# Modernising case finding for $\alpha_1$-antitrypsin deficiency by DNA sequencing of COPD patients

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**Commenting on the use of DNA sequencing in helping to reduce the reported under-diagnosing of alpha-1-antitrypsin deficiency** https://bit.ly/2ZPZbjx

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Alpha-1-antitrypsin deficiency (AATD) is a hereditary metabolic disorder caused by mutations in the SERPINA1 gene that result in a reduction in the serum concentration of the protein alpha-1-antitrypsin (AAT), and a predisposition to COPD [1]. AAT functions as an inhibitor of neutrophil elastase (and other proteases) and is essential in maintaining a balance of protease and antiprotease activity in the lungs [2]. Imbalance causes an escalating cycle of inflammation and degradation of lung tissue that, over time, leads to COPD [3].

The condition is autosomal co-dominant which means both inherited alleles are presented in the phenotype, such that being homozygous for pathogenic alleles results in more severe disease than being heterozygous, with one pathogenic allele [4]. Clinical severity relates to AAT serum level; individuals who are homozygous have lower levels and higher risk for worse COPD outcomes [5]. There have been approximately 100 allele variants identified for the SERPINA1 gene that relate to AATD, the most common allele type is designated M and correlates to normal antiprotease activity. The proteins produced by these alleles are traditionally given the prefix Pi for proteinase inhibitor followed by the letters for the allele, *i.e.* PiMM [6]. Typical alleles associated with severe deficiency and thus the most adverse pathology are designated S and Z, the most pathogenic. Null variants due to mutations in the gene resulting in a stop codon being present have also been described in the literature; these have no detectable AAT serum concentrations [2]. A meta-analysis of epidemiological studies estimated that approximately 2.8% of COPD patients may have AATD [7], though it remains underdiagnosed [2].

Diagnosis of AATD has traditionally relied upon three main pillars: measuring serum concentration of AAT, identification of pathogenic SERPINA1 genotypes and detection of functionally deficient AAT protein variants [8]. Serum concentration measurement on its own has a low sensitivity for detecting AATD and is often used alongside qualifying the AATD phenotype using migration patterns in an electrophoresis gel. Although this method has been seen as the gold standard, the European Respiratory Society and American Thoracic Society (ERS/ATS) have increasingly advocated for genotype to identify AATD [2, 9]. They noted four potential purposes for adopting this approach: identifying those with the deficiency, identifying those predisposed to clinically significant disease due to possessing severely deficient

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alleles, identifying those who are asymptomatic carriers for deficiency and general population screening. The advantage of genotype screening is that it would allow clinicians to make more informed treatment decisions, for example who to manage with AAT augmentation, based on a more personalised risk prediction.

The research carried out by Gupta *et al.* [10] is important in that it starts to make the case for DNA sequencing as a primary means of diagnosing AATD. The authors used Sanger sequencing to sequence exons 2 through 5 on the SERPINA1 gene to identify AATD alleles in 1359 patients within the Canadian Cohort of Obstructive Lung Disease (CanCOLD) including those with COPD, those classed as at-risk of COPD and those free of airway obstruction. The authors aimed to evaluate AATD allele frequencies within CanCOLD and to assess the relationship to COPD symptoms. They used *in silico* statistical software, along with the Combined Annotation Dependent Depletion (CADD) framework to assess the deleterious phenotypic effects of the genotypes identified and used a public archive of reports of the relationships among human variations and phenotypes, called ClinVar, to gauge likely clinical outcomes. The authors also measured AAT serum levels and had access to a range of pulmonary function tests and computed tomography data from patients. 34 genetic variants were ascertained, including three previously undescribed.

The most common alleles were M1 (45.8%), M1 $Ala^{213}$ (17.5%) and M2 (14.6%), with 91.9% of alleles sequenced being of normal effect. The most frequent deficiency allele found was S (5.74%), followed by Z (1.69%) and then F (0.2%) and I (0.2%). In terms of genotype frequencies, two subjects were PiZZ and were already confirmed AATD cases. Another two subjects were identified as PiSZ and were also counted as severely deficient, though this may not be appropriate, since there is debate in the literature about whether the clinical risk attributable solely to the genetic mutation is similar [11, 12]. In terms of heterozygosity of deficient alleles, 40 subjects carried a single Z and 143 a single S allele. The authors detected several rare variants in their sequencing, including six subjects who carried F and six who carried I alleles, including one PiIS. Very rare deficient alleles described were two $P_{Lowell}$, one $M_{Procida}$, one $M_{Wurzburg}$, one $S_{Munich}$ and one M3.Pro255Thr. Overall, the authors found 220 deficient alleles out of 2178 (8.1%) and 210 of the 1359 subjects (15.5%) were carriers of at least one deficient allele, including very rare mutants (*e.g.* $M_{Procida}$). This equates to four (0.29%) subjects with "severe" AATD, higher than the reported frequency of 0.02–0.05% [13] and suggests that the sequencing approach found more patients than prior work, potentially revealing advantages in terms of diagnostic accuracy.

To investigate their second aim, the authors clustered the 77 genotypes they described into four groups based on the predicted effects each variant has on AAT activity. Group 1 was for no deficiency and had 1149 PiMM subjects. Group 2, for mild deficiency, contained 147 subjects with either PiMS or PiMI. Group 3 contained 59 subjects with a wide range of genotypes usually described as carriers of AATD including PiMZ, PiMF, and very rare variants of M and S. Group 4 comprised the four diagnosed PiZZ an PiSZ AATD cases. Significant differences were found in AAT serum levels between the groups (ANOVA $p<0.001$), as expected, with progressively lower levels from group 1 to group 4. Diffusion capacity for carbon monoxide ($D_{LCO}$) and percentage of lung voxels below −950 Hounsfield units (LAA-950) varied across the groups, driven by those with severe deficiency as significant p-values were found only between group 4 and the other groups ($D_{LCO}$, p=0.005 and LAA-950, p=0.014).

A key strength of the study was the nature of the CanCOLD cohort, which meant that identified individuals had, or were at risk of, COPD; this means that AATD cases found were more likely to be clinically relevant. Effectively their process was the difference between case-finding and screening for AATD, in the sense that only at-risk individuals were approached, rather than the whole population. Notably, case-finding for COPD, without inclusion of genetic data, has been shown to ascertain more cases [14] and to be cost-effective [15], implying that the more refined approach of case finding might be more appropriate for AATD as well. Advances in sequencing technology have potentially made this a route that could be practical for wider use, though questions would remain about cost and value, especially in countries that do not have access to augmentation therapy routinely, where it might be argued that sequencing would not change management for the individual. The counter argument might be that making a diagnosis could make a patient more likely to adopt healthy behaviours (*e.g.* smoking cessation [16]) and could aid other family members by identifying a wider group at risk of disease. A potential area for improvement within the manuscript is the choice of method to group the genotypes, which was based mainly on the predicted effects on pathogenicity. Whilst the authors concede their approach is imperfect it might have strengthened the work if an already established approach for pathogenicity prediction had been used [17]. Furthermore, as CanCOLD is a study of the general population, a demographic breakdown of the subjects could have been useful to assess any associations between deficient alleles and ethnicity, considering Canada's sizeable indigenous population [18]. Historically, AATD has been thought of as a disease of Caucasian people, but there is growing understanding that this is not the case [19], and novel data on racial groups indigenous to Canada could have been generated.

With respect to their two aims the authors accomplished their objectives. They identified 34 genetic variants in the SERPINA1 gene including some very rare and novel variants. The effects of these variants on COPD phenotype were consistent with greater emphysema among genotypes that code for more severe deficiency, and of some emphysema within PiMZ heterozygotes (lower $D_{LCO}$ ($p<0.001$) relative to mild/no deficiency groups). The research carried out by the authors aligns well with the four purposes for DNA sequencing as described by ERS/ATS, in that it identified those with the deficiency, as well as carriers and those at risk of developing clinically significant disease.

The authors effectively argue the clinical, scientific and economic reasons for the use of DNA sequencing. The question for us as a European respiratory community now, is whether we are ready for the potential increase in AATD cases that their results imply we would identify by adopting it.

Conflict of interest: M. Quinn has nothing to disclose. A.M. Turner has nothing to disclose.

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