ACUTE LUNG INJURY

Vascular endothelial growth factor gene polymorphism and acute respiratory distress syndrome

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See end of article for authors’ affiliations

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A acute respiratory distress syndrome (ARDS), the most extreme form of acute lung injury, continues to have a significant mortality of at least 35% of patients despite improvements in the management of sepsis and ventilatory support. Death in these patients is usually secondary to the physiological derangement of multiorgan rather than respiratory failure per se.

Non-cardiogenic pulmonary oedema is a characteristic feature of ARDS. The potent effects of vascular endothelial growth factor (VEGF) on vascular endothelium as both a feature of ARDS. The potent effects of VEGF are among those polymorphic genes with a potential role in ARDS, genetic polymorphism being a potential explanation for the low incidence of ARDS within the large population. Non-cardiogenic pulmonary oedema is a characteristic feature of ARDS. The potent effects of VEGF on vascular endothelium as both a feature of ARDS. The potent effects of VEGF are among those polymorphic genes with a potential role in ARDS, genetic polymorphism being a potential explanation for the low incidence of ARDS within the large population.

Background: Non-cardiogenic pulmonary oedema is a characteristic feature of the acute respiratory distress syndrome (ARDS). The properties of vascular endothelial growth factor (VEGF) as a potent vascular permeagen and mitogen have led to investigation of its potential role in this condition. Lower VEGF plasma levels have been linked to the presence of the T allele in the +936 CT polymorphism. We hypothesised that the presence of the T allele would be associated with the development and severity of ARDS.

Methods: A cohort of 137 normal subjects, 117 ventilated patients with ARDS, and 103 “at risk” of ARDS were genotyped for the VEGF+936 CT polymorphism. The severity of physiological disturbance and mortality was determined in the ventilated cohorts.

Results: The CT and TT genotype frequencies were increased in ARDS patients compared with both normal subjects (OR 2.01, 95% CI 1.13 to 3.58, p = 0.02) and those “at risk” (OR 2.05, 95% CI 1.02 to 2.20, p = 0.03). In patients with ARDS but not those “at risk”, CT and TT genotypes were associated with a higher mean APACHE III score [80.9 (4.3) vs 69.3 (2.9), p < 0.05].

Conclusion: These data support a role for VEGF in the pathogenesis of ARDS and its associated physiological derangement.
platelet count of <81 x 10^9/L. All subjects were of North European origin and reflected the general population. The protocol was approved by the North Bristol NHS Trust local research ethics committee and patients or their surrogates gave informed consent.

**Clinical data**

Murray Lung Injury, Acute Physiology and Chronic Health Evaluation II (APACHE II), Acute Physiology and Chronic Health Evaluation III (APACHE III), and Simplified Acute Physiology (SAPS II) scores were recorded for each ICU patient. The Murray Lung Injury score is an accepted degree of pulmonary injury and oxygenation whereas APACHE II, APACHE III and SAPS II scores indicate the degree of generalised physiological disturbance. Twenty eight and 60 day mortality were also recorded.

**DNA extraction**

Genomic DNA was extracted from whole blood using a standard phenol-free high salt method as previously described. Induced heteroduplex generator (IHG) analysis for VEGF gene polymorphism and ARDS

**Table 1** Baseline characteristics of study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Mean (SE) age (years)</th>
<th>Sex (F:M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>137</td>
<td>52 (1.7)</td>
<td>66-64</td>
</tr>
<tr>
<td>&quot;At risk&quot;</td>
<td>103</td>
<td>64 (1.4)</td>
<td>43-58</td>
</tr>
<tr>
<td>ARDS</td>
<td>112</td>
<td>61 (1.4)</td>
<td>44-64</td>
</tr>
</tbody>
</table>

**RESULTS**

**Baseline characteristics**

Table 1 shows baseline characteristics for normal subjects, ventilated “at risk” patients, and patients with ARDS. The risk factor profiles of the “at risk” and ARDS cohorts are shown in table 2. The “at risk” and ARDS ventilated cohorts were also matched in terms of generalised physiology severity scores (APACHE II, APACHE III and SAPS II), but Murray Lung Injury scores were higher in the ARDS cohort as expected (2.84 (0.06) v 1.36 (0.08), p<0.001).

**CT, TT genotype and T allele frequencies in patient groups**

Table 3 shows the genotype and allele frequencies for the three different groups. For all samples, genotype distribution was in Hardy-Weinberg equilibrium ($\chi^2 = 1.42$, p = 0.23 for normal; $\chi^2 = 0.729$, p = 0.39 for “at risk”; and $\chi^2 = 0.137$, p = 0.71 for ARDS). CT and TT genotypes occurred significantly more frequently in the ARDS group than in the normal group (OR 2.01, 95% CI 1.13 to 3.58, p = 0.02) and in those “at risk” (OR 2.05, 95% CI 1.02 to 2.20, p = 0.03). The polymorphic T allele occurred significantly more frequently in the ARDS group (OR 1.77, 95% CI 1.06 to 2.91, p = 0.04) than in the normal and the ventilated “at risk” groups (OR 1.82, 95% CI 1.04 to 3.18, p = 0.04).

**CT, TT genotypes and mortality (28 and 60 day)**

Table 4 shows 28 and 60 day mortality according to disease group and genotype. There were no significant differences in mortality between the “at risk” and ARDS cohorts as a whole (28 and 60 day mortality rates 27.2% v 36.6%, 30.1% v 38.4%, respectively). Twenty eight and 60 day mortality rates did not

**Table 2** Risk factor profiles for ventilated cohorts

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>&quot;At risk&quot;</th>
<th>ARDS</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis (combined)</td>
<td>72 (49.9)</td>
<td>99 (84.6)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Sepsis (chest)</td>
<td>37 (35.9)</td>
<td>61 (52.1)</td>
<td>0.21</td>
</tr>
<tr>
<td>Sepsis (abdominal)</td>
<td>22 (21.4)</td>
<td>30 (25.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Sepsis (unknown site)</td>
<td>10 (9.7)</td>
<td>6 (5.1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Sepsis (nervous system)</td>
<td>3 (2.9)</td>
<td>2 (1.7)</td>
<td>0.65</td>
</tr>
<tr>
<td>Massive transfusion</td>
<td>22 (21.4)</td>
<td>7 (6.0)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>5 (4.9)</td>
<td>9 (7.7)</td>
<td>0.42</td>
</tr>
<tr>
<td>Inhalational injury</td>
<td>4 (3.9)</td>
<td>2 (1.7)</td>
<td>0.42</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>117</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are given as number (%). *Statistically significant (Fisher’s exact test).
differ between CT/TT and CC genotypes in “at risk” (OR 1.19, 95% CI 0.41 to 3.48, p = 0.78 and OR 1.32, 95% CI 0.47 to 3.72, p = 0.59, respectively) or ARDS cohorts (OR 1.81, 95% CI 0.80 to 4.05, p = 0.21 and OR 1.90, 95% CI 0.85 to 4.23, p = 0.15, respectively).

CT, TT genotypes and physiological scores

Table 5 shows the ICU severity scores according to disease group and genotype. There was no association between genotypes and Lung Injury score, APACHE II or SAPS II scores. However, ARDS patients with CT or TT genotypes had significantly higher APACHE III scores than those with CC genotypes (80.9 (4.3) v 69.3 (2.9), p<0.05).

**DISCUSSION**

This study suggests an association between a specific allele (the VEGF+936 T allele) and susceptibility to ARDS and its associated physiological disturbance (APACHE III score). This supports previously published data suggesting a role for VEGF in ARDS.3,4

We attempted to minimise genetic confounding by rigorous phenotypic classification using an accepted definition of ARDS and “at risk” subjects. A ventilated “at risk” cohort was used for comparison to exclude the possibility of a false association with critical illness. In addition, prospective recruitment to each cohort was undertaken over the same time period reducing the possibility of recruitment bias as a cause of chance variation in genotype frequencies. The cohorts were all of North European origin to remove the possibility of altered genotype frequencies in different ethnicities. A higher VEGF+936 CT genotype frequency has been reported in a Japanese cohort than would be expected in a normal white population.1

There are some limitations to this study. In any genetic epidemiological study the cohort size is key and our sample size is modest compared with other similar studies, although consistent with our initial power calculation. Furthermore, the higher proportion of patients with sepsis and lower proportion of transfusion-related injury in the ARDS cohort and age differences limit the strength of our conclusions. However, the polymorphism under study is associated with a functional effect on the gene product and our results are biologically plausible.

In assessing the relationship between the VEGF polymorphism and other ARDS parameters, only the APACHE III physiological score was associated. This system uses statistical modelling techniques to weight and select the variables and multiple logistic regression to estimate risk of death, unlike the other scoring systems which use a more subjective method with weights and variables selected by expert opinion. There is some evidence to suggest that the APACHE III is a superior prognostic model.15 However, further larger studies are required to confirm the absolute specificity of this association.

So why is there an apparent relation between the T allele, ARDS susceptibility, and severity of physiological dysfunction? Untested hypotheses include reduced VEGF expression via cellular gene enhancer effects or changes in VEGF isoform or receptor expression. These hypotheses would assume VEGF to have a protective role in recovery from lung injury and hence a more important effect of this polymorphism in the lung. It is possible the VEGF+936 CT polymorphism might be in linkage disequilibrium with another functional polymorphism, although this has yet to be demonstrated.

In vitro studies have confirmed that VEGF is abundant in the lung, especially in alveolar epithelium including A549 cells and preliminary data in primary human cultured type 2 alveolar epithelial cells (AE2 cells), suggesting it is the predominant pulmonary source of VEGF.16-19 Several lines of in vitro evidence point to a possible role for VEGF in repair and recovery following injury. Exogenous VEGF has been shown to act as a growth factor on human fetal pulmonary epithelial cells and is capable of restoring the ability of A549 cells to express VEGF in an acid exposure cellular model of injury, raising the possibility of an autocrine function in the lung.20-24 This has been described in specialised epithelial cells in other tissues.22

Animal models have contributed conflicting evidence for the role of VEGF in the lung. Some studies suggest a possible role in mediating lung injury. Adenoviral delivery of VEGF165 to the lung causes non-cardiogenic pulmonary oedema. High tidal volume ventilation strategies in an acid induced murine model of lung injury increase VEGF receptor 2 (VEGFR2) expression although intrapulmonary VEGF expression per se was unchanged.21-24 However, other studies suggest a role in recovery from lung injury and a possible survival function for

| Table 3 | Genotype and allele frequencies |
|-----------|-----------------|-----------------|-----------------|
| **Group** | **No** | **Genotype frequencies (%)** | **Allele frequencies (%)** |
| | | **CT, TT** | **CC** | **T** | **C** |
| | | **(polymorphic)** | **(normal)** | **(polymorphic)** | **(normal)** |
| Normal | 137 | 27 (19.7) | 110 (80.3) | 30 (10.9) | 244 (89.1) |
| “At risk” | 103 | 20 (19.4) | 83 (80.6) | 22 (10.7) | 184 (89.3) |
| ARDS | 112 | 37 (33.0)* | 75 (67.0) | 40 (17.9) | 184 (82.1) |
| *OR 2.01, 95% CI 1.13 to 3.58, p=0.02 (ARDS v Normal); OR 2.05, 95% CI 1.02 to 2.09, p=0.03 (ARDS v ARDS) |

| Table 4 | 28 and 60 day mortality |
|-----------|-----------------|-----------------|
| **Group** | **N** | **Genotype** | **28 day mortality (%)** | **60 day mortality (%)** |
| | | | | |
| “At risk” | 103 | All | 28/103 (27.2) | 31/103 (30.1) |
| 20 | CT, TT | 6/20 (30.0) | 7/20 (35.0) |
| 83 | CC | 22/83 (26.5) | 24/83 (28.9) |
| ARDS | 112 | All | 41/112 (36.6) | 43/112 (38.4) |
| 37 | CT, TT | 17/37 (45.9)* | 18/37 (48.6) |
| 75 | CC | 24/75 (32.0)* | 25/75 (33.3)* |
| *OR 1.81, 95% CI 0.80 to 4.05, p=0.21. |
| †OR 1.90, 95% CI 0.85 to 4.23, p=0.15. |
Table 5  ICU severity scores

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>ICU severity score</th>
<th>Murray Lung Injury</th>
<th>SAPS II</th>
<th>APACHE II</th>
<th>APACHE III†</th>
</tr>
</thead>
<tbody>
<tr>
<td>“At risk”</td>
<td>All</td>
<td>1.36 (0.08)</td>
<td>42.2 (1.31)</td>
<td>17.0 (0.78)</td>
<td>67.9 (2.34)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT, TT</td>
<td>1.21 (0.20)</td>
<td>39.9 (2.85)</td>
<td>18.0 (1.42)</td>
<td>62.2 (4.87)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1.40 (0.09)</td>
<td>42.8 (1.47)</td>
<td>17.0 (0.78)</td>
<td>69.3 (2.65)</td>
<td></td>
</tr>
<tr>
<td>ARDS</td>
<td>All</td>
<td>2.84 (0.06)</td>
<td>45.7 (1.32)</td>
<td>19.0 (0.75)</td>
<td>73.0 (2.47)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT, TT</td>
<td>2.74 (0.11)</td>
<td>46.8 (2.63)</td>
<td>19.0 (1.30)</td>
<td>80.9 (4.33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>2.90 (0.07)</td>
<td>45.2 (1.51)</td>
<td>18.5 (0.92)</td>
<td>69.3 (2.92)</td>
<td></td>
</tr>
</tbody>
</table>

Severity scores are expressed as mean (SE) values.
Data for APACHE II were log10 transformed to normalise before statistical analysis.
p<0.05 (Bonferroni) ARDS CT, TT v ARDS CC (two way ANOVA, p = 0.02).

REFERENCES


LUNG ALERT

Prostanoid DP receptor polymorphisms may cause changes in susceptibility to asthma


Prostaglandins have been implicated in the pathophysiology of asthma. The prostanoid DP receptor is expressed on the surface of airway eosinophils and mast cells. This group has studied the gene for the prostanoid DP receptor (PTGDR) and its relationship with the asthma phenotype.

Screening revealed four new single nucleotide polymorphisms (SNPs) in addition to the two previously reported SNPs in the areas of the gene promoter region that bind transcription factors. The frequencies of the four most common SNPs were determined by restriction fragment length polymorphism (RFLP) analysis in patients with mild to moderate asthma and controls. Genotypic analysis showed an increased risk of asthma associated with the T-549C SNP (odds ratio (OR) >2.0; p<0.05) for white and black ethnic groups. The T allele of the C-441T SNP was significantly more common in white subjects with asthma (OR >1.8; p<0.02); this association did not reach significance in the black population. Black patients carrying both the C-441T and T-549C alleles were more likely to have asthma (OR 24.36; p = 0.01). Patients who had at least one copy of the haplotype with low transcriptional efficiency were under-represented in the asthma group (OR 0.32 for blacks, 0.55 for whites; p<0.05). There was no association between any of the SNPs and total IgE.

Certain genetic variants that impair the expression of PTGDR seem to reduce susceptibility to asthma. This study supports the hypothesis that PTGDR is one of a number of genes that determine susceptibility to developing the asthma phenotype.

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