes should benefit from IV-AAT persist. IV-AAT is costly and involves weekly administration of a plasma product. Much of the risk stratification has been centred around the long-accepted hypothesis of a "putative protective threshold" of 11 µM (0.57 g·L^{-1}) AAT in serum. This hypothesis has become central to the paradigm of AATD care, although its derivation and accuracy for defining risk of disease remain unclear.

We reviewed the literature and examined the association between the 11 µM threshold and clinical outcomes to provide context and insight into the issues surrounding this topic.

We found no data demonstrating an increased risk of COPD dependent on the 11 µM threshold. Moreover, an abundance of recent clinical data examining this threshold refutes the hypothesis. Conversely, the use of 11 µM as a treatment target in appropriate ZZ individuals is supported by clinical evidence, although more refined dosing regimens are being explored.

Continued use of the 11 µM threshold as a determinant of clinical risk is questionable, perpetuates inappropriate AAT-augmentation practices, may drive increased healthcare expenditure and should not be used as an indicator for commencing treatment.

Genotype represents a more proven indicator of risk, with ZZ and rare ZZ-equivalent genotypes independently associated with COPD. New and better risk assessment models are needed to provide individuals diagnosed with AATD with reliable risk estimation and optimised treatment goals.

Introduction

Alpha-1 antitrypsin deficiency (AATD) is the only readily identifiable monogenic cause of COPD, most common in populations of European descent. To date, the only condition-specific intervention approved for the treatment of emphysema related to AATD is augmentation with intravenous AAT purified from pooled plasma (IV-AAT) [1], first approved by the United States Food and Drug Administration (FDA) in 1987. There is ongoing debate regarding which individuals should be prescribed IV-AAT, though it has long been proposed that those with AAT levels <11 µM (equivalent to 0.57 g·L^{-1}), known as the "putative protective threshold", are at increased risk of COPD.

Deficiency of the serine protease-inhibitor AAT results in decreased antiprotease activity, excessive elastin degradation and ultimately emphysema. Approximately 90% of individuals in populations of European descent are homozygous for the normal "wild-type" Pi*M allele (genotype MM) of the *SERPINA1* gene (14q32.1) which encodes AAT. The most prevalent deficiency-related alleles are Pi*S and the more severe

Pi*Z. Co-dominant inheritance of these alleles results in a range of genotypes, where each additional mutated allele leads to greater plasma deficiency (*i.e.* AAT levels in MM>MS>MZ>SZ>ZZ) (figure 1). While ZZ-AATD is rare, with prevalence estimated between 1/2500 and 1/5000 [2–7], genotypes such as MZ and SZ are far more common, present in up to 1/25 of the general population [7].

Pulmonologists will frequently encounter patients with a diagnosis of AATD in routine practice. Determining the significance and the need for treatment can be challenging for those not routinely dealing with AATD. The ZZ genotype of AATD has repeatedly been shown to be strongly associated with the development of COPD. The cohort-level trend in lung function among never-smoking ZZ individuals is towards accelerated decline relative to the usual (non-AATD) never-smoker population [8–10]. Conversely, never-smoker MZ and SZ individuals do not demonstrate accelerated decline relative to the non-AATD never-smoker population, suggesting equivalence with healthy controls, and require the addition of cofactors such as smoking to activate an increased risk of COPD [10–13]. From this point of view, genotype-defined severity presents a coherent and logical approach, with ZZ-AATD pre-disposing to COPD regardless of smoking, and MZ and SZ genotypes carrying an increased risk of COPD which is dependent on smoking (table 1).

Despite this, for more than three decades the definition of "severe" AAT deficiency has been based on the belief that individuals with circulating levels <11 µM of AAT are at increased risk of disease, although the exact derivation or accuracy of this proposed threshold has never been substantiated. Numerous guidelines and statements support the use of the <11 µM threshold as a classifier of severe AATD [14, 15]. Consequently, whether genotype or AAT levels should be used to guide indication for treatment in AATD remains unclear. The issue therefore raises two questions: 1) does the 11 µM provide additional discriminative utility in predicting risk of worse pulmonary outcomes; and 2) if not, what are the implications of using 11 µM as the chosen target concentration in augmentation therapy?

We review the evidence to provide clarity on a historically misunderstood topic which has become central to the accepted risk paradigm of AATD.

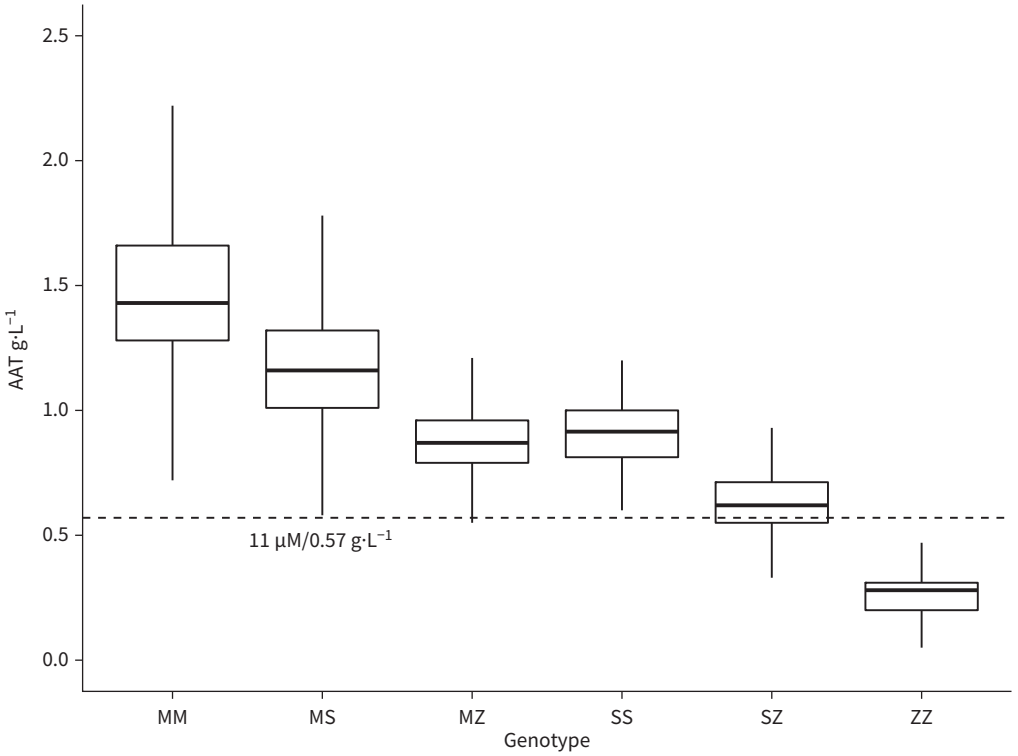

FIGURE 1 Common alpha-1 antitrypsin (AAT) genotypes and associated levels. Only the SZ genotype is routinely associated with levels that straddle the 11 µM (0.57 g·L^{-1}) threshold. Data from the Irish Targeted Detection Programme: M (n=12 363), MS (n=1905), MZ (n=3139), SS (n=102), SZ (n=340), ZZ (n=329).

TABLE 1 Summary of clinical evidence pertaining to common alpha-1 antitrypsin deficiency (AATD) genotypes

	MM	MS	MZ	SZ	ZZ
AAT levels <11 µM (expected proportion)	0%	0%	0%[#]	~40%[¶]	100%
Accelerated FEV_1 decline in never-smokers relative to MM	Ref.	No	No	No	Yes
Accelerated FEV_1 decline in smokers relative to MM smokers	Ref.	No	Yes	Yes	Yes
Typical predominance of emphysema in individuals diagnosed with COPD	Upper zone	Upper zone	Upper zone	Upper zone	Lower zone
Clinical trial evidence supporting use of IV-AAT for treatment of emphysema	No	No	No	No	Yes

FEV_1: forced expiratory volume in 1 s; IV-AAT: intravenous purified pooled human alpha-1 antitrypsin; Ref.: reference population. [#]: estimate based on 19 (0.006%) out of 3139 MZ individuals tested through the Irish National Targeted Detection Programme. Individuals with hepatic disease and synthetic failure may present with AAT levels <11 µM. [¶]: based on nonacute-phase AAT levels as demonstrated by Franciosi *et al.* [13].

Data for this review were identified by searches of MEDLINE (PubMed) and references from relevant articles published between January 1970 and April 2021, using the search terms "AATD", "alpha-1 antitrypsin deficiency" and "putative protective threshold" with no filter for original publication language. A secondary search of references was performed to identify manuscripts which specifically examined the clinical risk of disease associated with the MZ, SZ and ZZ genotypes, as well as the clinical and biochemical outcomes associated with the 11 µM threshold of AAT.

30 years ago: origins and plausibility of the 11 µM threshold

The exact origins of the putative protective threshold are unclear and no formal publication of its derivation exists. In a review published in 1989 [16], the group who originally defined the threshold stated that SS-genotype levels were typically between 13 and 19 µM and individuals with this genotype were not at risk of emphysema, while some SZ individuals, with levels of 6–11 µM, develop emphysema. However, at this stage, 2 years after FDA approval for IV-AAT and the seminal paper describing its use as a therapeutic target for ZZ-AATD [17], no high-quality studies of the SZ genotype had yet been performed and no evidence of increased risk of emphysema had been demonstrated. Subsequently, in 1991 the same group, using a more reliable standard for measuring AAT, refined their estimated range of SZ-AATD levels to 10–23 µM, suggesting that 11 µM approximated the lower 10th centile [18] (figure 1), although recent data suggest that 11 µM (interquartile range 9.6–13 µM) is closer to the 40th centile for SZ when levels are measured without acute-phase bias [13]. Nonetheless, on the basis that lung disease had not been reported as commonly in SZ as in ZZ-AATD, it was postulated that the protective threshold of AAT lies within the SZ range, a genotype associated with higher levels of AAT than ZZ, but lower than MZ. This level of AAT was adopted as the threshold for inclusion in the National Heart, Lung, and Blood Institute AATD registry [8]. In 1999, 12 years after FDA approval of IV-AAT, *in vitro* work by Campbell *et al.* [19] examined the effects of antiprotease insufficiency in AATD by comparing the area of proteolysis surrounding neutrophils drawn from individuals with differing AAT genotypes. Although the authors concluded that a significant increase in proteolytic area was associated with serum concentrations of AAT <10 µM, this effect was most clearly seen below concentrations of ~7 µM (figure 2a) and little difference was seen between non-ZZ genotypes (figure 2b). Since then, the 11 µM putative protective threshold has become a cornerstone of the risk paradigm in AATD, having been proposed as a categorical threshold for disease risk [20], an indication for consideration of IV-AAT therapy [21] and the therapeutic target for said therapy [17] (aiming to achieve weekly nadir plasma AAT levels >11 µM).

Hypothesis, confounders and relevance to clinical practice

The benefit of restoring the AAT levels of ZZ individuals [17] to those seen in a genotype which is typically not associated with lung disease in the absence of smoking (*i.e.* SZ) is fundamentally plausible, and moreover is supported by clinical evidence as demonstrated in the RAPID study [1], where a relative reduction in the rate of decline in lung density of 34% between those receiving IV-AAT *versus* placebo (when measured at total lung capacity) was seen. These results confirmed the hypotheses of AATD researchers, that augmenting levels in appropriate ZZ individuals to >11 µM resulted in measurable benefits.

Even among ZZ individuals, it is difficult to accurately predict the expected clinical course, with a significant number of individuals retaining good health. A ZZ genotype alone does not therefore automatically constitute a need for instituting augmentation therapy. Determining which individuals require treatment is complex, even when considering only ZZs and rare equivalent genotypes. Ideally, a method

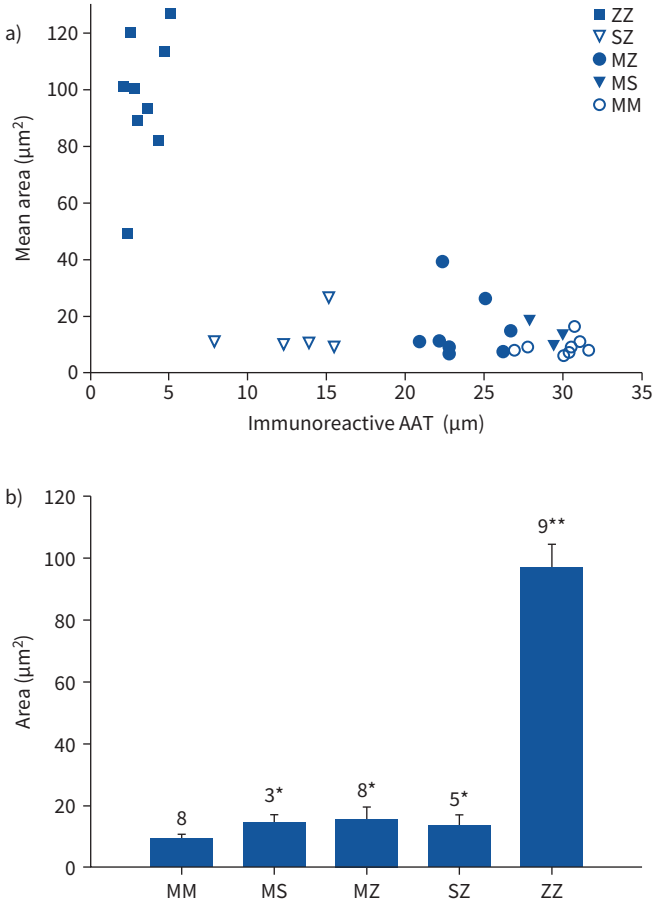

FIGURE 2 a) Size of quantum proteolytic events as a function of alpha-1 antitrypsin (AAT) concentrations in serum from donors: ZZ (n=9), SZ (n=5), MZ (n=8), MS (n=3) and MM (n=8). A significant increase in mean area of proteolytic events is seen below ~7 µM. b) Size of quantum proteolytic events represented by genotype cohorts. Reproduced from [19] with permission.

for determining or predicting "rapid-decliners" would be optimal, although recent data suggest that even in retrospective studies this is not as easy as one might expect [22]. In real-world practice, exactly how long an individual would need to be followed, how many measurements would be sufficient to robustly define a trend and what parameters should be met to indicate a need for initiating treatment remain undefined. As a result, the decision to treat remains in large part based on patients meeting the licensing indication of the various IV-AAT formulations (typically AAT levels <11 µM and forced expiratory volume in 1 s (FEV_1) 35–70% pred) and is at the discretion of clinicians. Ideally, these decisions would be best taken in centres with extensive expertise in AATD care, particularly when dealing with rare and uncommonly encountered genotypes such as Z/null and null/null mutations.

Nevertheless, no studies have ever demonstrated an innately increased risk of disease associated with the 11 µM threshold, or for that matter that SZ or MZ individuals benefit from IV-AAT, as no studies to support the latter practice have been performed. The nuance here is crucial.

The suggestion that levels below 11 µM result in an increased risk of disease in any genotype has led to frequent administration of IV-AAT to more common genotypes such as SZ and increasingly, MZ individuals [23] despite consensus on a lack of clinical evidence [15] and longstanding calls to avoid this practice, especially in the latter cohort [24]. Disconcertingly, treatment of even mildly deficient MS individuals with IV-AAT is seen in clinical practice [25] despite widespread recognition of no observed increased risk of COPD in this genotype [26, 27].

Individuals with the ZZ genotype do not achieve levels above the 11 µM threshold (aside from during an extreme acute phase response), and MZ heterozygotes do not typically present with levels below it.

Consequently, the need to define risk of disease based on a putative protective threshold of 11 µM of AAT is largely a moot point outside of the SZ genotype, given that it is the only one to typically result in plasma levels either above or below 11 µM (figure 1). Furthermore, a growing number of studies examining the SZ genotype have provided the opportunity to finally examine the accuracy of the 11 µM threshold as a classifier of risk within this very genotype.

Clinical evidence for a risk threshold at 11 µM

Our review found no studies which demonstrated an increased association of lung disease in non-ZZ individuals with levels below 11 µM, reaffirming the "putative" in putative protective threshold. Furthermore, while a large multicentre randomised controlled trial of IV-AAT *versus* placebo [1] and subsequent open-label extension study [28] demonstrated the benefits of augmenting circulating AAT levels to >11 µM in AATD, the study population included only two SZ and one MZ participants out of 180 (predominantly ZZ) individuals. As such, the applicability of these findings to heterozygous individuals is highly questionable. Conversely, a number of studies have provided results which collectively refute the 11 µM hypothesis of risk in nonsmoking non-ZZ genotypes.

In 1996 Turino *et al.* [29] reported one of the first large evaluations of 50 SZ individuals comparing them to ZZ-AATD. Interestingly, SZ individuals with levels <11 µM demonstrated better spirometry and diffusion capacity than those with levels above the threshold. Furthermore, in 2009, Holme *et al.* [30] compared the clinical features of 63 SZ and 63 ZZ individuals. They reported that 13 SZ individuals had levels <11 µM threshold, but that both the Medical Research Council score and 36-item short form physical summary score indicated better activity status in these individuals than the subjects with a level >11 µM. Moreover, they reported "no other differences in any variable studied, including computed tomography (CT) scan appearance and densitometry findings, between the two groups".

In 2015, Green *et al.* [31] compared a cohort of 126 SZs to 699 ZZs and 316 non-AATD individuals with COPD. In analyses examining the AAT level as a predictor of outcome, a level <11 µM was found to correlate with development of emphysema and lower zone predominant disease on CT, but only when including 699 ZZ individuals, all of whom would have AAT levels well below this threshold, thus undoubtedly confounding the relevance of the result to the SZ cohort. When the authors restricted the analyses of the interaction of AAT level and pack-years smoked on FEV_1 to only include SZ individuals (removing the confounding effect of the ZZ genotype), they found no significant effect, leaving them to determine that "specific studies in PiSZ patients would be required to determine this with confidence".

Our group recently examined the effect of the 11 µM threshold on clinical outcomes in the first prospective study of 82 SZ individuals [13] and subsequently again in a large registry study [10]. In the former, we found no evidence of an effect of AAT levels or the 11 µM threshold on outcomes in the SZ-genotype, no effect on spirometry for the interaction of AAT levels and pack-years smoked, and no effect for levels <11 µM on the rate of longitudinal spirometry decline in 60 SZs with median 60 months' follow-up. In our registry analysis of 117 SZ individuals, we found no association between AAT levels <11 µM and pulmonary function. Furthermore, we have shown that a significant proportion (~40%) of SZ individuals have levels of AAT <11 µM, suggesting that many individuals may be labelled "at-risk" on the basis of a threshold that lacks any supporting evidence. Overall, the data demonstrate no increased risk of lung disease attributable to the SZ genotype in never-smokers. Summarily, these data suggest a significant similarity between SZ and the moderate-deficiency MZ genotype, for which augmentation therapy is not recommended [14, 15].

Strikingly similar findings have been shown in a large-scale analysis [32] of AATD individuals studied in the UK Biobank [33], in which 867 SZ individuals and nearly 17 000 MZ individuals demonstrated minimal differences across a number of variables, and at whole-cohort level demonstrated near-parity to ~400 000 wild-type MMs (*e.g.* mean FEV_1/forced vital capacity (FVC) ratio=0.77 for MM *versus* 0.77 for MZ *versus* 0.77 for SZ and mean FEV_1 of 94% predicted for MM *versus* 94% predicted for MZ *versus* 95% predicted for SZ; p=nonsignificant for all comparisons). Further evidence that the risk of COPD is not linearly correlated to AAT levels was seen in this study. In analyses examining the total population (smokers and nonsmokers combined), the odds ratio for FEV_1/FVC <0.7 relative to MM was 1.1 for MZ (95% CI 1.0–1.1), 1.3 for SZ (95% CI 1.0–1.6) and 8.8 for ZZ (95% CI 5.8–13.3); a disproportionate order of magnitude greater for ZZ *versus* MZ/SZ than their respective levels would lead us to expect (figure 1). Similar findings were demonstrated in genotype-dependent all-cause mortality in the same study (figure 3), where the ZZ-genotype was associated with worse survival than MM (hazard ratio 2.4, 95% CI 1.2–4.6; $p=9.9\times10^{-3}$). These findings echo those reported in a Swedish study of 1339 ZZ patients followed for 18 years, though in those data never-smokers identified through screening (*i.e.* non-index

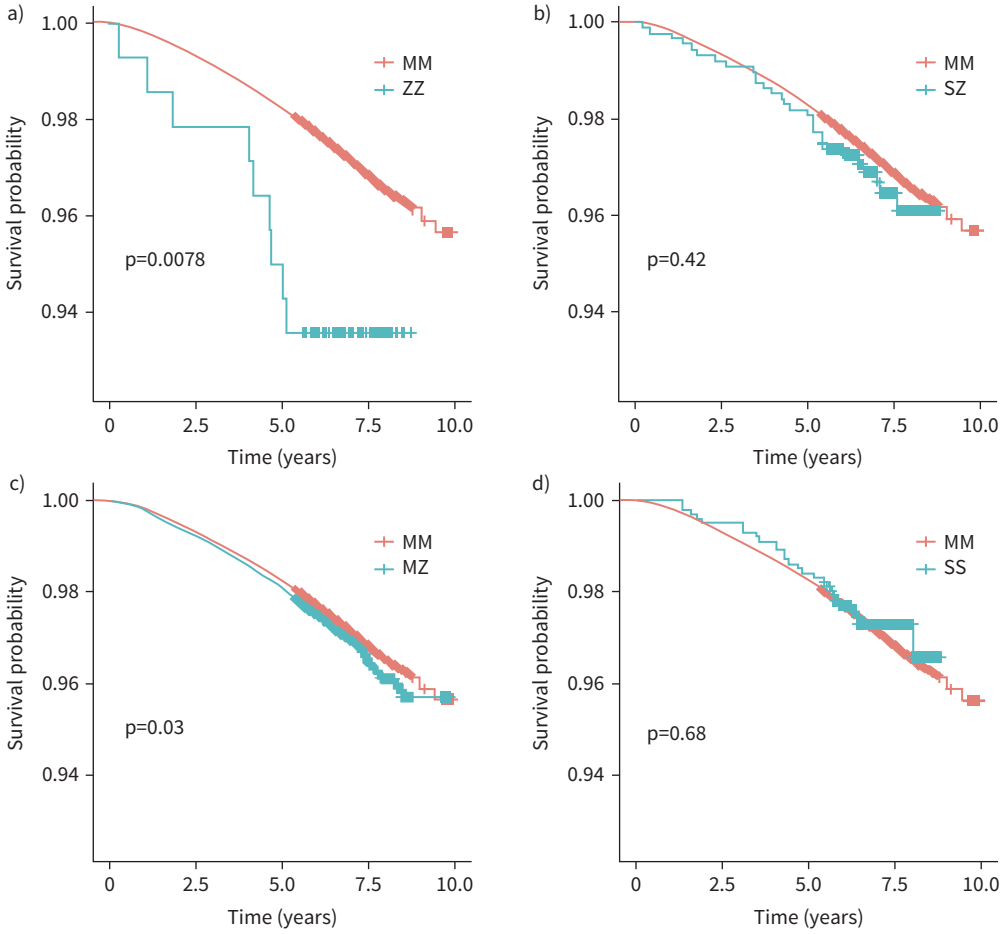

FIGURE 3 Survival curves of all-cause mortality stratified by *SERPINA1* genotypes in the UK Biobank. a) ZZ *versus* MM genotypes; b) SZ *versus* MM genotypes; c) MZ *versus* MM genotypes; d) SS *versus* MM genotypes. All p-values were calculated by log-rank test. Reproduced with permission [32].

cases) did not demonstrate a statistically significantly increased standardised mortality rate (1.20, 95% CI 0.44–2.63) [34].

Furthermore, we have previously reported that visually defined emphysema is typically not detected on CT chest scans of never-smoking MZ or SZ individuals, irrespective of levels above or below the threshold [10, 13]. Moreover, we have reported that visually defined emphysema distribution in SZ individuals is upper-lobe predominant as opposed to the lower-zone predominance seen in ZZ individuals, suggesting a phenotype more comparable with usual COPD. These findings support those reported by Green *et al.* [31] in 2015, where CT imaging in SZ individuals compared to ZZs demonstrated less emphysema and upper-lobe predominance as defined by the ratio of upper zone:lower zone voxel index at −910 HU. Taken together, the current evidence suggests that the typical concentrations of AAT seen in MZ and SZ individuals, even when <11 µM, are sufficient for preserving clinically normal lung physiology, with an increased risk of COPD only demonstrated in the setting of smoking (table 1).

The increased risk of COPD for MZ and SZ smokers relative to MM smokers has now been demonstrated repeatedly, highlighting the importance of encouraging smoking cessation in both moderate and severe AATD genotypes, especially given that the former individuals may be less likely to quit [35–37]. Tobacco smoking has been shown *in vitro* to reduce the antiprotease capacity of AAT [38], adding an acquired qualitative deficiency to the existing quantitative deficiency. This process occurs due to oxidation of the AAT molecule, with subsequent loss of anti-elastase capacity [39]. Consequently, IV-AATD therapy is not recommended for active smokers [14] as the efficacy of the therapy itself could be significantly reduced as a result of ongoing oxidation of the exogenous AAT protein. Importantly, our group has previously shown

that the plasma anti-elastase capacities of never-smoking SZ and former-smoking SZ individuals do not differ [13], suggesting that AAT activity in plasma normalises following the removal of tobacco smoke exposure, further reinforcing that smoking cessation should be the primary focus of care in AATD.

Whether use of IV-AAT is justifiable to slow lung function decline in non-ZZ individuals in whom COPD has already been diagnosed remains unclear. While there is a lack of data to compare the rate of decline between SZ-COPD and usual-COPD, data from SPIROMICS [27] suggests that the 3-year rate of FEV_1 decline did not differ significantly between 74 MZ and 1411 MM participants. Similar findings have been reported in other longitudinal population studies [40]. Given the clinical similarities demonstrated between MZs and SZs in the literature published to date an assumption of difference between the two in terms of decline among individuals with COPD would seem questionable.

A number of hypothetical benefits arising from treating heterozygous individuals with IV-AAT could be proposed [41], assuming the pathology is attributable to AATD. IV-AAT has been shown to modulate a wide range of immune responses [42] including pathways affecting neutrophil chemotaxis, interleukin-8 [43], tumour necrosis factor-α [44, 45], leukotriene B4 [46], and polymers of Z-AAT have been shown to have toxic gain-of-function effects [47–49]. Indeed, the emerging utility of IV-AAT to treat a range of illnesses seen in non-AATD (*i.e.* MM) individuals has been described [50, 51], suggesting that its role is that of more than just an antiprotease. However, if the increased inflammatory response to cigarette smoking in MZ and SZ-AATD is due to retention of intracellular Z-AAT rather than decreased levels, administration of exogenous AAT may not be expected to be of significant benefit. Nonetheless, for these hypotheses to translate to practice, prospective evidence of clinical benefit on pre-specified outcomes should be a pre-requisite.

Implications of adherence to the 11 µM threshold as a determinant of risk

While some may argue that prescribing IV-AAT to patients with moderate AATD genotypes could be justified on the basis of patient need or hypothetical benefit, the implications of the proliferation of a high-cost purified plasma therapy should be considered. Augmentation therapy comes with a time cost to the individual, has the potential to interfere with work schedules and may generally impact on the individual's own health perceptions. Furthermore, potential consequences related to repeated intravenous cannulation, fluid volume administration and adverse effects associated with plasma products should be considered. For individuals with moderate genotypes, where no proven benefit from IV-AAT has been shown, there is a real risk of causing inconvenience or harm, rather than providing an overall benefit. IV-AAT therapy carries significant cost implications to patients themselves (if the treatment is self-funded), the insurer or the health system that covers the cost of the treatment. Multiple studies have assessed the financial implications of augmentation therapy [52–57] as well as the cost of comorbidities associated with AATD [58]. IV-AAT costs equate to approximately USD 80 000 per patient annually [52]. The implications for individuals receiving treatment are not inconsequential. For those with lifetime insurance caps, a sizeable depletion could occur in a matter of short years, potentially impacting their ability to access other therapies. Augmentation therapy contributes significantly to the overall costs of AATD treatment, with the mean±SD annual healthcare costs in one US study found to be USD 122 936±96 036 for those receiving IV-AAT augmentation compared to USD 21 100±57 291 for those not receiving the treatment [52]. A 2003 cost–utility analysis assessing weekly infusions of Prolastin (Grifols) dosed weekly at 60 $mg \cdot kg^{-1}$ found that the annual cost associated with administration of augmentation therapy was ~EUR 45 000 for a 70-kg patient with a calculated cost per quality-adjusted life-year (QALY) gained of ~EUR 170 000 [59]. Of note, no subgroup analysis was performed to estimate the cost–benefits in non-ZZ genotypes. Furthermore, a 2016 cost-utility analysis by the All Wales Medicines Strategy Group [60] performed using data from RAPID [1] found that for treatment using Respreeza (CSL Behring) the cost per QALY gained was GBP 277 183 (~EUR 320 000).

The gradual shift in practice towards treating more common non-ZZ genotypes consequently has the potential to amplify AATD-associated costs significantly. Much of this shift can plausibly be attributed to the persisting acceptance of the 11 µM threshold. In the United States of America alone it can be estimated that a minimum of 1000 SZ and MZ (and even MS) individuals receive regular IV-AAT therapy [23, 25, 30], suggesting that the cost of treating individuals with these genotypes may easily exceed USD 80 million annually. Whether the best interests of patients with moderate AAT genotypes are truly being served by initiating IV-AAT should therefore be a point of greater scrutiny.

Moreover, the fact that previous studies did not subgroup outcomes by genotype, but rather simply used the threshold of 11 µM to define severity, raises a significant issue. As the recent evidence demonstrates that the AAT levels seen in SZ and MZ individuals are sufficient to preserve lung function in the absence

of smoking [10, 13], it stands to reason that had non-ZZ patients been excluded from these analyses, a more favourable cost per QALY gained might been demonstrated for those with truly severe AATD genotypes. Similar detrimental biases may have been inadvertently introduced into all previous studies, clinical or economic, which assessed the efficacy of IV-AAT therapy, due to the practice of using the putative protective threshold of 11 µM as an inclusion criterion, given that the current evidence suggests that moderately deficient individuals may have fared no worse without augmentation therapy in the first place.

As with all rare diseases, performing cost-effectiveness analyses in AATD is challenging. Furthermore, in real-world practice, clinicians understandably feel responsible for instituting an appropriate management plan for individuals presenting with abnormal AAT genotypes. To this end, a 2020 study examining claims databases of privately insured and Medicare Advantage enrolees found that implementation of an AATD disease management programme resulted in a reduction in cost in the management of patients with AATD over a 5-year period, attributable to a reduction in the number of exacerbations, emergency-room and specialist visits [56]. While the study did not compare AATD by genotypes specifically, it would stand that individuals with moderate genotypes may in the first instance be better served by the implementation of a disease management programme rather than resorting to IV-AAT.

Accuracy of the 11 µM threshold as a therapeutic target: opportunity for progress

Acceptance that the 11 µM threshold is indeed a slightly flawed hypothesis opens the way for improved understanding of how best to treat individuals with severe AATD. While the RAPID [1] and RAPID-OLE [28] studies demonstrated clinical efficacy for IV-AAT in reducing the rate of lung density loss in individuals with severe AATD, the treatment target of 11 µM was determined by an acceptance that this threshold was accurate, something that cannot now be taken to be true. AAT is an acute-phase protein, which rises and falls, irrespective of genotype, in the setting of inflammation, and consequently regulates immune response. The long-held assumption that a static, universal target AAT concentration is the best target for treatment has likely hampered efforts to refine treatment efficacy as it reduces the framing of AATD-related pathogenesis to a one-dimensional problem: insufficient antiprotease activity. In reality, the mechanisms that drive disease in AATD are more complex and depend upon the multidimensional interactions of antiprotease deficiency [61, 62], exaggerated and dysregulated neutrophil chemotaxis [43, 49] and immune dysfunction [42, 44] as well as genetic, epigenetic and environmental cofactors such as smoking. Indeed, even when examining the best-recognised pathogenic mechanism in AATD, antiprotease deficiency, it is worth noting that the S and Z isomers of AAT demonstrate fundamentally different antiprotease capacities [61] and association rates with neutrophil elastase and proteinase-3 [62], with S-AAT being more comparable to normal M-AAT than Z. Consequently, antiprotease activity would be likely to differ significantly between SZ and ZZ plasma, even at theoretical equivalent concentrations.

Cumulatively, these factors disproportionately affect ZZ-AATD, in large part explaining the nonlinear correlation between quantitatively determined deficiency and disease penetrance across the AATD genotypes. By extension, they also explain why a threshold for benefit in one genotype does not have to contiguous with a threshold for risk in another (*i.e.* the fact that treating ZZs to an 11 µM threshold has shown clinical benefits in ZZs is not incongruous with the absence of risk in <11 µM SZs). By comparing risk based on level, we fail to compare like with like.

Recognition of these facts, and a shift towards an individually determined risk estimation which captures the magnitude of inflammation and immune dysfunction could provide the opportunity to target dose-adjusted treatment in the appropriate individuals. Recently, the biological plausibility of this very hypothesis was demonstrated in pilot clinical trial of double-dose IV-AATD [63], while evidence from RAPID-OLE [28] suggested that higher trough levels of AAT might improve outcomes. The optimal dosing and target for any given individual may indeed therefore be higher than 11 µM.

Finally, novel specific therapies for AATD may on the horizon, including gene therapy [64–67], small-interfering RNAs [68–70] (which block hepatic synthesis of polymerogenic Z AAT) and small-molecule compounds which help Z-AAT exit the cell. Establishing a robust method for determining which patients will truly benefit from treatment will be essential. Based on the evidence to date, continued use of the putative protective threshold of 11 µM will not serve patients or healthcare systems well in this regard.

Discussion

There is now no evidence to support the hypothesis of the putative protective threshold of 11 µM as a categorical predictor of risk in AATD; indeed, the data in non-ZZ genotypes refute it. This conclusion should not be interpreted as suggesting that augmenting AAT concentrations to >11 µM in ZZ individuals

(and equivalent rarer genotypes) lacks efficacy. IV-AAT remains the only therapy proven to reduce the rate of lung tissue loss in severe AATD genotypes [1, 28] and has demonstrated effectiveness in treating rare manifestations of AATD, such as panniculitis [71], as well as having a potential role as a therapeutic immunomodulator in certain conditions even outside of AATD [50, 51]. Moreover, abrupt withdrawal of established augmentation therapy has been associated with significant clinical deterioration in ZZ individuals [72]. Taken together, these findings do indeed suggest that although 11 µM has served us well thus far for treating COPD in severe AATD, a more optimal target for augmentation may be achievable and may vary by individual on the basis of their inflammatory burden and response to therapy. Establishing agreed and robust means of characterising inflammatory burden in ZZ, and perhaps even specific subsets of heterozygous AAT-deficient individuals, could pave the way for much more refined therapies. This should be an area of particular interest going forward.

In practice, when determining risk of lung disease in AATD, genotype remains the best predictor, with the ZZ genotype significantly more at risk, independent of cigarette smoking [9, 10]. While a proportion of individuals with moderate AATD may be predisposed to worse lung outcomes, the appropriateness of resorting to IV-AAT to address this is questionable, given that these individuals also exist in non-AATD populations. These findings probably reflect the effects of environmental, genetic and epigenetic causes [73]. Consequently, the practice of prescribing IV-AAT to SZ, MZ and other moderate forms of AATD should be discouraged given the absence of data supporting either a need or a benefit in these individuals.

While the rarity of genotypes such a ZZ and Z/null mutations may have historically hampered the powering of studies analysing the effect of IV-AAT on traditional clinical end-points such as FEV_1, the same argument cannot be made for the far more common heterozygous genotypes, suggesting satisfactory data examining clinically meaningful end-points could be generated prospectively to test effectiveness of therapies in these groups if required.

Conclusion

The use of the 11 µM threshold as a determinant of clinical risk in AATD is highly questionable and should not be used as an indicator for commencing treatment. Genotype-based risk is more accurate and robust. Smoking cessation remains the most important intervention in AATD. Decisions regarding treatment with IV-AAT, especially in the setting of rare and atypical genotypes, should be made at centres with significant expertise. Treating ZZ individuals to a target of 11 µM AAT has shown clinical benefits, though more biologically appropriate targets should be explored. Individuals diagnosed with AATD deserve a better risk stratification paradigm than the putative protective threshold, so as to provide them with optimal care and, when appropriate, reassurance. Improvements in clinical phenotyping, biomarker discovery, computational analysis, and polygenic risk modelling [32] may provide new insights in this area, but will require significant international collaborative efforts to generate appropriate and carefully selected study populations [74].

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