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Genetic profiling for disease stratification in chronic obstructive pulmonary disease and asthma

Senani N.H. Rathnayake[a], *Maarten Van den Berge*[b,c], *and Alen Faiz*[a,b,c]

Purpose of review
In asthma and chronic obstructive pulmonary disease (COPD), the movement towards genetic profiling with a push towards 'personalized medicine' has been hindered by complex environment–gene interactions and lack of tools to identify clear causal genetic traits. In this review, we will discuss the need for genetic profiling in asthma and COPD, what methods are currently used in the clinics and the recent finding using new sequencing methods.

Recent findings
Over the past 10–15 years, genome-wide association studies analysis of common variants has provide little in the way of new genetic profiling markers for asthma and COPD. Whole exome/genome sequencing has provided a new method to identify lowly abundant alleles, which might have a much higher impact. Although, low population numbers due to high costs has hindered early studies, recent studies have reached genome wide significance.

Summary
The use of genetic profiling of COPD in the clinic is current limited to the identification of Alpha-1 antitrypsin deficiency, while being absent in asthma. Advances in sequencing technology provide new avenues to identify disease causes or therapy response altering variants that in the short-term will allow for the development of screening procedures for disease to identify patients at risk of developing asthma or COPD.

Keywords
asthma, chronic obstructive pulmonary disease, whole exome sequencing, whole genome sequencing

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is expected to become the third leading cause of death worldwide by 2030 [1] and along with asthma affecting ~300 million individuals worldwide [2]. Genetics can be used as a first step in understanding the underlying pathology of asthma and COPD and provides important avenues towards treating individuals using 'personalized medicines'. Both diseases are characterized by chronic airway inflammation, while asthma is specifically associated with airway hyperresponsiveness and airway remodeling, while COPD is associated with chronic airway obstruction that is caused by (small) airways diseases and emphysema [3]. The underlying mechanisms of both diseases remain unclear, and both are considered complex genetic (multigene) diseases.

Genetics can be used in asthma and COPD for two main proposes:

(1) to highlight subgroups within a disease that predict long-term outcomes

(2) to predict response to current therapies – pharmacogenomics

Although the use of genetics is a common practice for simple single gene diseases such as cystic fibrosis, its use in complex genetic diseases such as asthma and COPD has proven to be more difficult. The movement towards phenotyping respiratory disease and 'personalized medicine' has been hindered by factors such as complex environment–gene interactions [4], heterogeneity in disease traits and a

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lack of tools to identify clear causal genetic traits. The breakthrough genetic tool of choice when investigating complex genetic diseases was the hypothesis-free method of genome-wide association studies (GWAS), which measures 100 000–1 000 000 polymorphisms. However, recent advances in sequencing technology have allowed for whole exome/genome sequencing. In this review, we will discuss the need for genetic profiling in asthma and COPD, what methods are currently used in the clinics, the benefits and limitations of GWAS and finally the use of whole exome/genome sequencing in identifying variants associated with presence of disease and response to current medications.

WHY IS GENETIC PROFILING NEEDED IN ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE?

There is currently no pharmacological treatment available that prevents the accelerated decline in lung function that occurs in COPD patients. Although it is widely accepted that smoking is the main risk factor for COPD, only ~20–30% of smokers will ultimately develop the disease [5] indicating a possible genetic factor contributing to smoke susceptibility. A small subset of smokers will develop severe COPD at an early age and with relatively few pack-years smoking [5]. This severe early-onset COPD accounts for a significant part of the total personal, societal and economic burden attributed to COPD [5]. The question arises as to why these patients are particularly susceptible to the adverse effects of smoking. In this context, the findings of Silverman et al. [5] are of particular interest, investigating 44 patients with severe early-onset COPD (SEO-COPD) defined by severe airflow obstruction [i.e., Forced Expiratory Volume in 1 s (FEV_1) <40% predicted] and age less than 53 years. They showed that smoking first-degree relatives of these patients had a significantly lower FEV_1 than controls of the same age and smoking habits, suggesting genetic factors to play a role in COPD development. The latter is in line with findings of case–control and twin studies where it is estimated that 40–60% of the risk for development of COPD is genetically determined [6,7]. Although COPD has been defined as a complex genetic disease, mutations in a single gene SERPINA1 have been linked to have a causal role in the development of COPD in a subset of patients [8]. Depending on the variant it can lead to Alpha-1 antitrypsin (AAT) deficiency with the most severe (i.e. homozygous Z or null genotype and Z/null genotype) resulting in little to no expression of AAT, these variants are considered rare in the general public but found to cause up to 10% of the cases of severe early-onset COPD [9].

Similarly, asthma is another complex genetic disease believed to be regulated by a large number of underlying mechanisms, which contribute to the overall pathology. It was previously thought that common diseases such as asthma and COPD are caused by common alleles [10]. However, genetic studies have revealed that this is only the case in a handful of diseases. It is now increasingly recognized that multiple rare DNA sequence variations, each with relatively high penetrance, may also be major contributors to genetic susceptibility of clinical subphenotypes of common diseases [10]. However, despite the estimated 35–95% heritability [11,12], no single one gene has been able to explain the presence of asthma in a subgroup of patients. Despite numerous studies focused on identifying genes related to asthma, our understanding of the importance of the genetic background remains limited.

Current therapies for both asthma and COPD focus on suppressing the inflammation and relaxation of the airways following acute constriction. However, not all patients respond to the same extent to these therapies and it is currently believed that genetics may contribute to the variation in response.

WHERE ARE WE NOW?

AAT deficiency screening is currently the only genetic method widely used in clinical practice to phenotype COPD patients [13]. A diagnosis of AAT deficiency is made when serum levels of AAT are low ($<0.5\,g\,l^{-1}$), genotyping then allows a rapid and precise identification/exclusion of S and Z alleles and other variants, where specific primers are available, while sequencing remains necessary for those cases where a null variant or a deficient variant other than S or Z is suspected [13]. Patients with verified AAT deficiency can receive augmentation therapy

(purified human AAT), which slows down the rate of lung density decline measured by CT in severe AAT-deficient patients [14–16]. Recently, Stanley *et al.* [17] showed an association with mutations in telomerase genes: telomerase reverse transcriptase (TERT) and telomerase RNA (TR), with smokers with severe emphysema. Interestingly, the variants identified in this study were previously found to be associated with idiopathic pulmonary fibrosis (IPF) [18,19], a rare lung disease associated with uncontrolled tissue repair. Although telomerase mutation screening has been adopted in the IPF field it has yet to be implemented in COPD with severe emphysema [20]. Telomerase mutations have been found to be associated with co-morbidities such as liver disease and complications during lung transplantation due to increased risk for serious toxicities of immunosuppressive treatments, because of limited reserves of cells in the bone marrow [17,20]. The latter is extremely relevant to severe COPD as lung transplantation is usually the only effective intervention. Currently no genetic markers are used to stratify asthmatic patients in the clinical practice. However, GWAS have identified single nucleotide polymorphisms (SNPs) associated with the presence of disease [21].

BENEFITS AND LIMITATIONS FOR GENOME-WIDE ASSOCIATION STUDY

GWAS is a hypothesis-free method to identify genetic components associated with asthma and COPD. Although GWAS has had some success in identifying genetic risk factors for both asthma and COPD with small effects [22–39], the technique is prone to miss lowly abundant alleles, which might have a much higher impact. Most disease causing mutations are lowly abundant in the general population due to selective pressure. These so-called 'rare alleles' are carried by less than 1% of the population and are usually not explored on GWAS platforms. Furthermore, identified SNPs usually sit within large linkage disequilibrium blocks encompassing multiple genes making it difficult to identify which gene is the causal one. To overcome this problem, expression quantitative trait loci (eQTL) analysis can be applied associating genetic variants with gene expression [21,40,41]. A clear example of this was the finding that rs7216389 an SNP associated with childhood asthma [32] located within ORMDL3 gene was found to have a more significant effect on the expression of downstream gene GSDMA in an eQTL analysis conducted in lung tissue [42]. Another limitation of GWAS platforms is that pre-exiting knowledge of variants is required before they can be assessed. Therefore, the discovery of rare or de-novo genetic variants cannot be measured using this platform.

WHOLE EXOME SEQUENCING AND WHOLE GENOME SEQUENCING IN ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Whole exome sequencing (WES) involves sequencing the coding region of DNA (exons), while whole genome sequencing (WGS) involves sequencing the whole genome. Both methods allow for the discovery of nonannotated SNPs and de-novo mutations thus overcoming some of the major limitations of the current DNA chip platforms. However, due to the high costs of these methods the number of included subjects has remained limited and therefore studies so far usually focussed on the extreme cases of disease or pedigree analyses (Table 1).

DeWan *et al.* [44] conducted the first WES in asthma using a pedigree segregation study design. WES was conducted on a single family of four children (two asthmatic and two healthy) and both parents (asthmatic mother and healthy father). In this analysis, the authors identified 10 novel variants exclusive in the asthmatic mother and asthmatic children. Based on function enrichment scores and association with asthma, three of the 10 variants were highlighted in the following genes (*PDE4DIP*, *CBLB* and *KALRN*). Of interest, the two asthmatic children had more de-novo mutations than their healthy siblings, although this finding should be considered with caution as this study was only conducted on a single family.

Campbell *et al.* [45] conducted one of the first WGS focusing on asthma on 16 individuals (asthma ($n = 8$), healthy controls ($n = 6$), one with BHR, and one with asthma symptoms) from asthma-enriched and asthma-depleted families. In this study they focused on copy number variants (CNVs) containing duplications or deletions of more than 50 bp. None of the measured CNVs survived the multiple testing correction, possibly due to the sample size. Based on nominal *P* values the authors identified a CNV near *NEDD4L*, which increased the risk of asthma in their population.

Quao *et al.* [46] carried out the first WES study on EOS-COPD. This study contained 347 subjects in 49 extended pedigrees (GOLD 2–4 ($n = 155$), GOLD 1 ($n = 16$), healthy controls ($n = 148$) and preserved ratio impaired spirometry, FEV1 less than 80% predicted and FEV1/FVC at least 0.7 ($n = 28$)) and identified 69 genes with predicted mutations that segregated with severe COPD in at least two pedigrees. Four genes (*DNAH8*, *ALCAM*, *RARS* and *GBF1*) had an increase in rare high impact mutations in a

Table 1. An overview of the current whole exome/genome sequencing results in asthma and chronic obstructive pulmonary disease

Region/gene/SNP	Closest gene	Type of analysis	Method	Disease	Study design	Study
PDE4DIP FCRL6 AIM2 ZBTB37 IER5 CBLB CCDC80 KALRN GALNTL6 COMMD5		Single variant analysis focused on Nonsense mutations	WES	Asthma	Pedigree	[38]
ALCAM RARS DNAH8 GBF1		Gene based	WES	COPD	Pedigree (347 subjects in 49 extended pedigrees)	[40]
Chr19:53430000–53459999	ZNF816	Region based	WGS	COPD	Case (n=64) Control (n=65)	[41]
ZNF816		Gene based				
PTPRO		Gene based Only nonsynonymous variants				
rs1512281 rs12914385	HHIP CHRNA5	Single variant	WGS	COPD	Case (n=821) Control (n=973)	[42]
Chr16:7738639–77741680	NUDT7	Region based				
Chr5:163473111–163479934	LOC101927835	Region based rare variants				
rs17834628 rs10746419 rs13437006 rs1565749 rs17048684 rs16995064 rs66544720 rs6926020 rs60163793	LINC01194 MIR205HG HACE1 ASB7 LINC00613 PLCB1 AEBP2 HACE1 MN1	Single variant Meta-analysis Only common variants	WGS	Asthma BDR	Puerto Ricans Case (n=244) Control (n=239) Mexicans Case (n=240) Control (n=243) African Americans Case (n=242) Control (n=233)	[43]
4:73478000–73479000 **8:97926000–97927000**	ADAMTS3 SDC2	Region based All populations combined				
No significant genes		CNV analysis	WGS	Asthma	Case (n=8) Control (n=8)	[39]

Bold indicates genes or SNPs considered genome-wide significant by respective study. BDR, bronchodilator drug response, BDR, bronchodilator drug response, CNV, copy number variants, WES, whole exome sequencing, WGS, whole genome sequencing.

secondary validation cohort (204 cases with severe COPD and 195 resistant smokers) however; these results were not significant. It was concluded by the authors that although rare deleterious coding variants may increase risk for COPD, multiple genes are likely to contribute to COPD susceptibility.

The first WGS study in COPD was published by Radder *et al.* [47] was conducted in a case control design focusing on heavy smokers with (n=64) or without (n=65) to severe COPD with emphysema. This study identified a 30kB region (Chr19:53430000–53459999) with a suggestive association with emphysema ($P < 10^{-5}$). This region included the genes ZNF816 and ZNF321P. A subsequent analysis identified ZNF816 as being the top gene associated with emphysema in a gene-based test (4.52×10^{-6}). Finally, PTPRO was identified to have a suggestive P value $<10^{-5}$ for an association with emphysema in an analysis including only nonsynonymous variants across the exome. The top nonsynonymous variant identified in PTPRO was also found to be significantly associated with obstruction and emphysema in a larger cohort (n=686). The main limitation of this study was

the restricted number of samples, which was the main contributor that lead to no genome-wide significant associations being identified.

The largest WGS to date was published by Prokopenko *et al.* [48▪▪] consisting of 821 patients with severe COPD and 973 controls. Soler Artigas *et al.* [49] performed a single variant and grouped variant analysis. In the single variant analysis, a region near *HHIP* had the strongest association with COPD. A number of previously identified regions also approached significance which were located in proximity to *CHRNA5* and *TNS1*, respectively. Of interest, variants in *SERPINA6/SERPINA1* were associated with COPD in a subgroup analysis of only in African American individuals. The authors then attempted to replicate findings of Radder *et al.* [47] in their cohort at a gene-based and region-based association, but neither *ZNF816* or *PTPRO* was significant in their populations. Furthermore, the four reported nonsynonymous variants in *PTPRO*, gave nonsignificant or opposite effects. These results indicated a lack of replication between small and large WGS studies.

PHARMACOGENOMICS ON CURRENT ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE MEDICATIONS

In both asthma and COPD, there have been attempts to use genetics to separate individuals based on treatment response. This concept is known as pharmacogenomics and is the basis of 'personalized medicine'. A number of GWAS analyses have looked at the pharmacogenomics of β2-agonists, ICS, leukotrienes and montelukast [43,50–56].

Recently, Mac *et al.* [57▪▪] conducted the largest WGS pharmacogenomics study in asthma to date using data from 1441 children with asthma focusing on high or low β2-agonists (albuterol) bronchodilator drug response. This study focused on common variants and region based analysis on common/rare variants, analyses were conducted stratified for population or combined. The initial analysis of common variants showed rs17834628 to be associated with bronchodilator drug response at a genome-wide level in a meta-analysis of the three populations. A number of other SNPs had suggestive significance ($P < 10^{-5}$), including rs28450894, which is located in close proximity to NF-kB. The regional analysis identified five regions associated with bronchodilator drug response at a genome wide level, two (Chr1 : 114177000–114178000 (*MAGI3* intron 9), and Chr11 : 27507000–27508000) in the Mexican population, one (Chr19 : 10424000–10425000) in the African American population and two (Chr4 : 73478000–73479000 (*FDX1L*, intron 3) and Chr8 : 97926000–97927000 (*CPQ* intron 4)) in the combined analysis.

CONCLUSION

The use of genetic profiling of COPD in the clinic is current limited to the identification of AAT deficiency, while genetic profiling it is current absent in asthma. Over the past 10–15 years, GWAS analysis of common variants has provide little in the way of new clinical genetic profiling markers. However, advances in sequencing technologies have led to better methods of identifying rare potentially disease causing variants. As the price of these technologies, decreases and the population size of studies increases, we are positioned like never before to identify disease causes or therapy response altering variants that in the short-term will allow for the development of screening procedures for disease to identify patients at risk of developing asthma or COPD. In the long term, we anticipate similar to A1AT augmentation/replacement therapy can be used to treat patients with medications that can compensate for the mutations that we identify, pushing forward the idea of personalized medicine.

Conflicts of interest

All authors have no conflicts of interest.

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