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WHY IS DISEASE PENETRATION SO VARIABLE IN AATD? THE CONTRIBUTION OF ENVIRONMENTAL FACTORS

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Abbreviations

AATD	Alpha 1 antitrypsin deficiency	VGDF	Vapours, gases, dust and fumes
COPD	Chronic obstructive pulmonary disease	BHR	Bronchial hyperresponsiveness
NE	Neutrophil elastase	TLCO	Transfer factor of the lung for carbon monoxide
FEV1	Forced expiratory volume in one second	FVC	Forced vital capacity
KCO	Carbon monoxide transfer coefficient (TLCO/alveolar volume)	VC	Vital capacity
CT	Computerised tomography		
PM10	Particulates with diameter <10µM		

ABSTRACT

Environmental influences on clinical phenotype in AATD include cigarette smoke, occupational exposures, airway/sputum bacteria and outdoor air pollution. This narrative review describes the impact of the major environmental exposures and summarises their effect on clinical phenotype and outcomes. In general patients with AATD are more susceptible to pulmonary damage as a result of relatively unopposed action of neutrophil elastase, in the context of neutrophilic inflammation stimulated by environmental factors. However the amount of phenotypic variability explicable by environmental factors is insufficient to account for the wide range of clinical presentations observed, suggesting that a combination of genetic and environmental factors are likely to be responsible.

INTRODUCTION

Alpha 1 antitrypsin deficiency (AATD) is the only widely accepted genetic risk factor for chronic obstructive pulmonary disease (COPD) and emphysema, but there is wide variability in clinical presentation (1). Many factors are likely contribute to this, and to interact with genetic factors in the classical 'disease susceptibility + environment' manner that is common to many chronic diseases. Environmental factors are a particularly attractive area for clinicians to focus on as they are potentially modifiable, hence influence our discussions with patients about their current exposures, and how they might alter them to minimise risk of disease. In order to discuss which environmental factor may be important to AATD patients, we first need to think about what our environment actually is, and which manifestations of AATD such exposures may modify. The dictionary definition of environment is 'the external conditions in general affecting the life, existence, or properties of an organism or object'(2); in this sense any inhaled or ingested agent would be considered part of the environment, since it exists external to the patient, so we need to consider which of these affect pathology in AATD.

Conceptually social factors would also be considered part of the environment, though these are poorly studied for their relationship to pathology independent of their relationship to exposures within that environment. For example, low socio-economic status is associated with higher rates of cigarette smoking (3), and health detriments may occur from smoking, but could be influenced by other coexisting factors in the social environment. This narrative review will therefore focus on those external influences which have been studied to a reasonable degree in AATD, and affect the lung, namely cigarette smoking, air pollution and occupation. We will then consider whether

airway microbiota can be considered within the dictionary definition of environmental factors relevant to AATD or not. Finally, the environmental influences on disease outside the lung will be discussed briefly.

EFFECTS OF CIGARETTE SMOKE

In AATD one of the main drivers of lung pathology is protease imbalance, mediated by the relative deficiency of AAT, and hence unopposed action of neutrophil elastase (NE) and other proteases (4). The mechanism by which this occurs is illustrated in figure 1. Whenever a neutrophil enters the lung there is an obligate area of damage associated with the release of NE, which in AATD is larger than in individuals with normal AAT levels since it is unopposed. Smoking stimulates neutrophilic inflammation in the lung, and hence the amount of NE mediated damage to the lung in AATD is larger than in smokers who have normal AAT levels. Once COPD is present this also influences neutrophil transit in the lung (5), hence there is the possibility of exacerbating pathogenic processes dependent on the neutrophil, though it is possible neutrophil transit changes are exclusive to usual COPD, as in vitro work suggest PiZZ neutrophils have normal chemotactic activity(6). Aging may modify the effect of smoke exposure on neutrophilic inflammation in mice(7), and it is possible this effect exists in man, given that smoke exposure in older age appears more deleterious(8). In addition smoke exposure may alter the inherent immunological and oxidative properties of AAT in a detrimental way (9, 10), and in polymeric forms of AATD may drive polymerisation(10). Polymers themselves drive inflammation (11), hence the effects of smoking in AATD may be further magnified by secondary accumulation of polymers.

This wealth of mechanistic data supporting harmful effects of cigarette smoke is supported by clinical observations. Patients with AATD who smoke, or have smoked, are more likely to exhibit COPD and emphysema, and the relevant physiological markers of these conditions, specifically lower FEV1 and gas transfer coefficient (KCO), than those with less or no smoke exposure(12, 13). The prevalence of chronic bronchitis appeared unrelated to smoke exposure in our data (12); this is perhaps surprising since early onset chronic bronchitis outside AATD is associated with smoke exposure(14). The AATD genotypes have variable levels of risk dependant on enzyme levels. PiMM individuals have normal levels of AAT and thus smoking results in the same risk of COPD as normal individuals (15). PiMZ are at a slightly increased risk of emphysema (16), but do not develop lung disease in the absence of smoking (17); FEV1/FVC ratio and FEV1% predicted were significantly decreased compared to PiMM individuals, with a higher rate of FEV1 decline in PiMZ ever-smokers. The following discussion references studies primarily relating to people with the PiSZ and PiZZ genotypes who may develop lung disease in the absence of smoking.

Smoking also influences lung function decline, with the most marked harm coming from current cigarette use (18-21); effect sizes of current smoking have varied in the studies that reported it, but are invariably damaging. The amount smoked in terms of pack years is also relevant, in that threshold effects have been reported in both American and UK data for patients with PiZZ AATD(18, 22), whereby it seems that after 20 pack years there is no longer a relationship between FEV1 and exposure. In patients who are PiSZ this threshold rises to 30 pack years(18), consistent with the higher AAT levels that such patients generally have, and the known relationship between greater area of obligate NE mediated damage and AAT level. Some caution must be exercised when

considering these putative thresholds as the data from which they were generated does show marked scatter, consistent with multiple influences (both genetic and environmental) on FEV1 - indeed the amount of variability in FEV1 explicable by pack years smoked was just 15% in UK data (12). The duration of smoking may also be relevant, as data from the German registry demonstrates that longer durations associate with faster subsequent FEV1 decline(23); teasing out the differences between cumulative exposure (pack years) from duration is clearly difficult and requires further research. Age at onset of smoke exposure may also be critical, as demonstrated by Mayer et al in a survey of patients with PiZZ AATD, which reported earlier onset of symptoms in those exposed to smoke passively in childhood(24). Passive smoke was also a risk factor for lung disease in an independent study, albeit of only 52 PiZZ patients(25). Whether age at onset is a risk independent of total amount or duration is unknown. Interestingly data, such as that from the Swedish registry(26), has reported that after smoking cessation FEV1 decline may return to that of a never smoker. This emphasises the importance of smoking cessation at any time of life in patients with AATD, and perhaps suggests that the relationship between pack years smoked and FEV1 is largely related to damage incurred during the period of active smoking.

Smoking may also relate to a higher exacerbation frequency, and concomitant deterioration in quality of life over time, as shown in the German registry (27, 28). Whilst relatively few studies have reported influences on exacerbations in AATD, the strong influence of smoking on exacerbation rate in COPD outwith AATD implies that this may be a true finding, irrespective of published replication in other cohorts.

Further research about smoking in AATD might focus on improving our understanding of specific phenotypic associations as that has been limited by lack of detailed

phenotyping in many AATD cohorts internationally, in particular there have been relatively few quantitative CT studies in AATD outside the context of clinical trials, when compared to usual COPD, and few studies that have data on small airways disease whether measured physiologically or radiologically. The COPDgene study emphasises the value of CT studies in progression of emphysema and air trapping(29). Radiographic progression was found to have variable correlation to FEV1 (29), and better association to rate of DLCO decline especially in severe disease (30). There is no data available on other inhaled smoke types, such as cannabis, heroin or other drugs of abuse, or of vaping with lung disease in AATD, but since these are known to be harmful in people with normal AAT levels (31), we can reasonably presume that they will be more harmful in AATD.

EFFECTS OF OUTDOOR AIR POLLUTION

There are a number of pollutants potentially relevant to lung disease risk, of which the most important in the general COPD literature are particulate matter and ozone. Studies of outdoor air pollution in AATD have been conducted by our group in the UK in PiZZ patients, and reported for PiMZ patients by a European group. In PiZZ AATD patients we found that a surrogate measure for lifelong exposure to ozone, namely current ozone level as determined by geocoding of pollution (geographical information system mapping, down to a 1km level, based on the patients' home address) related to lower FEV1 and KCO at enrolment to the cohort(32), after adjustment for cigarette smoke exposure and other relevant demographic features. The effect of ozone was relatively small, with the difference in ozone seen between urban and rural environments associating with a difference of 2% in KCO, and overall ozone exposure accounting for

1% of KCO variability. AAT levels in the lung are elevated after exposure to ozone (33), and AAT offers over 80% of pulmonary defence against ozone induced inflammation (34). This suggests a rationale for enhanced susceptibility to ozone in AATD. However the use of cross-sectional data had limitations, as was demonstrated by the data for PM10 (particulates with diameter $<10\mu\text{M}$) where current high levels appeared to relate to better lung function. At a biological level this seemed implausible, given that particulates stimulate neutrophilic inflammation to which patients with AATD would be more vulnerable in terms of lung damage. We therefore went on to study true cumulative exposure with respect to lung function decline; whilst the range of values seen for lung function decline was not as wide as the range seen in baseline data for each lung function parameter (thus potentially limiting power to detect pollution effects) the model was almost certainly more robust when considering accuracy of pollution data. This study showed that PM10 adversely affected FEV1 decline, and ozone related to decline in KCO(35). Once more effect sizes were small, with an increase of $1\mu\text{g}/\text{m}^3$ in PM10 exposure relating to an additional 3ml/year of loss in FEV1.

One study in the SAPALDIA cohort demonstrated in short term follow (2001-2003) that PM10 exposure did not appear to interact with AATD genotype with respect to FEV1 decline, however the only statistics generated were on PiMZ individuals in the study (comparing to PiMM as the reference)(36). This suggests that the effect of pollution on decline is lost in lower risk genotypes, or is so subtle as not to be detectable in a short period of follow up. This is consistent with the UK data from PiZZ patients and the concept that lower AAT levels carry corresponding increased risks from environmental insults. Confirming this, a study of a single large exposure to environmental dusts, after

the collapse of the world trade centre (9/11) demonstrated increased FEV1 decline in exposed PiMZ and PiSZ firefighters relative to normal genotypes(37).

THE INFLUENCE OF OCCUPATIONAL EXPOSURES

There are a number of occupational exposures which could stimulate pulmonary inflammation, and hence be of greater risk to patients with PiZZ AATD compared to normal individuals. Occupational exposure is often assessed in large epidemiological studies by use of self-reported exposure to vapours, gases, dusts and fumes (VGDF), which are relevant to development of airways disease. This is an accepted method of reporting such data, but potentially less accurate in comparison to a full occupational history, whereby the job the patient did is used to assign the level of risk. This is usually done via a job exposure matrix (38) which classifies jobs for their likely level of risk using Standard Occupational Classifications (eg SOC 2000) generated using a recognised system (eg CASCOT (39)). Whilst this is more accurate it is also much more time consuming, and relies on patients remembering all the jobs they have done.

An early study in 52 PiZZ patients identified that lower FEV1 was observed in those reporting VGDF exposure, albeit not statistically significant ($p=0.07$)(25). More recently exposure to VGDF (or an equivalent self-reported measure) has been assessed in AATD by Germany, Sweden, National Jewish Health and SAPALDIA, comprising 451 PiZZ, 6 PiSZ and 220 PiMZ subjects altogether (23, 36, 40-42). Results of these studies are summarised in table 1. In general VGDF exposure associated with increased symptomatology, reduced lung function and lung function decline (23). In PiMZ carriers an interaction was seen between genotype, smoking and VGDF exposure such that those carrying a Z allele, smoking and having VGDF exposure were more likely to exhibit

deterioration in their small airways (as measured by FEF₂₅₋₇₅)(36). In PiZZ patients agricultural employment in particular associated with lower FEV₁ and increased bronchial hyperresponsiveness (BHR) (41, 42); increased BHR was also seen in PiMZ subjects but the risk was approximately half that of a PiZZ person. In addition to farmers, those in the timber industry (e.g. wood trimmers, furniture making, sawmills) are at higher risk of allergic alveolitis and lung cancer (43). Whilst this study (43) was not specific to AATD it is probable the same, or enhanced, risks apply since studies have linked wood dust exposure to increased antibody secretion resulting in airway inflammation (44), which then may result in decreased FEV₁ and increased FEV₁ decline (45) in normal individuals. In any pro-inflammatory insult like this people with AATD are likely to be more at risk due to reduced inhibition of inflammation.

In our own data (previously published only in abstract form(46)) we took a different approach, and classified occupational risk using a job exposure matrix, from the full occupational history. Data was available on 379 PiZZ patients, and risk was allocated according to likelihood of exposure to a risk agent, determined by an occupational hygienist. If the intensity was likely to be less than 30% of the workplace exposure limit this was classed as low risk, with intensities above this being deemed high risk. Those who had never worked in an exposure prone job were classed as zero risk. Patients had worked for a mean of 19 years, and stopped work on average at age 48 years if cessation was due to lung disease. There were no differences in duration of working life or age when stopped work between risk groups. Characteristics of the patients are shown in table 2; no elements of lung function differed between risk groups. Whilst our results contradict others data there are several possible explanations for this; firstly it is possible our methods were more robust and specific to occupational exposure than other self-reported studies, in which VGDF exposure might have been overestimated due to recall

biases. Secondly, it is possible that the use of a general job exposure matrix rather than one specifically selecting airways disease could have influenced results; this would have been difficult to do, since many professions exposed to agents known to lead to interstitial or fibrotic disease (eg asbestos in building trade) also have exposure to agents known to influence airways disease (eg wood dusts in building trade). Thirdly it might be that confounding was influencing results; in order to assess the latter issue we looked specifically at lung function decline according to occupational risk, adjusting for both pollution, smoke exposure and other relevant covariates, and found enhanced KCO decline in high risk occupations relative to those with zero risk, with enhanced FEV1 decline also being seen in female workers in high risk jobs (47).

There have been few studies of non VGDF exposures in AATD. However one study of asbestos exposure reported an increased risk of asbestosis in carriers of the S or Z allele (48). The number of patients was small, and the mechanism less clear than it would be for airways disease, so further studies of fibrotic lung diseases in AATD patients would be required to say if there is enhanced risk of pulmonary pathology outside of the airway. Notably in our dataset reported above no subjects exhibited restrictive lung function or interstitial lung disease on HRCT, even though risk professions potentially linked to asbestos (eg construction work) were amongst the most common risk groups seen.

ARE AIRWAY BACTERIA A RELEVANT ENVIRONMENTAL FACTOR?

The presence of bacteria in the sputum or airway could be considered a relevant factor, since they are acquired from the environment around us and influence clinical phenotype. Bacterial colonisation of the airway may influence exacerbation rate, health

status(49) and subsequent lung function decline in usual COPD, and since bacterial events involve neutrophilic inflammation(49, 50) conceivably these effects would be enhanced in patients who have AATD. There is some evidence that this is the case, with exacerbating AATD patients exhibiting more inflammation than those with usual COPD (51), and those with colonisation exhibiting greater immune activation, as measured by free light chain level in the blood (52). However colonisation itself may be influenced by genetic modifiers in AATD (53), implying that gene-environment interaction exists and thus it may be difficult to separate phenotypic features due to exposure to infection, from those due to underlying genetic influences.

There is growing evidence that the bacteria we culture are not the only relevant part of the microbial environment, with studies in other airway diseases demonstrating changes in inflammation(54) and clinical phenotype dependent on type of micro-organisms, as measured by 16s DNA analysis(55, 56), and on outcome, specifically mortality(57). Ongoing analyses of the microbiome in AATD as part of the GRADS research programme (NCT01832220) will therefore be of interest in future, to aid understanding of this environmental factor.

ENVIRONMENTAL INFLUENCES INFLUENCING DISEASE OUTSIDE THE LUNG

Since ingested substances may be considered part of the environment, it is worth noting that alcohol is a recognised co-factor in development of liver fibrosis and cirrhosis when the Z allele is present(58). However, unlike smoke exposure in the lung where there is a synergistic interaction between genotype and environmental exposure the mechanism of AATD liver disease is not enhanced by alcohol, and the association is likely observed simply due to additive effects of environment and genotype. Nevertheless AATD

patients should be advised about the risks of alcohol to the liver, in order to minimise risk of long term complications.

CONCLUSIONS

Patients with AATD have enhanced susceptibility to inflammatory stimuli in the environment, predominantly cigarette smoke, although air pollution and occupational inhalational exposures also play a part. The degree of variability in respiratory presentation accounted for by environmental factors is difficult to accurately quantify but undeniably is linked to lung disease progression in AATD patients.

Reference	N & genotype	Exposure type	Clinical findings
Fahndrich et al. (20)	PiZZ N=100 FEV1 N=116 TLCO	<ul style="list-style-type: none"> Smoking Exacerbations Occupational dusts 	<ul style="list-style-type: none"> ↑ FEV1 decline associated with occupational dust exposure, shorter duration of smoking abstinence, and an increased frequency of exacerbations per year.
Mehta et al. (31)	PiMZ N=97	<ul style="list-style-type: none"> Pollution (PM10) Occupational VGDF 	<ul style="list-style-type: none"> No significant clinic findings for PM10 ↑ FEF25-75 & FEV1/FVC decline in VGDF exposed smokers v PiMM
Mayer et al. (35)	Pi*ZZ N=128	<ul style="list-style-type: none"> Smoking Occupational dust, fumes, smoke and gas 	<ul style="list-style-type: none"> ↑ mineral dust associated with cough, leaving employment due to breathlessness, ↓ FEV1 and ↓ FEV1/FVC Exposure to occupational fumes or smoke associated with ↓ FEV1/FVC ratio.
Piitulainen et al (36)	PiZZ N=205	<ul style="list-style-type: none"> Agricultural work Passive smoking Indoor pollution 	<ul style="list-style-type: none"> Agricultural work ≥10 years associated with wheezing and chronic bronchitis Exposure to kerosene heaters (not gas cookers) and agricultural work associated with ↓ FEV1% & VC Passive smoke ≥10 years associated with chronic bronchitis
Sigsgaard et al. (37)	PiZZ N=2 PiSZ N=6 PiSS N=4 PiMZ N=123	<ul style="list-style-type: none"> Farming work 	<ul style="list-style-type: none"> Increased odds ratio for BHR with PiSZ, SS, ZZ alleles (4.34) in farming school attendant group compared to control group.

Table 1: Summary of studies of occupational risk according to AATD genotype

	Level of occupational risk			p value
	Zero	Low	High	
Gender (%♂)	60.9	67.7	50.7	ns
Age	50.40 (0.8)	48.8 (1.3)	49.2 (1.3)	ns
Pack years	14 (0.2-26)	13.5 (5.3-24.5)	15.8 (0-24)	ns
FEV1 (% predicted)	36.7 (25.8-58.9)	39.9 (24.1-68.5)	34.7 (25.9-69.9)	ns
FEV1/FVC (%)	38.2 (29-53)	41(30.6-57)	39.1 (32-60.3)	ns
KCO (gas transfer coefficient) % predicted	68.9 (57.4-88.8)	69.9 (2.8)	66.1 (57-85.9)	ns
Bronchodilator reversibility (% of patients with reversibility present)	62.2	64.7	59.5	ns

Table 2: Characteristics of PiZZ patients studied using a job exposure matrix

Bronchodilator reversibility was defined as >12% improvement in FEV1 after administration of a nebulised bronchodilator, with an improvement which was also at least 200ml in volume

Figure 1: Mechanism of damage from neutrophilic inflammation in the AATD lung

The figure shows migration of neutrophils from the blood into the airspaces of the lung, with normal lung shown on the left and AATD on the right. Inflammation triggers normal immunological responses resulting in increased AAT release due to neutrophil recruitment and release of neutrophil elastase (NE). Smoking also releases reactive oxygen species (ROS), causing increased neutrophil recruitment and subsequent increase in NE and AAT. Different mutations in the SERPINA1 gene result in varying

amounts of AAT deficiency. Unopposed NE action causes alveolar destruction by proteolysis resulting in emphysema. This is illustrated by the cell on the right of the figure, where diffusion of active NE outwards from the cell is greater in AATD than a PiMM individual. In addition to this protease imbalance mechanism, polymerisation of AAT occurs in the presence of malformed AAT proteins and ROS. Polymerised AAT results in increased neutrophil recruitment and further alveolar inflammation. This may contribute rapid progression of emphysematous changes in AATD patients.

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