Prognostic Values of Nucleotide Polymorphism in ARDS: A Whole Exome Sequencing Association Study

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Research

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Abstract

**Background** Genetic locus were identified associated with ARDS outcome. Our goal was to explore the associations between genetic variants and outcome of ARDS, and the prognostic values of nucleotide polymorphism in ARDS.

**Methods** This was a single-center, prospective trial enrolling adult ARDS patients. After baseline data were collected, blood samples were drawn to perform whole exome sequencing, and single nucleotide polymorphism (SNP) / insertion-deletion to explore the quantitative and functional associations between genetic variants and ICU outcome. Then the lung injury burden (LIB), which was defined as the ratio of nonsynonymous SNP number per megabase of DNA, was used to evaluate its value in predicting outcome of ARDS.

**Results** A total of 105 ARDS patients were enrolled in the study, including 70 survivors and 35 nonsurvivors. Based on the analysis of a total of 65542 nonsynonymous SNP, LIB in survivors was significantly higher than nonsurvivors [1892 (1848 - 1942) /MB versus 1864 (1829 - 1910) /MB, p = 0.018], while GO analysis showed that 60 functions were correlated with ARDS outcome, KEGG enrichment analysis showed that SNP/InDels were enriched in 13 pathways. Several new SNPs were found potentially associated with ARDS outcome. Analysis of LIB was used to determine its outcome predicting ability, the area under the ROC curve of which was only 0.6103, and increase to 0.712 when combined with APACHE II score.

**Conclusions** Genetic variants are associated with ARDS outcome; however, their prognostic value still need to be verified by larger trials.

**Trial registration** [Clinicaltrials.gov](https://clinicaltrials.gov) NCT02644798. Registered 20 April 2015.

Background

Acute respiratory distress syndrome (ARDS) is characterized by the acute lung injury, associated with increased pulmonary vascular permeability and reduced aerated lung tissue[1]. With an extremely high hospital mortality rate among 35–46%[2], current therapeutic strategies to increase ARDS survival consist of support to prevent ventilator induced lung injury, to improve oxygenation and gas exchange, advances in etiology and pathology of ARDS are urging. Clinical factors and protocolized therapeutic strategy poorly explain the outcome of ARDS, the role of genetic locus in the pathogenesis of ARDS is increasingly recognized[3–6].

Numerous genetic variants were identified which are associated with the outcome of ARDS. Morrell[7] found that genetic variation in MAP3K1 associated with ventilator-free days in ARDS, while Wei[8] showed that the missense genetic variant in LRRC16A/CARMIL1 improved survival by attenuating platelet count decline in ARDS patients. However, as a heterogeneous disease with multiple and interactive pathogenic processes, the effect of genetics contributing differently[7–11], meanwhile, the racial and ethnic differences in mortality also exist[12, 13]. More than five categories of genes were found to associate with the outcome of ARDS: genes influencing immune regulation, genes influencing endothelial barrier function, genes influencing respiratory epithelial function, genes influencing coagulation, genes influencing injury and oxidative stress and so forth. Then a few genetic risk factors have been discovered by large-scale genotyping approaches, from in vivo or in vitro models...
of lung injury, which highlight the importance of identifying genetic biomarkers of outcome for ARDS to further improve stratification. The mutational landscape and variability at single nucleotide polymorphisms (SNP) with outcome of ARDS is unknown. By whole exome sequencing association study, our goal was to explore the associations between genetic variants and outcome of ARDS, and the prognostic values of nucleotide polymorphism in ARDS.

Materials And Methods

Setting

This was an investigator-initiated, single-center, prospective trial that was conducted in the intensive care unit of a tertiary care teaching hospital. The study protocol was approved by the Ethics Committee (Approval Number: 2015ZDSYLL014.0) of Zhongda Hospital, School of Medicine, Southeast University, and written informed consent was obtained from each patient or their next of kin. Trial registration: Clinicaltrials.gov NCT02644798. Registered 20 April 2015.

Patients

Adult ARDS (according to Berlin definition) patients were enrolled in the trial. The diagnostic criteria included (a) within one week of a known clinical insult or new or worsening respiratory symptoms; (b) chest imaging showing that bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules; (c) respiratory failure not fully explained by cardiac failure or fluid overload; and (d) arterial partial pressure of oxygen / fraction of inspiration oxygen (PaO₂/FiO₂ ratio, P/F ratio) less than or equal to 300 mmHg.

Data collection

Baseline-recorded data included demographic characteristics, comorbidities, and the origin and etiology of ARDS were collected by trained investigators. Predicted body weight was calculated by sex and height. Severity of illness was assessed with the Acute Physiology and Chronic Health Evaluation (APACHE) II score within 24 hours on enrollment. Sequential Organ Failure Assessment (SOFA) and Murray lung injury score within 24 hours on enrollment were also calculated.

Predisposing conditions of ARDS were collected, and subphenotypes of ARDS were determined. Severe ARDS group and non-severe group were divided according to the severity of lung injury (Berlin definition). Patients with risk factors of pneumonia (pulmonary sepsis), pulmonary contusion, inhalation and drowning were categorized as having pulmonary ARDS, whereas patients with risk factors of non-pulmonary sepsis or pancreatitis were categorized as having extrapulmonary ARDS. Patients with sepsis on enrollment after enrolled were recorded as ARDS with sepsis. Patients with shock on enrollment were recorded as ARDS with shock. Sepsis was defined by Sepsis 3.0.

Peripheral blood samples were drawn. Prognosis was recorded as the survivors and non-survivors in ICU.

Methods
Whole-exome sequencing was performed by the sequencing platform Illumina, the data were compared with reference genome UCSC hg19. Firstly, genomic DNA was isolated from the peripheral blood samples taken from individuals by following the manufacturer’s standard procedure using QIAamp DNA Blood kits (Qiagen, Hilden, Germany). Then exome sequence capture was performed on SureSelect Human All Exon V6 (Agilent). DNA library was subjected to 2 × 150 bp paired-end massively parallel sequencing using a HiSeq2000 Sequencing System (Illumina, San Diego, CA, USA). Before variant calling, sequence alignment files were generated to duplicate removal, local realignment around known Indels and base quality recalibration using the Genome Analysis Toolkit (GATK)[14]. Variations that included single-nucleotide variants (SNVs) and small insertions or deletions (Indels) were identified using both the VarScan 2.2.7 software package (http://www.ncbi.nlm.nih.gov/pubmed/22300766)[15] as well as the variant quality score recalibration (VQSR) protocol in GATK, and further filtered using a recommended threshold value (mapping quality > 30, base quality > 15, and read numbers > 3). Then, SNP available at dbSNP130 (hg19) as well as those reported by the 1000 Genomes Project were filtered out from the output files using the ANNOVAR (http://nar.oxfordjournals.org/content/38/16/e164)[16].

After identifying a newly number of coding SNPs potentially associated with ARDS, SNP/InDel were tested by plink method to understand the difference between the outcome and subphenotypes of ARDS. While detecting the number and function of nonsynonymous SNV, the lung injury burden (LIB) was calculated by the ratio of nonsynonymous SNP number per megabase (MB) of DNA. The area under the receiver operating characteristic curve (ROC) was used for evaluating the predictive values of LIB in predicting outcome and subphenotype of patients with ARDS.

Statistics

Data were presented as number (%) for categorical variables, and median (interquartile range) for continuous variables. Fisher’s exact test or χ2 test were used for categorical variables, and Student’s t-test or Mann-Whitney U test were used for continuous variables, as appropriate. The value of predictive ability was evaluated by the area under the curve (AUC) in the receiver operating characteristic (ROC) analysis. A p value < 0.05 was considered statistically significant. Statistical analyses were carried out by the SPSS 16.0 software (IBM, Somers, NY).

Results

There were 105 patients enrolled in the study, including 70 survivors and 35 non-survivors. The characteristics of outcome and subphenotype are presented in Table 1. The median age was 59 years old, while the median APACHE II score was 23, the median SOFA score and Murray lung injury score was 9 and 2.7, respectively. Among them, 91 patients were categorized as having pulmonary ARDS, 89 patients were diagnosed as sepsis and 66 patients as shock on enrollment.
### Table 1
Baseline patient characteristics and subphenotypes of ARDS

|                                | Patients (n = 105) |
|--------------------------------|--------------------|
| Age, year                      | 59 (46–73)         |
| Sex, male/female               | 83/22              |
| APACHE II score                | 23 (17–27)         |
| SOFA score                     | 9 (6–12)           |
| Murray lung injury score       | 2.7 (2.1–3.3)      |
| Severe ARDS, n (%)             | 52 (50)            |
| Pulmonary ARDS, n (%)          | 91 (87)            |
| ARDS combined with sepsis on enrollment, n (%) | 89 (85) |
| ARDS combined with shock on enrollment, n (%) | 66 (63) |
| Non-survivors in ICU, n (%)    | 35 (34)            |

APACHE II score, Acute Physiology and Chronic Health Evaluation II score; SOFA score, Sequential Organ Failure Assessment score. Data presented as median (interquartile range).

### Snp/indel Data By Whole-exome Sequencing

By whole-exome sequencing, the number of SNP/InDel were 471131 (Table 2). Among them, 120830 SNP/InDel were in exonic region. The number of nonsynonymous SNV were 65542, with 436 of frameshift-insertion for InDel and 897 of frameshift-deletion for InDel. GO analysis showed that 52 functions were correlated with ARDS development ($p < 0.01$), and KEGG enrichment analysis showed that these SNP/InDel were in 10 pathways, such as cGMP-PKG signaling pathway, Platelet activation ($p < 0.05$).
### Table 2
Functional type of SNP/InDel count by whole-exome sequencing

| Functional type             | SNP/InDel Count |
|-----------------------------|-----------------|
| Nonsynonymous SNV           | 64452           |
| Synonymous SNV              | 49590           |
| Unknown                     | 2613            |
| Frameshift insertion        | 436             |
| Frameshift deletion         | 897             |
| Nonframeshift insertion     | 792             |
| Nonframeshift deletion      | 1269            |
| Stopgain                    | 1125            |
| Stoploss                    | 66              |

### Snp/indel Data Between Ards Patients With Different Outcome

LIB was tested to determine the quantitative differences between survivors and non-survivors. LIB of survivors was significantly higher than non-survivors [1892 (1848–1942) /MB versus 1864 (1829–1910) /MB, \( p = 0.018 \)].

GO and KEGG analysis were performed to determine the functional difference of SNP with outcome. GO analysis showed that 60 functions were correlated with ARDS outcome \( (p < 0.01) \) (Fig. 1), and KEGG enrichment analysis showed these SNP/InDel were in 13 pathways (Table 3), such as ECM-receptor interaction pathway, Platelet activation pathway and cGMP-PKG signaling pathway \( (p < 0.01) \).
## Association Of Genetic Polymorphisms With Ards Outcome

To identify the novel SNPs which associated with ARDS outcome, the genotype distribution in different gene were summarized in Table 4, conformed to Hardy-Weinberg equilibrium. Although no strong evidence of stratification has been reported, several SNPs which potentially associated with ARDS outcome were found (Fig. 2).

### Table 3
KEGG enrichment analysis for SNP/InDel data between different ARDS outcome

| KEGG enrichment pathway                              | Number of genes | p value  |
|------------------------------------------------------|-----------------|----------|
| ECM-receptor interaction                             | 29              | 0.00144  |
| Purine metabolism                                    | 48              | 0.00279  |
| Protein digestion and absorption                     | 29              | 0.00336  |
| Platelet activation                                  | 36              | 0.00336  |
| Calcium signaling pathway                            | 48              | 0.00336  |
| Thyroid hormone signaling pathway                    | 34              | 0.00367  |
| Insulin secretion                                    | 27              | 0.00386  |
| Oxytocin signaling pathway                           | 41              | 0.00495  |
| Phospholipase D signaling pathway                    | 39              | 0.00685  |
| cGMP-PKG signaling pathway                           | 42              | 0.00782  |
| Glutamatergic synapse                                | 32              | 0.00782  |
| Adrenergic signaling in cardiomyocytes               | 38              | 0.00807  |
| Long-term depression                                 | 20              | 0.00821  |
| SNP       | Model     | Genotype | Case | Control | OR (95% CI)       | p value     | Gene    |
|-----------|-----------|----------|------|---------|-------------------|-------------|---------|
| rs3735041 | Codominant| G/G      | 11   | 2       | 24.57(4.45–135.7) | 0.0002402  | AKR1B10 |
| rs3735041 | Recessive | A/A-A/G  | 24   | 67      | 16.17(3.293–79.4) | 0.0006081  | AKR1B10 |
| rs10904402| Dominant  | A/A      | 21   | 18      | 0.2144(0.08731–0.5264) | 0.0007792  | AKR1C3  |
| rs10904402| Additive  | -        | -    | -       | 0.2955(0.1446–0.6039) | 0.0008289  | AKR1C3  |
| rs12239311| Additive  | -        | -    | -       | 4.247(2.021–79.233)  | 0.0001349  | CFAP57  |
| rs12239311| Dominant  | C/C      | 10   | 46      | 5.935(2.331–15.11)  | 0.0001882  | CFAP57  |
| rs11210812| Additive  | -        | -    | -       | 4.098(1.94–8.655)    | 0.0002179  | CFAP57  |
| rs11210805| Additive  | -        | -    | -       | 3.876(1.824–8.237)   | 0.0004277  | CFAP57  |
| rs513009  | Additive  | -        | -    | -       | 3.876(1.824–8.237)   | 0.0004277  | CFAP57  |
| rs2453412 | Additive  | -        | -    | -       | 3.876(1.824–8.237)   | 0.0004277  | CFAP57  |
| rs612626  | Additive  | -        | -    | -       | 3.876(1.824–8.237)   | 0.0004277  | CFAP57  |
| rs616045  | Additive  | -        | -    | -       | 4.006(1.844–8.703)   | 0.0004544  | CFAP57  |
| rs663824  | Additive  | -        | -    | -       | 3.839(1.808–8.153)   | 0.0004634  | CFAP57  |
| rs598336  | Additive  | -        | -    | -       | 3.839(1.808–8.153)   | 0.0004634  | CFAP57  |
| rs75528102| Additive  | -        | -    | -       | 3.797(1.763–8.179)   | 0.0006557  | CFAP57  |
| rs663336  | Additive  | -        | -    | -       | 3.797(1.763–8.179)   | 0.0006557  | CFAP57  |
| rs499839  | Codominant| A/C      | 25   | 23      | 5.999(2.265–15.89)   | 0.0003115  | CLSTN2  |
| rs499839  | Dominant  | A/A      | 8    | 42      | 5.603(2.144–14.65)   | 0.0004392  | CLSTN2  |
| rs533657  | Additive  | -        | -    | -       | 3.966(1.772–8.877)   | 0.0008043  | CLSTN2  |
| SNP            | Model     | Genotype | Case | Control | OR (95% CI)               | p value  | Gene  |
|---------------|-----------|----------|------|---------|---------------------------|----------|-------|
| rs495109      | Codominant| T/A      | 22   | 20      | 4.58(1.85–11.32)          | 0.0009866| CLSTN2|
| rs589819      | Additive  | -        | -    | -       | 3.797(1.763–8.179)        | 0.0006557| FAM183A|
| rs626842      | Additive  | -        | -    | -       | 3.659(1.735–7.717)        | 0.0006571| FAM183A|
| rs60192064    | Additive  | -        | -    | -       | 3.606(1.686–7.712)        | 0.0009434| FAM183A|
| rs2453416     | Additive  | -        | -    | -       | 3.606(1.686–7.712)        | 0.0009434| FAM183A|
| rs291083      | Dominant  | A/A      | 14   | 53      | 4.97(2.047–12.06)         | 0.0003956| FCMR  |
| rs167082      | Dominant  | C/C      | 14   | 53      | 4.97(2.047–12.06)         | 0.0003956| FCMR  |
| rs291083      | Additive  | -        | -    | -       | 4.099(1.848–9.093)        | 0.0005207| FCMR  |
| rs167082      | Additive  | -        | -    | -       | 4.099(1.848–9.093)        | 0.0005207| FCMR  |
| rs4645915     | Additive  | -        | -    | -       | 4.586(1.944–10.82)        | 0.0005038| KAT5  |
| rs4244812     | Codominant| A/A      | 10   | 4       | 12.36(2.809–54.38)        | 0.0008799| KAT5  |
| rs4645915     | Dominant  | T/T      | 13   | 50      | 4.523(1.851–11.05)        | 0.0009311| KAT5  |
| rs2274344     | Dominant  | T/T      | 20   | 62      | 6.589(2.35–18.47)         | 0.0003372| MIPEP |
| rs2274344     | Additive  | -        | -    | -       | 6.589(2.35–18.47)         | 0.0003372| MIPEP |
| rs12866705    | Dominant  | T/T      | 21   | 62      | 5.802(2.057–16.36)        | 0.0008895| MIPEP |
| rs12866705    | Additive  | -        | -    | -       | 5.802(2.057–16.36)        | 0.0008895| MIPEP |
| rs17007214    | Dominant  | C/C      | 31   | 35      | 0.1298(0.04112–0.4094)    | 0.0004959| MYF5  |
| rs10862184    | Dominant  | T/T      | 31   | 35      | 0.1298(0.04112–0.4094)    | 0.0004959| MYF5  |
| rs17007214    | Additive  | -        | -    | -       | 0.1456(0.04786–0.4428)    | 0.0006859| MYF5  |
| rs10862184    | Additive  | -        | -    | -       | 0.1456(0.04786–0.4428)    | 0.0006859| MYF5  |
| SNP          | Model     | Genotype | Case | Control | OR (95% CI)          | p value   | Gene  |
|--------------|-----------|----------|------|---------|----------------------|-----------|-------|
| rs2227294    | Codominant| G/G      | 13   | 4       | 15.65(3.737–65.54)   | 0.0001672 | MYL3  |
| rs2227294    | Additive  | -        | -    | -       | 3.548(1.789–7.038)   | 0.0002891 | MYL3  |
| rs2227294    | Recessive | T/T-T/G  | 22   | 65      | 10.24(2.88–36.42)    | 0.0003253 | MYL3  |
| rs875956     | Codominant| C/T      | 15   | 9       | 6.269(2.259–17.39)   | 0.0004228 | PIEZO2|
| rs875956     | Additive  | -        | -    | -       | 3.585(1.749–7.347)   | 0.000487  | PIEZO2|
| rs73943314   | Dominant  | T/T      | 16   | 55      | 4.699(1.929–11.45)   | 0.0006602 | PIEZO2|
| rs9954308    | Dominant  | T/T      | 16   | 55      | 4.699(1.929–11.45)   | 0.0006602 | PIEZO2|
| rs2277858    | Dominant  | T/T      | 15   | 53      | 4.561(1.888–11.02)   | 0.0007457 | PIEZO2|
| rs2291822    | Dominant  | A/A      | 19   | 14      | 0.2147(0.0879–0.5243)| 0.0007319 | TLL1  |
| rs2291822    | Additive  | -        | -    | -       | 0.3165(0.1614–0.6208)| 0.0008169 | TLL1  |
| rs1061495    | Dominant  | T/T      | 20   | 61      | 5.805(2.124–15.86)   | 0.0006064 | TNC   |
| rs1061495    | Dominant  | T/T      | 20   | 61      | 5.805(2.124–15.86)   | 0.0006064 | TNC   |
| rs2274750    | Dominant  | C/C      | 22   | 63      | 6.827(2.23–20.9)     | 0.0007659 | TNC   |
| rs2274750    | Additive  | -        | -    | -       | 6.827(2.23–20.9)     | 0.0007659 | TNC   |
| rs79003972   | Dominant  | C/C      | 22   | 62      | 6.712(2.194–20.54)   | 0.000848  | TNC   |
| rs79003972   | Additive  | -        | -    | -       | 6.712(2.194–20.54)   | 0.000848  | TNC   |
| rs2094794    | Dominant  | C/C      | 22   | 63      | 7.476(2.274–24.57)   | 0.0009218 | TNC   |
| rs2094794    | Additive  | -        | -    | -       | 7.476(2.274–24.57)   | 0.0009218 | TNC   |
| rs58968019   | Additive  | -        | -    | -       | 7.044(2.464–20.14)   | 0.0002694 | UBXN4 |
| rs16831997   | Dominant  | C/C      | 20   | 61      | 5.937(2.142–16.45)   | 0.0006149 | UBXN4 |
| SNP       | Model  | Genotype | Case | Control | OR (95% CI)       | p value    | Gene |
|-----------|--------|----------|------|---------|-------------------|------------|------|
| rs16831997| Additive| -        | -    | -       | 5.937(2.142–16.45) | 0.0006149  | UBXN4|
| rs4851890 | Codominant| T/C     | 24   | 21      | 5.373(2.048–14.1)  | 0.0006357  | VWA3B|
| rs6967385 | Dominant| T/T      | 21   | 19      | 0.2065(0.08322–0.5126) | 0.0006723  | VWDE |
| rs6967385 | Codominant| T/G     | 8    | 37      | 0.1735(0.06266–0.4801) | 0.0007455  | VWDE |

**The value of LIB in predicting outcome for ARDS patients**

To determine the ability of LIB to predict ARDS outcome, analysis was carried out on LIB, P/F ratio, APACHE II score, SOFA score and Murray lung injury score with the area under the ROC curve of 0.6103 ($p = 0.0807$), 0.568 ($p = 0.3124$), 0.6763 ($p = 0.0053$), 0.6204 ($p = 0.1002$), 0.6614 ($p = 0.0581$), respectively. The predicting value of LIB could increase to 0.712 ($p = 0.001$) when combined with APACHE II score.

**Snp/indel Data Between Ards Patients With Different Subphenotypes**

ARDS patients were divided into different subphenotypes (online supplemental material).

Severe ARDS group and non-severe group were divided according to the severity of lung injury. Compared with non-severe group, LIB was lower in severe ARDS group, with the ROC of predictive value of 0.727 ($p < 0.0001$). GO analysis showed that 25 functions were correlated with ARDS severity ($p < 0.01$), and KEGG enrichment analysis showed that these SNP/InDel were in 4 pathways, such as PI3K-Akt signaling pathway, ECM-receptor interaction ($p < 0.05$).

ARDS patients were divided into pulmonary ARDS and extrapulmonary ARDS group. LIB was not significantly altered between the pulmonary and extrapulmonary ARDS. GO analysis showed that 19 functions were correlated with pulmonary and extrapulmonary ARDS ($p < 0.01$), and KEGG enrichment analysis showed that these SNP/InDel were in 8 pathways, such as ECM-receptor interaction ($p < 0.05$).

ARDS patients were divided into ARDS combined with sepsis and ARDS without sepsis on enrollment. Compared with patients without sepsis, the LIB was lower in ARDS combined with sepsis, with the ROC of predictive value of 0.6803 ($p = 0.0084$). GO analysis showed that 24 functions were correlated with ARDS combined with sepsis ($p < 0.01$), and KEGG enrichment analysis showed that these SNP/InDel were in 3 pathways, such as ECM-receptor interaction, Focal adhesion ($p < 0.05$).

ARDS patients were divided into ARDS combined with shock and ARDS without shock on enrollment. Compared with patients without shock, the LIB was lower in ARDS combined with shock, with the ROC of predictive value of 0.6915 ($p = 0.0008$). GO analysis showed that 46 functions were correlated with ARDS combined with shock.
(p < 0.01), and KEGG enrichment analysis showed that these SNP/InDel were in 10 pathways, such as cAMP signaling pathway, ECM-receptor interaction (p < 0.05).

Discussion

In this single-center, prospective trial which enrolled adult ARDS patients, whole exome sequencing was performed to understand the difference between the ICU prognosis of ARDS. The highlight of the study is the integrated framework of genetic variability of ARDS displayed through ARDS survivors and non-survivors. As defined by lung injury burden, the mutational landscape of ARDS showed the overall genetic variability between survivors and non-survivors, while the detailed specific genetic polymorphisms which have an influence on outcome which finally showed genetic factors play a role in the outcome of ARDS.

As the role of genetics in the pathogenesis of ARDS is increasingly recognized, numerous genes and genetic variants were identified to proclaim their association with outcome of ARDS. However, most were single genetic polymorphisms, little studies focus on the whole mutational landscape and its influence on ARDS outcome. To build an integrated framework, we classified different categories of genes, and try to observe their association with the outcome of ARDS, which are genes influencing immune regulation, genes influencing endothelial barrier function, genes influencing respiratory epithelial function, genes influencing coagulation, genes influencing injury and oxidative stress and so forth. However, as multiple different pathogenic processes, all these genes could interrelate.

Tumor mutation burden (TMB) is a marker which calculated as the nonsynonymous mutation number of per MB of DNA in tumor tissue\cite{17}. High TMB often correlates with a higher probability of tumor neoantigens, which could be recognized by lymphocytes \cite{18–19}, so it is hypothesized that the tumors with the highest TMB might be more likely to respond to immune checkpoint blockade therapy. Previous studies showed that patients with high TMB response better to immune checkpoint blockade therapy \cite{20–22} and might have a better outcome \cite{23}. However, little data observed the mutation burden in ARDS, which might make a rough estimate on the whole mutational landscape of ARDS. In this study, we found when combined with clinical characteristics, burden could predict prognosis of ARDS.

We acknowledge some limitations in our study. Firstly, there was no validation group to study the association of functional SNPs with ARDS outcome which found by whole exome sequencing. Secondly, functional studies are needed to evaluate the mechanisms that underlie the associations between all the genetic variants and ARDS outcome and the mediating pathway. Thirdly, there was no healthy control group. In addition, the findings in the study were mainly pertinent to patients in single center who developed ARDS, and should be validated before generalization in cohorts.

Conclusions

Genetic variants are associated with ARDS outcome; however, their prognostic value still need to be verified by larger trials.

Abbreviations
APACHE, acute physiology and chronic health evaluation; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; PEEP, positive end-expiratory pressure; SD, standard deviation; SNP, single nucleotide polymorphism; SOFA, sequential organ failure assessment; TMB, tumor mutation burden.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee (Approval Number: 2015ZDSYLL014.0) of Zhongda Hospital, School of Medicine, Southeast University, and written informed consent was obtained from each patient or their next of kin.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due [Other papers are still preparing] but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions: JYX participated in the design of the study, carried out the analysis and interpretation of data, participated in drafting, editing and submitting the manuscript. ARL, JFX and SSM participated in drafting the manuscript and made substantial contributions to data analysis. ZSW made substantial contributions to data acquisition and statistical analysis. XXQ and CHL carried out the analysis. SQL contributed to the design and coordination of the study. CSY contributed to the design and coordination of the study. LL contributed to the design and coordination of the study. YZH contributed to the design and coordination of the study. FMG contributed to the design and coordination of the study. YY was responsible for conception and design and revising the manuscript for important intellectual content. HBQ was responsible for conception and design and revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Figures
Figure 1

GO analysis showed that the Top 30 of the 60 functions were correlated with ARDS outcome. BP, Biological process; CC, Cellular component; MF, Molecular function.

Figure 2

Genome-wide association study showed that several SNPs potentially associated with ARDS outcome. A. The QQ-plots of the results of whole exome sequencing of ARDS obtained in the analyses. B. Corresponding Manhattan plots for the same analysis on the left panel.

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