α_1-antitrypsin PI*SZ genotype: a SERPINA1 deficiency haplotype with uncertain clinical and therapeutic implications

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Despite its high prevalence and being one of the first genotypes identified in the field of α_1-antitrypsin deficiency, the PI*SZ genotype has been little studied, and many unknowns regarding its clinical significance remain unanswered. https://bit.ly/3aut4ZE

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After the discovery of lung emphysema associated with α_1-antitrypsin (AAT) deficiency (AATD) in 1963 by Laurell and Eriksson [1], basic and clinical advances in the AATD field have been dramatic and relatively fast [2]. It was soon confirmed that AATD was an inherited codominant recessive condition, but it took seven more years to associate AATD-related lung emphysema with neutrophil elastase [3, 4], and between 7 to 10 years to discover its causal relationship with childhood and adult liver cirrhosis [5, 6], and with neutrophilic panniculitis [7]. In the 1970s, isoelectric focusing (IEF) became the technique of choice for diagnosis, capable of identifying more than 30 AAT variants [8]. In the 1980s, the AAT gene was sequenced and cloned, its locus located on the long arm of chromosome 14 [9], and the structure of the protein revealed [10]. In 1987, the US Food and Drug Administration approved augmentation therapy for pulmonary emphysema associated with AATD [11]. This fact was crucial to promote the creation of powerful associations and patient registries aimed to enhance diagnosis and research, and to rationalise the use of augmentation therapy. In the 1990s, granulomatosis with polyangiitis (also known as Wegener's granulomatosis) was added to the list of diseases related to AATD [12]. In parallel, in the field of basic sciences, researchers from Malmö, Sweden, and other laboratories first observed the polymerisation of AAT protein, and later Lomas *et al.* [13, 14] demonstrated the polymerisation of AAT-Z proteins, and developed the hypothesis of AATD and other serpinopathies pathogenesis. In 1997, the World Health Organization, and later many other scientific societies, recommended investigating AATD in all COPD and blood relatives of index cases, as well as in individuals with other AATD-related pathologies [15]. Moving to basic sciences, DNA sequencing and PCR techniques came into play, increasing the number of known AAT variants by over a hundred. At the end of the 20th century, the computed tomography scan began to be used to measure lung density in several clinical trials, finally demonstrating the efficacy of augmentation therapy to slow emphysema progression [16]. Currently, inhaled AAT is a reality [17], the

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attainment of recombinant AAT suitable to be applied to humans seems feasible, and various modalities of gene therapy are taking firm steps with the purpose of "getting the cure of the Alphas".

However, despite these remarkable achievements, there remain many unanswered questions on genetics, pathophysiology, penetrance, natural history, prognosis, therapy or, among others, risk associated with other non-ZZ genotypes, such as the PI*SZ type, the subject matter of this editorial.

In this issue of the *European Respiratory Journal*, the report by McElvaney *et al.* [18] is timely for health professionals, AATD patients and their families The estimated number of those with the PI*SZ haplotype worldwide is 1.5 million individuals in countries with reliable data [19]. This PI*SZ haplotype is not uncommon in white individuals of European ancestry living in Europe and in countries to where they migrated or their ancestors colonised (figures 1 and 2).

Despite being known since the beginning of the AATD discovery, the risk for lung disease of individuals with the PI*SZ genotype has not been well established yet, partly due to its less frequent clinical detection, underreporting in registries, and lack of studies with sufficient statistical power to reach sound conclusions. The PI*SZ associated risk to develop COPD has been described as slightly increased, especially in smokers. But in absence of smoking or exposure to other toxic factors, its risk has been quantified as low or practically insignificant. In addition, its causal relationship with liver diseases is even less defined, although in any case is much smaller than in PI*ZZ individuals. In addition, its causal relationship with pathologies other than lung emphysema and liver cirrhosis is much less well documented [20]. Therefore, although it seems likely that a small proportion of PI*SZ individuals may have an increased risk for lung and liver diseases, definitive evidence to sustain this assertion is lacking, and consequently the ambiguity of the available statements makes it difficult for practising physicians to feel confident enough to make decisions or give accurate advice when faced with PI*SZ individuals in clinical practice [18].

AAT proteinase inhibitor (PI*) SZ genotype (PI*SZ) results from a double mutation in the SERPINA1 (serine protease inhibitor, family A, member 1) gene, which changes its normal nucleotide sequence by that of the two most common AATD alleles: "S" (Glu264Val) and "Z" (Glu342Lys) [21]. These mutated alleles encode abnormally constituted "S" and "Z" glycoproteins that polymerise into the rough endoplasmic reticulum of the producing cells (mainly hepatocytes), forming homopolymers "S-S" and "Z-Z", as well as heteropolymers "S-Z", that accumulate within the liver without being secreted, thus provoking a decreased concentration of AAT in blood and tissues [22].

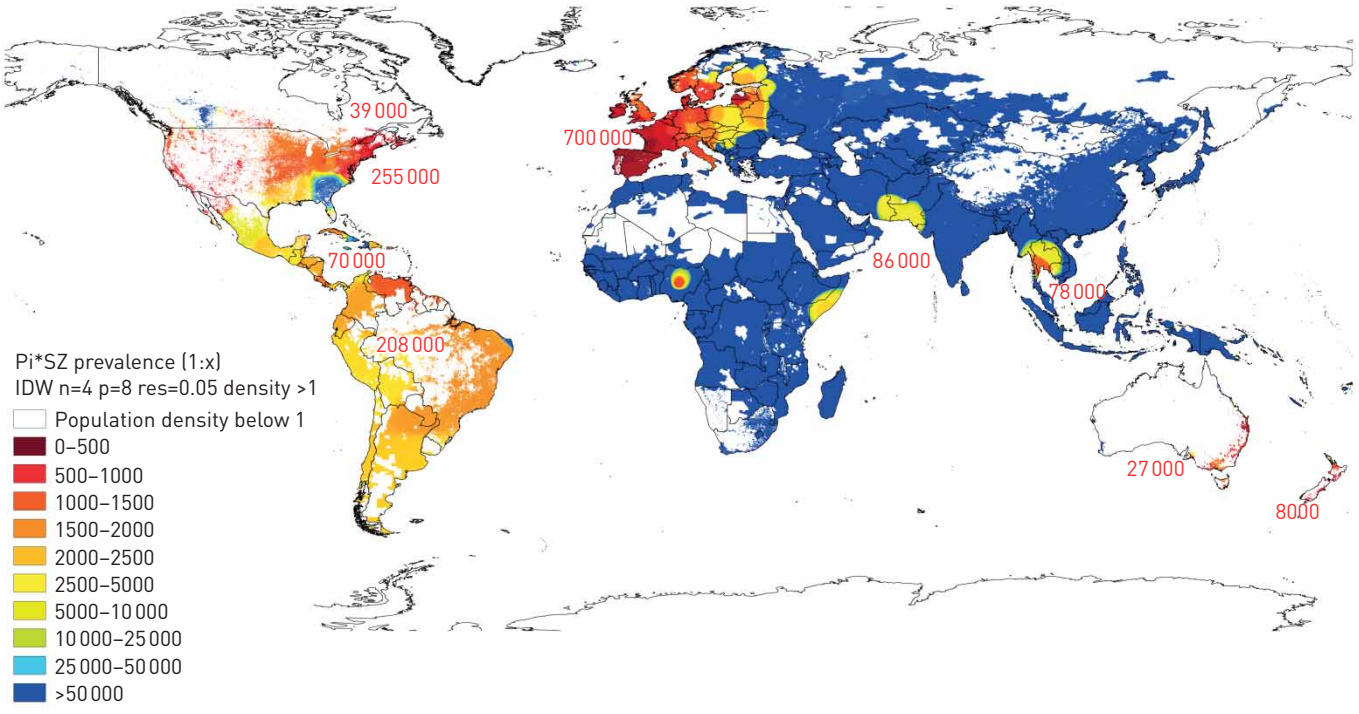

FIGURE 1 Inverse distance weighting (IDW) interpolation map of prevalence and number of PI*SZ genotypes in the world. It has been estimated that there may be approximately 1.5 million individuals PI*SZ genotypes in the world. Almost half of them (approx. 700 000) are in Europe, and the rest in America (approx. 600 000), Australia and New Zealand (roughly 35 000), in addition to about 165 000 irregularly distributed throughout Africa and Asia. Figure and text adapted from [19] with permission.

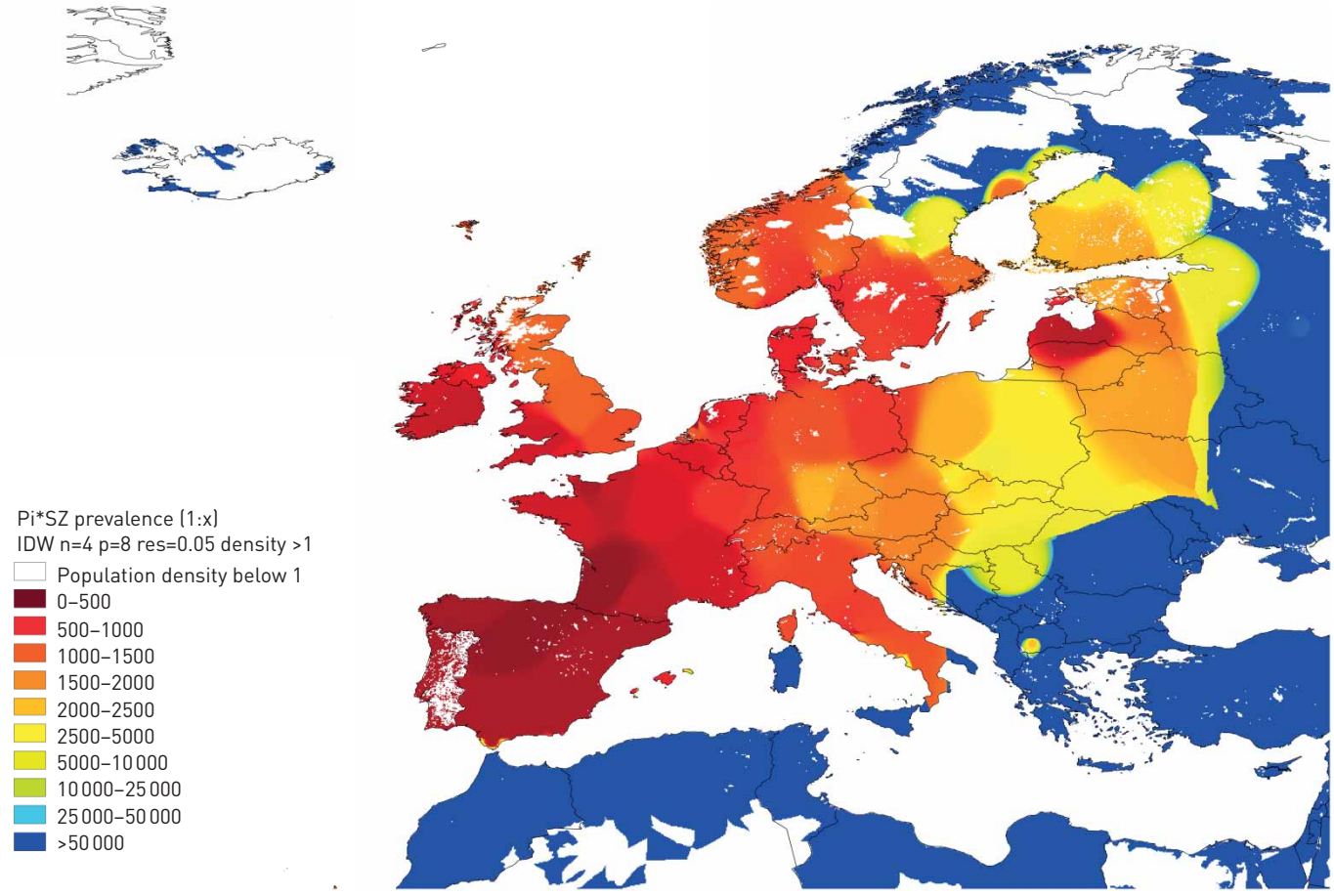

FIGURE 2 Inverse distance weighting (IDW) interpolation map of PI*SZ prevalence in Europe. Prevalence is represented by a colour-gradient "red (high)–blue (low)" scale; areas with population density below 1 inhabitant per km^2 appear in white. The highest PI*SZ prevalence is in Portugal (1:205), followed by Spain (1:278), Latvia (1:354), France (1:413), Republic of Ireland (1:424), Belgium (1:551), the Netherlands (1:617), and UK (1:900), and it is over 1 per 1000 in the remaining European countries, reaching the minimum prevalence of almost 1:25 000 in Russia. By individual countries, the highest number of PI*SZ are in Spain (174 822), followed by France (161 680), UK (73 973), Italy (64 137), Germany (60 396) and Portugal (52 836). Pi*SZ numbers in other countries are very variable, but always much lower than in the aforementioned. Figure and text adapted from [19] with permission.

Compared to the normal "M" protein, the mutated "Z" molecule has profound conformational changes causing a loss of around 80% of its inhibitory capacity, and a massive intrahepatic polymerisation (of about 85–90%) of mutated molecules, resulting in very low serum concentrations of 10–15% [23]. On the contrary, compared to the "Z", the structural alterations of the "S" protein are much less pronounced, keeping its antielastase capacity practically unchanged, its polymerisation tendency less pronounced (approximately 55% of molecules), and serum levels moderately reduced to around 45% [24].

Reported PI*SZ serum levels differ between studies [25], although at least 10–20% express serum values below the theoretical "protection threshold" of 11 $\mu M \cdot L^{-1}$ (equivalent to 57 $mg \cdot dL^{-1}$ measured by nephelometry), raising doubts about whether they should be considered at increased risk of developing AATD-related diseases [12]. However, since AAT is an acute phase reactant whose production can double during inflammation and its local production by lung resident cells can be multiplied up to 11 times [20], it is assumed that in these circumstances SZ levels may exceed the "protective threshold" reaching sufficient concentrations to neutralise neutrophil serine proteases and other inflammatory mediators released by microorganisms and activated cells.

Interestingly enough, it has been shown that the composition of circulating AAT in AATD heterozygotes is inversely proportional to the amount of liver polymerisation/retention of each type of AAT. For example, in a recent study the percentage of "Z" AAT in MZ individuals was only 18%, because it was mostly retained intracellularly, and that of "S" protein in MS subjects was just 37% for a similar reason [26]. In this regard, although the composition of circulating AAT in PI*SZ patients has not been sufficiently studied, it could be approximately of 70% "S" and 30% "Z". This would mean that some PI*SZ

individuals with AAT levels around or below the lower limit, having around of two-thirds of circulating AAT of "S" type (*i.e.*, efficient) and a third of "Z" type (*i.e.*, poorly efficient) may actually be below the "protective threshold", and therefore at increased risk, especially if they suffer from frequent or intense inflammatory respiratory exacerbations, or if AAT is inactivated by tobacco smoke. However, it must be taken into account that AATD is not a disease, but a complex monogenic inherited condition that can promote the development of diseases, especially when other environmental and/or genetic factors, mostly poorly understood, associate with this condition. Thus, the empirically assigned 'protective threshold' value of 11 $\mu M \cdot L^{-1}$ (or 57 $mg \cdot dL^{-1}$), though it may provide some clinical guidance in defining risk, it may do so inaccurately or even erroneously [12].

Augmentation therapy has been approved for adults with emphysema associated with severe AATD (*i.e.* homo and heterozygous combinations of Z, S, null and rare alleles) showing AAT levels below the putative "protective level", and impaired lung function. But while the US Alpha 1 Foundation guidelines does not exclude from AAT therapy PI*SZ individuals that meet these or similar criteria [27], the European Respiratory Society statement does not support this indication, arguing lack of supporting evidence [28].

Apart from this unresolved question, the authors of the manuscript identified other several open research questions concerning to the natural history, prognosis, genetic penetrance, monitoring requirements, and other unmet needs that deserve more research. At present, according to the expert panel, the lack of studies with results of high-quality evidence hinders the implementation of accurate medical recommendations to grade the effectiveness of an intervention aimed at ensuring proper control. Thus, in order to adequately address the limitations identified by the international panel of experts, long-term longitudinal follow-up studies of larger samples of PI*SZ individuals may be needed to obtain reliable clinical, epidemiological, analytical, radiological and functional data from index and non-index cases, to identify risk factors, progression rate and healthcare costs, and assist in collection of other specific data to advance the scientific and medical knowledge about PI*SZ genotypes.

Augmentation therapy for PI*SZ patients requires further study; it will be necessary to perform well-designed randomised controlled clinical trials to verify tolerance, safety and effectiveness of AAT therapy, and to find the appropriate dose of exogenous "M" protein that added to the partially efficient circulating native SZ protein provides sufficiently high protective levels against proteinases in blood and alveolar fluid.

In this context, the European Alpha-1 Clinical Research Collaboration (EARCO), a newly created patient registry designed to elaborate a powerful standardised database for the follow-up of patients with AATD, will include a significant number of PI*SZ individuals. This will facilitate collection of the most-needed clinical data, which is expected to answer the open research questions raised by this expert panel [29, 30].

Conflict of interest: I. Blanco has nothing to disclose. I. Diego has nothing to disclose.

References

1 Laurell CB, Eriksson S. The electrophoretic alpha1-globulin pattern of serum in alpha1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963; 15: 132–140.
2 de Serres F, Blanco I. Role of alpha-1 antitrypsin in human health and disease. *J Intern Med* 2014; 276: 311–335.
3 Janoff A, Scherer J. Mediators of inflammation in leukocyte lysosomes. IX. Elastinolytic activity in granules of human polymorphonuclear leukocytes. *J Exp Med* 1968; 128: 1137–1155.
4 Turino G, Senior RM, Garg BD, *et al.* Serum elastase inhibitor deficiency and alpha 1-antitrypsin deficiency in patients with obstructive emphysema. *Science* 1969; 165: 709–711.
5 Sharp HL, Bridges RA, Krivit W, *et al.* Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognized inherited disorder. *J Lab Clin Med* 1969; 73: 934–939.
6 Berg NO, Eriksson S. Liver disease in adults with alpha-1 -antitrypsin deficiency. *N Engl J Med* 1972; 287: 1264–1267.
7 Warter J, Storck D, Grosshans E, *et al.* Syndrome de Weber-Christian associe a un deficit en alpha-I-antitrypsine. Enquete familiale [Weber-Christian syndrome associated with an alpha-1 antitrypsin deficiency. Familial investigation]. *Ann Med Interne (Paris)* 1972; 123: 877–882.
8 Long GL, Chandra T, Woo SL, *et al.* Complete sequence of the cDNA for human alpha 1-antitrypsin and the gene for the S variant. *Biochemistry* 1984; 23: 4828–4837.
9 Allen RC, Harley RA, Talamo RC. A new method for determination of alpha-1-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slabs. *Am J Clin Pathol* 1974; 62: 732–739.
10 Loebermann H, Tokuoka R, Deisenhofer J, *et al.* Human alpha 1-proteinase inhibitor. Crystal structure analysis of two crystal modifications, molecular model and preliminary analysis of the implications for function. *J Mol Biol* 1984; 177: 531–557.
11 Wewers MD, Casolaro MA, Sellers SE, *et al.* Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med* 1987; 316: 1055–1062.
12 Stoller JK, Lacbawan FL, Aboussouan LS. Alpha-1 antitrypsin deficiency. *In*: Pagon RA, Adam MP, Ardinger HH, *et al.*, eds. GeneReviews. Seattle, University of Washington, 1993–2017 [updated January 19, 2017].

13 Lomas DA, Evans DL, Finch JT, *et al.* The mechanism of Z alpha 1-antitrypsin accumulation in the liver. *Nature* 1992; 357: 605–607.
14 Lomas DA, Carrell RW. Serpinopathies and the conformational dementias. *Nat Rev Genet* 2002; 3: 759–768.
15 Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting. *Bull World Health Organ* 1997; 75: 397–415.
16 Chapman KR, Burdon JG, Piitulainen E, *et al.* Intravenous augmentation treatment and lung density in severe α1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; 386: 360–368.
17 Stolk J, Tov N, Chapman KR, *et al.* Efficacy and safety of inhaled α1-antitrypsin in patients with severe α1-antitrypsin deficiency and frequent exacerbations of COPD. *Eur Respir J* 2019; 54: 1900673.
18 McElvaney GM, Sandhaus RA, Miravitlles M, *et al.* Clinical considerations in individuals with α_1-antitrypsin PI*SZ genotype. *Eur Respir J* 2020; 55: 1902410.
19 Blanco I, Bueno P, Diego I, *et al.* Alpha-1 antitrypsin Pi*SZ genotype: estimated prevalence and number of SZ subjects worldwide. *Int J Chron Obstruct Pulmon Dis* 2017; 12: 1683–1694.
20 American Thoracic Society, European Respiratory Society. American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. *Am J Respir Crit Care Med* 2003; 168: 818–900.
21 Nukiwa T, Brantly M, Garver R, *et al.* Evaluation of "at risk" alpha 1-antitrypsin genotype SZ with synthetic oligonucleotide gene probes. *J Clin Invest* 1986; 77: 528–537.
22 Teckman JH. Liver disease in alpha-1 antitrypsin deficiency: current understanding and future therapy. *COPD* 2013; 1: 35–43.
23 Sinden NJ, Baker MJ, Smith DJ, *et al.* α-1-antitrypsin variants and the proteinase/antiproteinase imbalance in chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 2015; 308: L179–L190.
24 Ogushi F, Hubbard RC, Fells GA, *et al.* Evaluation of the S-type of alpha-1-antitrypsin as an in vivo and in vitro inhibitor of neutrophil elastase. *Am Rev Respir Dis* 1988; 137: 364–370.
25 Bornhorst JA, Greene DN, Ashwood ER, *et al.* Alpha1-Antitrypsin phenotypes and associated serum protein concentrations in a large clinical population. *Chest* 2013; 143: 1000–1008.
26 Donato LJ, Karras RM, Katzmann JA, *et al.* Quantitation of circulating wild-type alpha-1-antitrypsin in heterozygous carriers of the S and Z deficiency alleles. *Respir Res* 2015; 16: 96.
27 Sandhaus RA, Turino G, Brantly ML, *et al.* The diagnosis and management of alpha-1 antitrypsin deficiency in the adult. *Chronic Obstr Pulm Dis* 2016; 3: 668–682.
28 Miravitlles M, Dirksen A, Ferrarotti I, *et al.* European Respiratory Society statement: diagnosis and treatment of pulmonary disease in alpha1- antitrypsin deficiency. *Eur Respir J* 2017; 50: 1700610.
29 Miravitlles M, Chorostowska-Wynimko J, Ferrarotti I, *et al.* The European Alpha-1 Research Collaboration (EARCO): a new ERS Clinical Research Collaboration to promote research in alpha-1 antitrypsin deficiency. *Eur Respir J* 2019; 53: 1900138.
30 Greulich T, Altraja A, Barrecheguren M, *et al.* Protocol for the EARCO Registry: a pan-European observational study in patients with α1-antitrypsin deficiency. *ERJ Open Res* 2020; 6: 00181–2019.