Examining genetic susceptibility in acute exacerbations of COPD

Emily S Wan

Acute exacerbations (AE) contribute significantly to morbidity and mortality, and account for a substantial proportion of the direct costs associated with chronic obstructive pulmonary disease (COPD). Although AE frequency generally increases with worsening spirometric airflow obstruction on the population level, considerable variation in the number of exacerbations experienced by individuals exists. Epidemiological studies support the existence of an ‘intrinsic susceptibility’ towards AE which is independent of the degree of impairment in forced expiratory volume in 1 s (FEV₁) on spirometry. This has fuelled the search for genetic variants which may contribute to differential susceptibility towards AEs.

An overview of studies investigating associations between genetic sequence variants and AE risk in COPD is shown in table 1. The vast majority of studies published to date have used the ‘candidate gene’ approach, whereby a limited number of variants in genes felt to be plausible contributors to exacerbation susceptibility are examined. Unfortunately, the body of knowledge arising from these investigations is often inconsistent. Non-replication or conflicting reports of association between studies are common, even when identical variants are interrogated (as exemplified by studies on the adrenoceptor beta 2 gene, ARDB2). This is likely due, at least in part, to chance findings, limited power from small study sizes and inadequate statistical rigour. Recent advances in genotyping, computing power and statistical methodologies have facilitated the widespread application of ‘hypothesis-free’ genomics-based studies of complex diseases and traits, yet studies of AE risk in COPD using this approach have been conspicuously absent from the literature.

A major limitation of hypothesis-free approaches, which exhaustively interrogate all the variants within a search space regardless of a priori assumptions regarding involvement, is the prerequisite for a well-defined phenotype. AEs of COPD, which are notoriously difficult and labour-intensive to characterise, do not meet this requirement. The diagnosis of an AE is fundamentally based on a patient’s subjective perception of worsening symptoms. There are currently no established biomarkers or gold standards to confirm or disprove the diagnosis of an AE. As shown in table 1, significant variability in the diagnostic instruments (ie, diary cards, self-report and medical records) and ascertainment methods (retrospective vs prospective) used to define and quantify AEs in different studies exists. Although research in the field has generally moved towards a medical utilisation-based consensus definition for AEs, the use of standardised diagnostic codes and/or medical records may still be limited by inaccurate coding or diagnostic uncertainty on the part of the healthcare provider caring for the patient. Thus, the lack of well-curated data on AEs in cohorts large enough to overcome the multiple testing burden inherent to genomics-based studies has limited the application of these methods in identifying genetic loci associated with AE risk.

Not surprisingly, the majority of our knowledge on the association between variants in the mannose-binding lectin 2 (MBL2) gene and AE risk derives from candidate gene studies. Given the central role of acute infections in precipitating AEs, the discovery of functional genetic variants in MBL2 which impact systemic levels of MBL, a protein involved in innate immunity, logically led to interest in the gene as a susceptibility locus. Analogous to studies of ARDB2, the results of candidate gene-based studies of MBL2 variants have been inconsistent. Early studies reported that genetic variants associated with MBL deficiency were correlated with increased exacerbation risk. Subsequently, several larger studies investigating systemic MBL protein levels (but not genotype directly) found no association with exacerbation risk. Adding to the fray, Dicker et al demonstrated that MBL protein selectively binds to (and presumably compromises clearance of) Haemophilus sp in vitro and that individuals with ‘sufficient’ genotypes have reduced bacterial diversity in induced sputum with an increased prevalence of colonisation with Haemophilus sp. It is notable, however, that the authors did not directly assess whether differential binding of bacteria by MBL2 genotype exists as only one (presumably wild type) recombinant MBL protein was examined. Several of the genetic sequence variants used to characterise deficiency states are missense mutations located in exonic regions which, in addition to impacting quantitative levels of MBL protein, may

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How should we weigh the findings reported by Dicker et al against the existing literature on the association between MBL2 variants and AE risk? The current study, which includes data from 1796 patients with spirometrically confirmed COPD, is considerably larger than previous genetic studies of MBL2 variants. The use of records-based approaches to identify and quantify AEs over a median follow-up period of 5.4 years in a single-payer medical system with minimal missing data is also considerably more rigorous than methods employed in previous studies. These strengths, combined with the use of statistical models which adjust for relevant covariates such as baseline lung function, comorbidities, and socioeconomic differences, lend credence to a finding which outright contradicts the existing literature on the topic—the counterintuitive link between genetic MBL deficiency with reduced exacerbation risk. The authors then proceed to support the validity of their epidemiological findings through the inclusion of functional and microbiome studies which explore the possible biological underpinnings of the association.

The prevailing theory that increased circulating levels of MBL protein, which is involved in the phagocytosis of pathogens and apoptotic cells, should be associated with protection from AE fails to consider potential differences in MBL function attributable to the local environment of the lungs. Increased oxidative stress in the airways has been postulated to contribute to enhanced generation of oxidised MBL (ox-MBL), a protein which impairs phagocytosis of selected pathogens below control levels. Thus, higher levels of circulating MBL in ‘sufficient’ states would lead to increased levels of ox-MBL and paradoxically result in reduced clearance and increased colonisation of the airways by selected pathogens. Dicker et al demonstrate that MBL protein selectively binds to (and presumably compromises clearance of) Haemophilus sp in vitro and that individuals with ‘sufficient’ genotypes have reduced bacterial diversity in induced sputum with an increased prevalence of colonisation with Haemophilus sp. It is notable, however, that the authors did not directly assess whether differential binding of bacteria by MBL2 genotype exists as only one (presumably wild type) recombinant MBL protein was examined. Several of the genetic sequence variants used to characterise deficiency states are missense mutations located in exonic regions which, in addition to impacting quantitative levels of MBL protein, may
Table 1 Genetic variants investigated for association with AE of COPD

<table>
<thead>
<tr>
<th>Gene</th>
<th>N</th>
<th>Variant(s)</th>
<th>Phenotype*</th>
<th>Study</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>206t</td>
<td>rs4646994</td>
<td>Hospitalisation records</td>
<td>Miak et al&lt;sup&gt;10&lt;/sup&gt;</td>
<td>Deletion variant protective among males</td>
</tr>
<tr>
<td>ADRB2</td>
<td>5125</td>
<td>rs1042713</td>
<td>Prospective moderate-to-severe AE (steroids/ antibiotic treatment)</td>
<td>Rabe et al&lt;sup&gt;11&lt;/sup&gt;</td>
<td>Pharmacogenetic study; Major allele of rs1042713 associated with decreased risk of AE in salmeterol-treated group</td>
</tr>
<tr>
<td>ADRB2</td>
<td>190t</td>
<td>rs1042713</td>
<td>'Frequent’ exacerbators (≥3 hospitalisations within 1 year) vs ‘Stable’ (0 in 2 years)</td>
<td>Vacc et al&lt;sup&gt;12&lt;/sup&gt;</td>
<td>No association reported</td>
</tr>
<tr>
<td>ADRB2</td>
<td>92</td>
<td>rs1042713</td>
<td>Self-reported exacerbations during the 12 months prior to enrolment</td>
<td>Emeny-Mksymiuk et al&lt;sup&gt;13&lt;/sup&gt;</td>
<td>Major allele of rs1042713 associated with increased risk of AE</td>
</tr>
<tr>
<td>EPHX1</td>
<td>219</td>
<td>rs1051740</td>
<td>Moderate-to-severe AE for 1 year with administration of oral N-acetylcysteine</td>
<td>Zhang et al&lt;sup&gt;14&lt;/sup&gt;</td>
<td>Pharmacogenetic study; ‘slow’ enzyme activity group with lower exacerbation rate than ‘fast activity’ group</td>
</tr>
<tr>
<td>F2R</td>
<td>203t</td>
<td>rs222744</td>
<td>Diary card exacerbations— dichotomised ‘frequent’ (≥3) vs ‘infrequent’ (&lt;3)</td>
<td>Plàtè et al&lt;sup&gt;15&lt;/sup&gt;</td>
<td>Minor allele protective for frequent exacerbations</td>
</tr>
<tr>
<td>GC</td>
<td>135t</td>
<td>rs4588</td>
<td>Diary card exacerbations (count)</td>
<td>Ishii et al&lt;sup&gt;16&lt;/sup&gt;</td>
<td>rs4588 variants associated with increased frequency of exacerbations</td>
</tr>
<tr>
<td>HMOX1</td>
<td>368</td>
<td>Long (&gt;32) dinucleotide repeats</td>
<td>Moderate-to-severe AE for 1 year with administration of oral N-acetylcysteine</td>
<td>Zhang et al&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Pharmacogenetic study; absence of long dinucleotide repeats protective</td>
</tr>
<tr>
<td>MBL2</td>
<td>200t</td>
<td>rs1800450</td>
<td>Hospital admissions by medical record review+telephone confirmation</td>
<td>Yang et al&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Minor allele associated with lower systemic MBL levels and increased risk for AE</td>
</tr>
<tr>
<td>MBL2</td>
<td>215</td>
<td>rs11003125</td>
<td>Hospital admissions by medical record review+telephone confirmation</td>
<td>Yang et al&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Minor allele associated with lower systemic MBL levels and increased risk for AE</td>
</tr>
<tr>
<td>MBL2</td>
<td>277</td>
<td>rs11003125</td>
<td>Moderate-to-severe AE by interview, medical records and public registry data. 'Frequent' (&gt;2/year) vs ‘infrequent’ (&lt;2/year)</td>
<td>Mandal et al&lt;sup&gt;6&lt;/sup&gt;</td>
<td>MBL2 deficiency haplotypes more common in ‘frequent’ exacerbators, however, no correlation with systemic MBL levels and exacerbation phenotypes</td>
</tr>
<tr>
<td>NR3C1</td>
<td>207t</td>
<td>rs56149945</td>
<td>'Unstable' (≥3 hospitalisations) vs ‘stable’</td>
<td>Schwabe et al&lt;sup&gt;18&lt;/sup&gt;</td>
<td>No association reported</td>
</tr>
<tr>
<td>SIGLEC9</td>
<td>135</td>
<td>rs2075803</td>
<td>Diary card exacerbations (count)</td>
<td>Ishii et al&lt;sup&gt;19&lt;/sup&gt;</td>
<td>rs2075803 variants associated with increased risk of AE. Did not replicate in larger study</td>
</tr>
<tr>
<td>SIGLEC14</td>
<td>135</td>
<td>Null allele</td>
<td>Prospective interviews—mild to severe AEs recorded</td>
<td>Angata et al&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Null allele associated with increased risk of AE. Did not replicate in larger study</td>
</tr>
<tr>
<td>SERPINA1</td>
<td>204t</td>
<td>11 478G→A</td>
<td>Diary card exacerbations— dichotomised ‘frequent’ (≥3) vs ‘infrequent’ (&lt;3)</td>
<td>Quint et al&lt;sup&gt;20&lt;/sup&gt;</td>
<td>No association reported</td>
</tr>
<tr>
<td>SFTP8</td>
<td>389</td>
<td>rs2118177</td>
<td>Emergency room visits and hospitalisations</td>
<td>Foreman et al&lt;sup&gt;21&lt;/sup&gt;</td>
<td>SFTP8 variants associated with AE. Variants in EPHX1, GSTP1, TGFB1, SERPINE2 also examined but demonstrated no associations</td>
</tr>
<tr>
<td>SFTP9</td>
<td>192t</td>
<td>rs911887</td>
<td>Emergency room visits and hospitalisations— dichotomised 'high' (≥2) vs 'low' (&lt;2)</td>
<td>Ou et al&lt;sup&gt;22&lt;/sup&gt;</td>
<td>No association with haplotypes reported</td>
</tr>
<tr>
<td>TNF</td>
<td>60t</td>
<td>rs1800629</td>
<td>Retrospective moderate-to-severe AE year prior to enrolment</td>
<td>Özdoğan et al&lt;sup&gt;24&lt;/sup&gt;</td>
<td>No association reported</td>
</tr>
</tbody>
</table>

*As published in methods section of manuscripts.
†Among studies which enrolled both COPD and controls, only number of COPD subjects included in exacerbation analysis are included in this table.
AE, acute exacerbation; COPD, chronic obstructive pulmonary disease; MBL, mannos-binding lectin.

contribute to qualitative differences in function which should be assessed. A careful assessment of the remaining limitations of the study by Dicker et al<sup>70</sup> is necessary to contextualise the findings. As noted by the authors, the COPD subjects included in Dicker et al<sup>70</sup> had relatively mild airflow limitation (mean FEV<sub>1</sub>, 78.0%–79.6% predicted) relative to subjects enrolled in most COPD genetic studies. In addition, the subgroup enrolled in the microbiome analysis of induced
sputum was modest, with a significant minority (29.8%) unable to provide any samples for analysis; this, in addition to consideration of local patterns of bacterial colonisation as well as potential differences in the microbiome of individuals with more advanced COPD should be examined in future studies. Finally, the current study was presumably conducted among an ethnically isolated subpopulation of European descent (Scotland) and replication in an independent cohort was not attempted. The impact of ancestry and population structure on these findings, as well as assessing whether the associations between MBL2 variants and AE risk are generalisable to non-European populations, represent potential future avenues of investigation.

There is a growing misconception that candidate gene studies are irrelevant in the age of genomics. The study by Dicker et al\(^7\) serves as an elegant argument against this by demonstrating that data from a well-designed candidate gene study can serve as one component of multiple, converging data streams which collectively support the association between MBL2 variants and AE susceptibility. It should also be noted that many of the most robust associations reported from genome studies (eg, the association between TGFβ2 and COPD disease status) were originally identified through candidate gene studies. Carefully conducted candidate gene studies will likely remain among the armamentarium of techniques used by researchers to explore the biology of complex disorders. However, due to the relative ease of conducting candidate gene studies, the literature will likely continue to be populated by reports of varying rigour; it will remain the collective responsibility of the scientific community to interpret and adjudicate the findings of such studies.

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**Competing interests**

None declared.

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