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Update on α_1-antitrypsin deficiency

α_1-Antitrypsin deficiency (AATD) is an inherited metabolic disorder in which mutations in the coding sequence of the *SERPINA1* gene prevent secretion of α_1-antitrypsin (α_1-AT) and cause predisposition to pulmonary and liver diseases. The heterogeneity of clinical manifestations in AATD is related to the complexity of biological function of α_1-AT. The role of smoking is crucial in the natural history of lung damage progression in severe AATD individuals, even if it also partly explains the heterogeneity in lung disease. Lung damage progression in AATD can also be related to body mass index, exacerbation rate, sex, environmental exposure and specific mutations of *SERPINA1*. Recent randomised controlled trials, together with previous observational work, have provided compelling evidence for the importance of early detection and intervention in order to enable patients to receive appropriate treatment and preserve functional lung tissue.

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α_1-Antitrypsin deficiency (AATD) is an inherited metabolic disorder in which mutations in the coding sequence of the *SERPINA1* gene, also known as the proteinase inhibitor (Pi) system, prevent secretion of α_1-antitrypsin (α_1-AT) and cause predisposition to pulmonary and liver diseases. In particular, AATD has been associated with the development of chronic obstructive pulmonary disease (COPD), characterised by permanent destruction of the alveolar distal unit of the terminal bronchioles (emphysema) and increased risk for infectious exacerbations [1].

Severe AATD is inherited as an autosomal recessive disorder with codominant expression, as each allele contributes 50% of the total circulating enzyme inhibitor. Severely reduced serum α_1-AT levels occur from the inheritance of two Pi-deficient alleles at the *SERPINA1* gene on chromosome 14 (14q32.1), with most cases resulting from homozygous inheritance of the Z allele (p.E366K; giving the genotype known as PiZZ), although more than 100 pathological variants have been so far identified.

Any individuals heterozygous for a pathological variant should not be simply labelled as "healthy carriers", since the risk of lung diseases in this category can vary largely, according to the gene mutation and environmental exposure.

Pathogenesis of AATD in the lungs

α_1-AT is a 52-kDa glycoprotein mainly synthesised and secreted by hepatocytes into the bloodstream. Nevertheless, lung tissue is the principal target of α_1-AT, since the protein is a serine-proteinase

inhibitor and it is crucial in maintaining protease–antiprotease homeostasis in the lungs.

The principal pathophysiological pathway is associated with neutrophil recruitment and the release of serine proteinases, especially neutrophil elastase, which causes collateral tissue damage due to inadequate α_1-AT protection. The role of inhibition that α_1-AT carries out towards neutrophil elastase is well known (figure 1a) [2]. The imbalance of the protease/antiprotease activity in favour of the neutrophil serine proteases can result in a self-perpetuating cycle of inflammation and respiratory tissue damage. Moreover, α_1-AT also inhibits two other serine proteinases, namely cathepsin G and proteinase 3, which are produced by neutrophils and cause lung damage. New findings have emerged on the role of α_1-AT in inhibiting a broader range of proteases, such as metalloproteases and cysteine-aspartic proteases [3]. Furthermore, α_1-AT may have other anti-inflammatory and immunomodulatory effects, including reduction of Toll-like receptor expression, reduction of neutrophil adherence to the endothelium, and reduction of selected proinflammatory cytokines in the lungs [4, 5]. In this picture, it is pretty clear what impact a deficiency or lack of α_1-AT could have on lung tissue protection (figure 1b).

Cigarette smoking is an additional risk factor, which accelerates the development of lung pathologies in individuals with AATD, as supported by the *pallid* mice model [6]. Oxidative modifications of α_1-AT are induced by components of cigarette smoke, as well as by oxidants and enzymes (*e.g.* myeloperoxidase) released by cells at sites of inflammation. Although oxidative modifications do not abolish the anti-inflammatory effects of α_1-AT [3], the oxidation of the P1 methionine (methionine 358 or methionine 351) to methionine sulfoxide significantly reduces the ability of α_1-AT to inhibit neutrophil elastase released by neutrophils during inflammatory processes in the lungs [7]. The oxidation of methionine in α_1-AT by oxidants released by cigarette smoke or inflammatory cells not only reduces the effective anti-elastase protection in the lungs, but also converts α_1-AT into a proinflammatory mediator; the oxidised α_1-AT, which is generated in the airways, interacts directly with epithelial cells to release chemokines that attract macrophages into the airways [8].

Recent studies have demonstrated that, even in α_1-AT non-deficient individuals, cigarette smoking disables the endothelial pro-survival effect of α_1-AT, which may contribute to chronic lung damage in susceptible individuals [9]. However, the lack of function of α_1-AT is not the only mechanism by which this protein contributes to lung impairment. The enhanced tendency of Z-type α_1-AT to combine in oligomeric assemblies is well documented in hepatocytes and it has been recently demonstrated in bronchial epithelial cells [10]. Polymeric forms of Z-type α_1-AT are less active as elastase inhibitors, and may also possess proinflammatory properties (figure 1c) [11]. Polymers secreted from hepatocytes can contribute to circulating polymers [12], which have also been found in lung lavage. Extracellular polymers are chemotactic and stimulatory for human neutrophils and may contribute to inflammatory neutrophil infiltration in the lungs [13]. Moreover, the observation that cigarette smoke accelerates polymerisation of Z-type α_1-AT by oxidative modifications [14] has linked two of the major prevailing hypotheses in COPD, namely oxidants and proteinases in Z-type α_1-AT-related emphysema, and added the new idea that the polymers in the lung could promote lung inflammation.

Similarly to what occurs in "usual" COPD, an important adaptive immune inflammation, comprising B, $CD4^+$ and $CD8^+$ lymphocytes and lymphoid follicles, is a prominent feature in AATD [15]. Baraldo *et al*. [15] showed that lymphoid follicles in AATD and usual COPD were

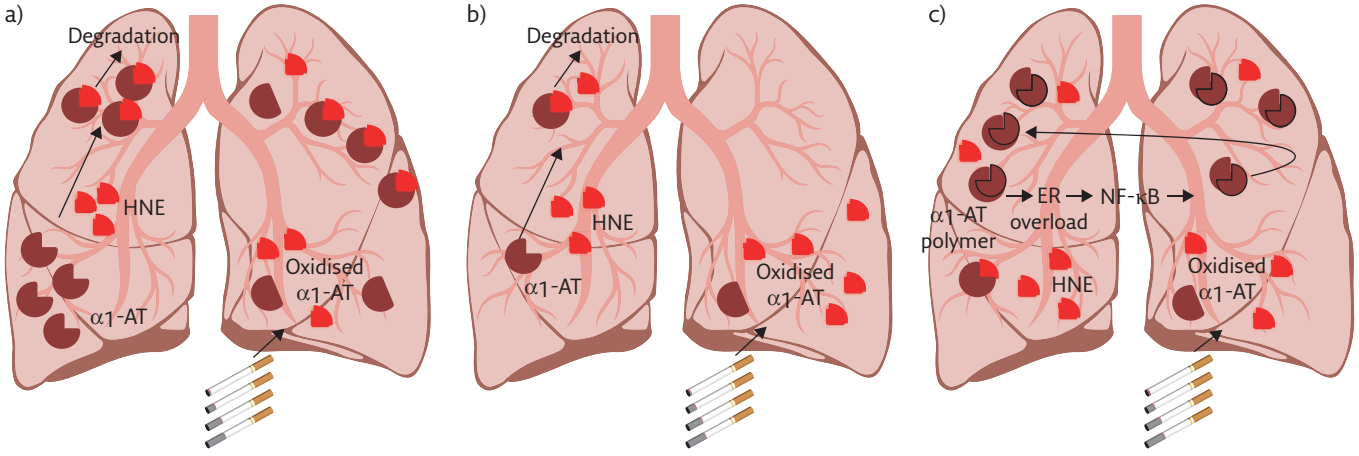

Figure 1 *Roles and functions of α_1-AT in lungs of a) individuals with normal levels of protein, b) patients with deficient or null mutations, and c) patients with deficient and polymerogenic mutations of the SERPINA1 gene. HNE: human neutrophil elastase; ER: endoplasmic reticulum.*

markedly increased when compared with control groups, and their number correlated negatively with the ratio of forced expiratory volume in 1 s (FEV1) to forced vital capacity (FVC). These results suggest that the extent of inflammation in AATD is similar to that found in severe usual COPD; thus, the inflammatory mechanisms involving the adaptive immune system, known to be important in usual COPD, seem also to be at play in AATD.

Another interesting role of α_1-AT polymers implies an increased tendency of Z-type α_1-AT towards hydrophobic interactions. In fact, α_1-AT polymers have a better propensity to bind fatty acids and upregulate the expression of angiopoietin-like protein 4 (Angptl4), thus suggesting a novel role for α_1-AT in lipid homeostasis and immune regulation [3].

Different α_1-AT variants have a different prognosis

There is considerable heterogeneity in clinical presentation among AATD patients, since this disorder predisposes to lung and liver disease, but it might also manifest with granulomatosis with polyangiitis and panniculitis. Various studies demonstrate that lower levels of α_1-AT are associated with a risk of HIV type 1 infection [16], type II diabetes mellitus [17], spontaneous abortions [18] and pre-eclampsia [19].

The clinical pulmonary manifestations are rather various, although the "classical" clinical phenotype still remains the basal panacinar emphysema; nevertheless, some patients display other radiological patterns, such as centrilobular emphysema and bronchiectasis. Signs and symptoms of pulmonary involvement with AATD closely resemble those of other patients with COPD. However, a very recent study on 500 severe AATD subjects has provided evidence that nearly 46% of all participants were diagnosed with either asthma or allergic disease [20], and a higher incidence of the main AATD alleles has recently been detected in pulmonary Langerhans cell histiocytosis [21].

When attempting to explain the heterogeneity of clinical manifestations, the complexity of biological function of α_1-AT is not negligible. Interactions of α_1-AT with other molecules may lead to degradation, complex formation, oxidation, self-assembly or other modifications. Genetic mutations together with cigarette smoke, which is known to induce post-translational modifications such as oxidation or polymerisation, may alter α_1-AT bidirectional intracellular traffic in endothelial cells and thus determine its functional bioavailability in certain lung compartments [22].

It is known that the stepwise reduction in plasma α_1-AT is associated with greater risks of spirometry-defined airway obstruction and COPD, and there is a clear association between α_1-AT concentration in plasma and common genotypes of *SERPINA1* [23]. Pathological α_1-AT variants are either "null", with no detectable levels of α_1-AT in serum and generally due to a premature stop codon, or "deficient", where a point mutation causes different grades of retention in the hepatocytes and, consequently, reduced levels of α_1-AT in plasma. The most common severely deficient variant is Z-type α_1-AT (p.E366K), but several other deficient variants, often referred to as "rare", have been identified over the last decades. The alleles bearing missense mutations result in conformationally altered proteins that have variable degrees of degradation/accumulation in the endoplasmic reticulum and different degrees of plasma deficiency. Nonrespiratory clinical manifestations, mainly associated with intracellular accumulation of α_1-AT polymers, are listed in table 1.

Table 1 *Nonrespiratory clinical manifestations of AATD*

Organ(s)	Clinical manifestation	Life stage
Liver	Prolonged jaundice after birth	Infant
	Hyperbilirubinaemia	Infant
	Abnormal liver enzymes	Infant/adult
	Cirrhosis	Adult
	Hepatocellular carcinoma	Adult
	Cholangiocellular carcinoma	Adult
Adipose tissue	Panniculitis	Young adult/adult
Small/medium-size vessels	Granulomatosis with polyangiitis	Adult

The progression of lung damage in severe AATD

Individuals with severe AATD have, as a consequence of pathological *SERPINA1* alleles inherited from both parents, α_1-AT plasma concentrations below the canonical protective threshold of 11 µM, corresponding to 50 mg·dL^{-1} or 0.5 g·L^{-1} [23]. Most of our knowledge about the natural history of severe AATD (PiZZ) has come from the follow-up data from a Swedish birth cohort [24]. The purpose of the Swedish neonatal screening was to determine the frequency of liver disease during the neonatal period in AATD; therefore, no details about lung diseases were reported in the first publication [24]. Anyway, lung clinical manifestations in AATD during infancy are still reported as case reports. Although respiratory symptoms do not usually appear during childhood, recent studies conducted in a cohort of children and teenagers diagnosed with severe or intermediate AATD have suggested that oxidative stress is a feature of severe AATD at an early stage, as indicated by lower total glutathione and reduced glutathione levels, decreased catalase activity and increased glutathione peroxidase activity [25], as well as reduced telomere length [26], in children and teenagers with severe AATD compared to controls.

The examination of the Swedish cohort at about 18 years of age reported essentially normal lung function [27], but high prevalence of asthma symptoms especially among ever-smokers was reported at 22 years of age [28]. At 35 years of age, the PiZZ ever-smokers had significantly lower transfer coefficient of the lung for carbon monoxide (*K*CO) values and 15th percentile density than the control subjects [29]. The prevalence of recurrent wheezing was significantly higher among the PiZZ ever-smokers than the PiZZ never-smokers, which could indicate early symptoms of COPD [30].

The UK Antitrypsin Deficiency Assessment and Programme for Treatment (ADAPT) programme demonstrated in a group of 101 PiZ individuals, mainly between the fourth and sixth decades of life, that FEV1 decline was greatest in those with moderately severe disease, and this showed associations with bronchodilator reversibility, body mass index and male sex and exacerbation rate [31]. *K*CO decline, conversely, was greatest in severe disease [31]. In the same cohort, decline of lung function was predicted by outdoor pollution, in particular exposure to ozone and particles with a 50% cut-off aerodynamic diameter of 10 µm (PM_{10}) [32]. An observational study in patients with severe AATD enrolled in the Spanish and Italian national registries showed that, among the three principal clinical phenotypes (emphysema, chronic bronchitis and asthma), patients with chronic bronchitis were younger, had more preserved lung function and lower tobacco consumption, whereas patients with asthma–COPD overlap were more frequently never-smokers and female [33]. The role of smoking is crucial in the natural history of lung damage progression in severe AATD individuals, even if it also partly explains the heterogeneity in lung disease. Cigarette smoking was the greatest predictor of impairment in FEV1 and diffusing capacity of the lung for carbon monoxide (*D*LCO) in the PiZZ cohorts (139 individuals) identified from the Irish National AATD Registry and (surprisingly) passive smoke exposure in childhood resulted in a greater total pack-year smoking history [34]. In severe AATD the impact of smoking is greater at the beginning of the habit, as demonstrated by the steeper decline in lung function with the first 20 pack-years of smoking compared with consequent consumption for PiZZ and PiSZ patients [33]. Later on, intensive (ex-) smokers had diminished differences in quality of life and exacerbation frequency between PiZZ and PiSZ individuals, as recently demonstrated by data from the German registry for individuals with AATD [35].

The PiZZ subjects identified by the neonatal Swedish programme had a significantly shorter survival time at the age of 35 years than the controls of the Swedish general population, yet the never-smoking PiZZ individuals had a similar life expectancy to the control never-smokers. Mortality due to respiratory disease was markedly increased in PiZZ smoker Swedish subjects compared with the age- and sex-matched Swedish population [30]. Lower body mass index has also been linked with greater progression of lung disease and mortality in PiZ patients [36].

The lung damage progression associated with intermediate AATD

According to the recently published European Respiratory Society statement on AATD, "Never-smoking PiMZ subjects do not have an increased risk for COPD" and "Smoking PiMZ and PiSZ subjects have an increased risk of COPD compared to smoking PiMM subjects" [37]. Data about lung damage progression in intermediate AATD, principally due to the presence in heterozygosity of Z or S alleles, are difficult to obtain because of scant screening programmes in the general population. Data from the Copenhagen City Heart Study and the SAPALDIA (Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults) epidemiological studies have partially filled this gap. Thanks to the population-based Danish cohort with 21-year follow-up data between the fourth and sixth decades of life and triple measurements of lung function, Dahl *et al.* [38] reported that the average annual decrease in FEV1 was 19% greater in persons with the PiMZ genotype and 169% greater in persons with the PiSZ *g*enotype, in comparison with a 21-mL average annual decrease in persons with the PiMM genotype. Studies based on the SAPALDIA cohort indicated that the PiMZ genotype was associated with an accelerated average annual decline in forced expiratory flow at 25–75% of FVC ($FEF_{25-75\%}$), in smoking and obesity subgroups from the general population, in comparison to the PiMM genotype [39]. Moreover, a statistically significant interaction ($p<0.0001$) was observed between the PiMZ genotype and high levels of exposure to vapours, gas, dusts and fumes (VGDF) on annual change in $FEF_{25-75\%}$ [40]. A similar interaction of statistical significance ($p=0.03$) was observed between the PiMZ genotype and high-level VGDF exposure on annual change in FEV1/FVC [40]. More recently, a robust family-based study to determine the risk of COPD in PiMZ individuals indicated that PiMZ heterozygotes are at an increased risk for impaired lung function and COPD, and cigarette smoke exposure exerts a significant modifier effect [41].

A comparison of lung function decline among PiMZ and PiZZ subjects has not yet been examined, although this matter does deserve attention.

The paradigm of null mutations

The identification of the exact molecular mechanisms underlying AATD would definitely help in the definition of different diagnostic risk levels

for the liver and lung disease displayed by varying AATD genotypes. For example, the identification of alleles associated with a high degree of α_1-AT polymerisation, such as Z or M_{malton}, would indicate the need for a more extensive liver evaluation, whereas null alleles do not indicate a risk for liver pathology. Regarding a possible relationship between the severity of lung disease and different α_1-AT genotypes, most of the data are focused on the Z mutation. Progression and severity of lung diseases are little considered in cohorts of AATD patients with rare pathological variants, mainly because their rareness. The only exceptions are null mutations, where interest is raised by the total absence of α_1-AT. As a consequence of the fact that serum levels of α_1-AT are correlated with the severity of the pulmonary phenotype, subjects with null mutations in homozygote fashion should be considered a subgroup at particularly high risk of emphysema within AATD. Among the lung function measurements, FEV1 as a percentage of predicted and *K*CO as a percentage of predicted were statistically lower in subjects who were null/null, in comparison to PiZZ [42]. Moreover, evidence of the recurrence of lung symptoms (dyspnoea, cough) and lung diseases (emphysema, asthma, chronic bronchitis) was reported in M/null subjects aged >45 years, irrespective of their smoking habit [43].

The simplification of the pathogenetic mechanisms of null mutations, since no polymer effect needs to be considered, is interesting in terms of lung damage progression. Null/null patients have a total absence of α_1-AT *in vivo*; therefore, their clinical follow-up would allow a comprehensive investigation of the role of α_1-AT as a serine-proteinase inhibitor.

Table 2 *Lung density and lung function measurements in RCTs and observational studies with augmentation therapy in AATD*

Study	Year	Treatment and comparator	Subjects n	Treatment duration years	Infusion frequency	Decline in lung density at TLC $g \cdot L^{-1} \cdot year^{-1}$	FEV1 decline $mL \cdot year^{-1}$
RCT *versus* placebo							
Dirksen [44]	1999	250 $mg \cdot kg^{-1}$ α_1-AT *versus* 625 $mg \cdot kg^{-1}$ albumin solution	58	3	Monthly	2.6 *versus* 1.5 (p=0.07)	59 *versus* 79 (p=0.25)
Dirksen [45]	2009	60 $mg \cdot kg^{-1}$ α_1-AT (Prolastin) *versus* 2% albumin solution	77	2	Weekly	1.4 *versus* 2.2 (p=0.06)	
Chapman [46]	2015	60 $mg \cdot kg^{-1}$ α_1-AT (Zemaira) *versus* lyophilised preparation	180	2	Weekly	1.5 *versus* 2.2 (p=0.03)	44.3 *versus* 44.9 (p=0.21)
Observational with control							
Seersholm [47]	1997	60 $mg \cdot kg^{-1}$ α_1-AT (Prolastin or Trypsone) *versus* no therapy	295	1	Weekly		53 *versus* 75 (p=0.02)
AATD registry group [48]	1998	60 $mg \cdot kg^{-1}$ α_1-AT (Prolastin) *versus* no regular therapy	1129	1–7	Weekly		73 *versus* 93 (p=0.01)
Wencker [49]	2001	60 $mg \cdot kg^{-1}$ α_1-AT *versus* data prior to infusion	96	1	Weekly		34 *versus* 49 (p=0.02)
Tonelli [50]	2009	Any dose regimen *versus* no therapy	164	3.5			37 *versus* 46 (p=0.05)

RCT: randomised controlled trial; TLC: total lung capacity.

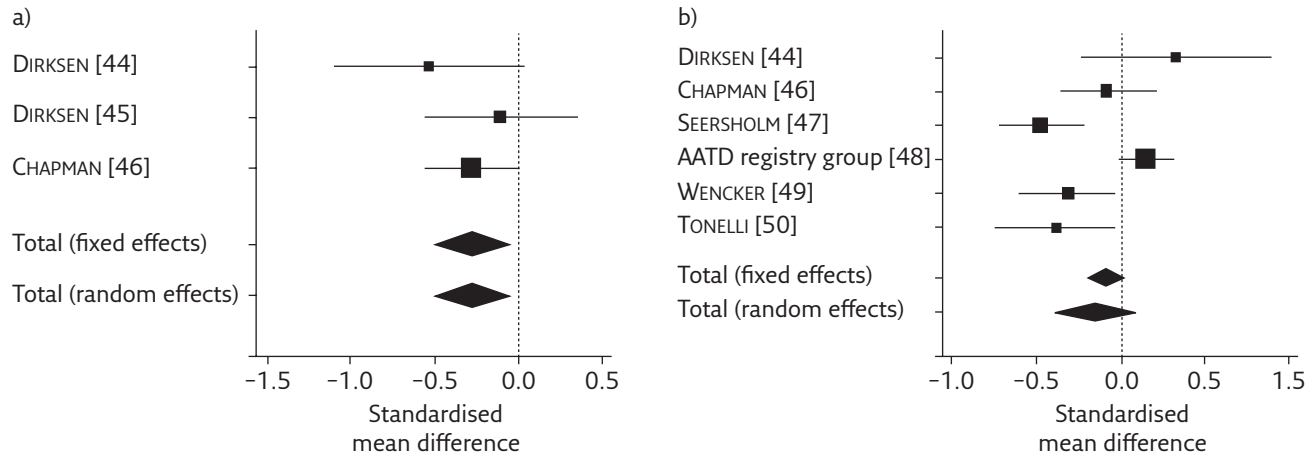

Figure 2 *Forest plot of studies included in the meta-analysis of a) CT scan density and b) FEV1 data.*

Augmentation therapy and lung damage progression: a meta-analysis

The current standard of care for patients affected by AATD-associated pulmonary emphysema is replacement therapy by weekly intravenous infusion of pooled human plasma purified α_1-AT. By the dose usually recommended (60 mg per kg bodyweight, weekly), the plasma level of α_1-AT is kept over the protective threshold (>50 mg·dL^{-1}) for the 7 days preceding the next infusion.

The appropriateness and utility of this therapy has been a topic of intense debate. There are some meta-analyses and reviews about it, and the recent European Respiratory Society statement on AATD dedicated to this matter an exhaustive analysis based on standard systematic review [37], which showed that intravenous augmentation therapy reduces the progression of emphysema as assessed by computed tomography (CT) densitometry. The reduction of the rate of lung density decline with augmentation therapy compared with placebo and the reduction of lung function decline in observational studies are reported in table 2. Because of expected between-study heterogeneity, a random effects model was employed for meta-analysis; pooled differences in density and FEV1 slopes were estimated using the META program (https://mathgen.stats.ox.ac.uk/genetics_software/meta/meta.html). Figure 2 presents the individual study and pooled density and FEV1 slopes and slope differences. Our meta-analysis failed to show a significant effect on FEV1 decline (standardised mean difference (SMD) –0.132 mL·year^{-1}, 95% CI –0.406 to 0.113 mL·year^{-1}; p=0.269), while it showed a positive effect on CT densitometry, with a density SMD of –0.275 g·L^{-1}·year^{-1} (95% CI –0.499 to –0.0506 g·L^{-1}·year^{-1}; p=0.016) between

Table 3 *Advice for severe and intermediate AATD*

Advice for severe AATD (homozygotes or compound heterozygotes)
Quit smoking
Avoid outdoor pollution and exposure to dust/irritants
Avoid alcohol and apply appropriate diet, in case of polymerogenic mutations
Test for AATD in first-degree relatives
Perform regular respiratory follow-up: lung function tests, lung imaging (if necessary)
Perform regular liver follow-up, in case of polymerogenic mutations: liver tests, abdominal ultrasounds, fibroscan
Give augmentation therapy, if necessary
Advice for intermediate AATD (heterozygotes)
Quit smoking
Avoid outdoor pollution and exposure to dust/irritants
Reduce alcohol and apply appropriate diet, in case of polymerogenic mutations
Perform regular respiratory follow-up: lung function tests, lung imaging (if necessary)
Perform regular liver follow-up, in case of polymerogenic mutations: liver tests, abdominal ultrasounds, fibroscan

Key points

- Lung tissue is the principal target of α_1-AT, since the protein is a serine-proteinase inhibitor and it is crucial in maintaining protease–antiprotease homeostasis in the lungs; α_1-AT has also anti-inflammatory and immunomodulatory effects.
- Clinical pulmonary manifestations in individuals with AATD are rather various, and include emphysema, chronic bronchitis, bronchiectasis and asthma; this heterogeneity in lung disease is only partly explained by exposure to known risk factors, such as cigarette smoke.
- In clinical practice, annual measurement of lung function, including post-bronchodilator FEV_1 and lung diffusion, provides information about disease progression. Lung damage in severe AATD is appreciable after the third decade of life and FEV_1 decline is associated with smoking, body mass index and exacerbation rate. The risk of lung diseases in individuals with intermediate AATD can vary largely, according to the gene mutation and environmental exposure.
- Several randomised clinical trials in severe AATD have shown that intravenous augmentation therapy reduces the progression of emphysema as assessed by CT densitometry.

active treatment and placebo. This confirms the results of a previous Cochrane systematic review [51]; however, statistical methods and conclusions were different. We state that the protective effect of augmentation therapy is primarily attributable to the blunted decline of lung density, because CT lung density reflects emphysema lung destruction (and thus disease severity) better than FEV_1 does. In contrast, changes in FEV_1 are less sensitive, so that several hundred patients would need to be randomised to capture a significant effect in a few years of follow-up. Although many of the observational studies showed a benefit of treatment on the rate of FEV_1 decline, the potential for bias is greater than in a randomised controlled trial, and the data should be interpreted with caution.

Conclusions

Early and precise diagnosis of AATD is essential to address healthy lifestyles, prevent clinical manifestations, set up an effective follow-up and apply appropriate therapy, in order to delay symptoms and slow down the progression of emphysema. Lifestyle, follow-up and therapy advice for both severe and intermediate AATD patients is summarised in table 3.

Conflict of interest

I. Ferrarotti reports grants, and personal fees for seminars and congress participation, from CSL Behring, outside the submitted work. All other authors have nothing to disclose.

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