Genetic susceptibility in acute lung injury and acute respiratory distress syndrome

Susceptibilidade genética na lesão pulmonar aguda e síndrome da angústia respiratória aguda

INTRODUCTION

Acute lung injury (ALI) and its major expression, acute respiratory distress syndrome (ARDS) are a common denominator for an array of illnesses able to trigger intensive pulmonary inflammatory response. These factors are considered primary when the lung injury starts at the epithelial end and secondary when starts at the alveolar-capillary barrier endothelial end.\(^1,2\) Epidemiological studies have shown considerable variability in ALI/ARDS incidence, with a yearly figures of 78.9 per 100,000 in the United States of America (USA),\(^3\) 13.5 in Scandinavian,\(^4\) 16 in Scotland\(^5\) and 28 in Australia.\(^6\) Nevertheless several risk factors may trigger the syndrome,\(^1,2\) its expression and mortality are very variable.\(^7\) One of the reasons explaining these differences may exactly reside on the impact of different risk factors on the inflammatory response.\(^8\)

In the recent years, several genomic studies have shown an association between critical illness and genetic determinants, leading to a deeper un-
understanding of pathophysiological mechanisms, particularly in sepsis/septic shock and ALI/ARDS. An important issue in ALI and ARDS is why some patients die from uncontrolled inflammation or sepsis, while others recover without major issues. This could be explained, at least partially, by that cellular events involved in inflammation mediation, tissue injury and repair, are controlled at a molecular level, and may not be completely explained without considering the genes and their products participating in this response.\(^{(9)}\) In experimental trials, for instance, it was shown that the genetic profile expression in lung injury is specific for the injury type. In rat models, dos Santos et al.\(^{(10)}\) documented that gene expression in ALI induced by LPS is different from that induced by alveolar hyperdistension. This change occurs early in ventilator induced lung injury (VILI) by high tidal volume, leading to proinflammatory mediators genes expression.\(^{(11)}\) There are evidences that humoral and cellular immune response is subject to polymorphic genetic control, which could explain the diversity of manifestations, outcomes and the risk of being chronic among patients with a same illness. This picture is due to gene polymorphisms, which are gene variations in at least 1% population.\(^{(12)}\)

In ALI/ARDS several candidate genes were associated to susceptibility, clinical course and outcomes. The role of these genes is related to inflammation, immu-

**Chart 1 – Acute lung injury/acute respiratory distress syndrome associated genes**

<table>
<thead>
<tr>
<th>Author</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>ALI/ARDS Association</th>
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</thead>
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<tr>
<td>Gong MN, et al.</td>
<td>TNF-(\alpha)</td>
<td>-308G&gt;A</td>
<td>↑ mortality</td>
</tr>
<tr>
<td></td>
<td>TNF-(\beta)</td>
<td>TNFB12</td>
<td>No mortality effect</td>
</tr>
<tr>
<td>Flores C, et al.</td>
<td>IL-6</td>
<td>six 5' IL-6 SNPs -1363 to +4835</td>
<td>Haplotype GGAAC ↑ susceptibility</td>
</tr>
<tr>
<td>Hildebrand F, et al.</td>
<td>IL-8</td>
<td>-251A&gt;T</td>
<td>↑ MV time</td>
</tr>
<tr>
<td>Gong MN, et al.</td>
<td>IL-10</td>
<td>-1082G&gt;A</td>
<td>Genotype GG ↑ susceptibility &lt; 52 years</td>
</tr>
<tr>
<td>Schroeder O, et al.</td>
<td>IL-10</td>
<td>-1082G&gt;A</td>
<td>↓ susceptibility</td>
</tr>
<tr>
<td>Gong MN, et al.</td>
<td>MBL-2</td>
<td>SNPs at 52, 54, 57 codons</td>
<td>Homozygotes 54BB ↑ susceptibility, severity, DMOS and mortality</td>
</tr>
<tr>
<td>Gao L, et al.</td>
<td>Myosin light chain</td>
<td>51 SNPs</td>
<td>Specific haplotypes gene 5' and 3' in different populations ↑ susceptibility</td>
</tr>
<tr>
<td></td>
<td>kinase (MYLK)</td>
<td></td>
<td>-1001G&gt;T ↑ susceptibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1543T&gt;C</td>
<td>-1543T&gt;C better outcome</td>
</tr>
<tr>
<td>Zhai R, et al.</td>
<td>IκB-(\alpha)</td>
<td>-881A&gt;G, -826C&gt;T,</td>
<td>Haplotype GTC ↑ ARDS risk in Caucasians, particularly male</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-297C&gt;T</td>
<td></td>
</tr>
<tr>
<td>Adamzik M, et al.</td>
<td>NFKB1</td>
<td>I/D -94ATTG</td>
<td>Affects ALI severity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No mortality effect</td>
</tr>
<tr>
<td>Arcaroli JJ, et al.</td>
<td>EC-SOD</td>
<td>rs4691, rs5321, rs5360,</td>
<td>Haplotype GCCT ↓ ALI and mortality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs5955, rs5982</td>
<td></td>
</tr>
<tr>
<td>Arcaroli JJ, et al.</td>
<td>Urokinase</td>
<td>rs2227562, rs2227564,</td>
<td>Haplotype CGCCCC Risk factor ↑ mortality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2227566, rs2227571, rs4065</td>
<td>↑ MV time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ mortality in heterozygotes</td>
</tr>
<tr>
<td>Marshall MP, et al.</td>
<td>ACE</td>
<td>I/D</td>
<td>Genotype II best survival likelihood; allele D not associated with ↑ mortality in Chinese</td>
</tr>
<tr>
<td>Jerng JS, et al.</td>
<td>ACE</td>
<td>I/D</td>
<td>No ARDS susceptibility or mortality association</td>
</tr>
<tr>
<td>Villar J, et al.</td>
<td>ACE</td>
<td>I/D</td>
<td>Genotype DD ↑ risk of death</td>
</tr>
<tr>
<td>Adamzik M, et al.</td>
<td>ACE</td>
<td>I/D</td>
<td>AGT -6A&gt;G no susceptibility of mortality change</td>
</tr>
<tr>
<td>Medford AR, et al.</td>
<td>VEGF</td>
<td>936C&gt;T</td>
<td>Genotypes CT and TT more frequently associated with ARDS risk of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ ARDS susceptibility</td>
</tr>
<tr>
<td>Gong MN, et al.</td>
<td>SFTPB</td>
<td>SNP no intron 4</td>
<td>↑ susceptibility in women</td>
</tr>
<tr>
<td>Lin Z, et al.</td>
<td>SP-A1, SP-A2, SP-B, SP-D</td>
<td>SP-B Thr131le</td>
<td>↑ primary ALI in women</td>
</tr>
<tr>
<td>Frerking I, et al.</td>
<td>CC16</td>
<td>-26G&gt;A</td>
<td>No susceptibility of outcome effect</td>
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</table>

ALI = acute lung injury; ARDS = acute respiratory distress syndrome.
ne response, vascular permeability, vascular tonus, repair, chemotaxis, cell motility and coagulation (Chart 1). From a practical standpoint, the intensivist wants to know if a genetic marker may identify some clues, such as which patients would be more susceptible to develop ALI/ARDS, which patients could have a better response to a given treatment, and individual patients’ prognosis.(7)

Considering that ALI/ARDS is consequence of another illnesses, has high mortality, involve long recovery and considerable costs, this study aims to review the main genes and polymorphic variations associated with ALI/ARDS incidence, morbidity and mortality.

This literature review was based on PubMed (National Library of Medicine and National Institute of Health – USA), LilACS and relevant references mentioned on articles extracted from the research. The search was made using the keywords acute lung injury, acute respiratory distress syndrome, adult acute respiratory distress syndrome, combined with genetic polymorphisms considering only adult patients. Sixty nine articles were found, and after excluding editorials, reviews and genetic polymorphism in other diseases, the 38 remainder studies were included in the review. Were also considered the relevant articles extracted from the references in the database selected articles.

STUDIES OF CANDIDATE GENES AND ALI/ARDS ASSOCIATION

As ALI/ARDS are not based on a single etiology, genomic association studies in this field is a challenge task. The main objectives of identifying genetic markers in this syndrome is the determination of the susceptibility, prognosis and patients who could benefit from a given treatment.(7) The conclusion of human genome sequencing, listed 1.4 million single nucleotide polymorphisms (SNP), most of the variations occurring in genome regions not encoding protein products.(13) A SNP is a DNA change which represents the variation in one single base and is used to describe inter-individuals genetic variation.(7) When these SNPs occur in protein encoding regions, they may affect the effectiveness of this protein or gene function.(14)

The study of a gene expression or function by its phenotype analysis is useful in genetic diseases with classical Mendelian inheritance. This method is not suitable in ALI/ARDS, as multiple interactions of different genes and countless risk factors interact. Due to this difficulty, candidate genes case-control studies are the most common approach in ALI/ARDS susceptibility related factors. In these studies, a genetic variation has its genotype determined in a population where phenotypic information is available (ALI/ARDS). If a correlation between the study genotype and phenotype exists, it is assumed that there is an association between the genetic variation and the illness.(7)

Another way to identify candidate genes is by the microarrays technique. This technique determines the degree of a given gene expression by measuring mRNA (messenger ribonucleic acid). In summary, it consists in preparing a recipient for the genes of interest DNA with (1) isolation from a tissue cells mRNA; (2) cDNA generation by reverse transcription; (3) cDNA hybridization with the array DNA; (4) generation of array images by laser assisted optical digitalization.(15) However, as ALI/ARDS is a complex condition, specific genes changes probably do not fully explain all physiological derangements. The wide phenotypic range, incomplete penetrancy, interactions from genetics and environment and, potential for loci heterogeneity may difficult the genetic evaluation this syndrome.(16) Additionally, there is no possibility of isolating the cells from the affected tissue during the ALI/ARDS to be informative in the microarrays study. The main genetic polymorphisms associated with ALI/ARDS are shown in chart 1, and a glossary of currently used terms in shown in chart 2.

GENES RELATED TO INFLAMMATION, IMMUNE RESPONSE, OXIDATIVE STRESS AND COAGULATION

Cytokines have a fundamental role in local and systemic inflammatory response secondary to infection or inflammatory condition. The tumoral necrosis factor α (TNF-α) is a key cytokine in the inflammatory cascade, and one of the main mediators for sepsis and ALI/ARDS. The gene encoding TNF-α is at the chromosome 6, near to other genes encoding other cytokines, with an essential role for the cell cycle.(12) The polymorphism -308G>A in the TNF-α promoter gene, and TNFB1/2 in the TNF-β gene are associated with increased TNF-α synthesis.(17) In a case-control study with 441 Caucasian controls and 212 cases admitted with ALI/ARDS risk factors, the authors identified an association between the allele -308A and 60 days mortality, being this association more distinct in patients below 67 years-old. The TNFB polymorphism did not show
association with mortality.\textsuperscript{(17)}

The association between interleukin (IL)-6, a potent proinflammatory cytokine and ALI/ARDS shows conflicting results, at least regarding the SNP -174G>C. Although some authors have found an association between IL-6 gene with ALI/ARDS susceptibility,\textsuperscript{(18)} this was not confirmed by others.\textsuperscript{(19)} However, studying 20 SNPs in IL-6 gene, Flores et al\textsuperscript{(19)} found an association between the haplotype GGAAC and ALI/ARDS occurrence.

Also, IL-8 is one important mediator in ALI/ARDS pathogenesis. In ARDS patients’ bronchoalveolar lavage (BAL), IL-8 levels are significantly increased and show association with ARDS development in the population at risk.\textsuperscript{(20)} In studies of the association between IL-8 gene SNP -251A>T and ALI/ARDS, those bearing the -251A allele had more IL-8 synthesis and patients with the genotype -251AA needed mechanic ventilation (MV) for a relatively longer time.\textsuperscript{(21)}

The SNP -1082GG genotype, in the IL-10 promoter gene is associated with reduced acute respiratory failure risk in trauma patients.\textsuperscript{(22)} In ARDS patients, the genotype -1082GG is associated with lower admission severity, lower organ dysfunction degree and mortality.\textsuperscript{(23)}

MBL (Mannose Binding Lectin) is a standard recognition protein, important in complement system and phagocytosis inducing opsonins activation.\textsuperscript{(24)} The MBL protein is encoded by the MBL-2 gene (mannose binding lectin-2), at the chromosome 10. The circulating MBL
levels depend on three SNPs on codons 52 (rs5030737), 54 (rs1800450) and 57 (rs1800451, on the exon 1 and one SNP in the -221 position (MBLXY; rs7096202). A case-control study evaluated 212 Caucasian ARDS patients and 442 controls, hypothesizing that the X allele of MBLXY polymorphism and the MBL2 gene codons 52, 54 and 57 variants D, B and C are associated with increased ARDS susceptibility and mortality. Homozygote for 54B allele variation (54BB) had increased admission severity, increased septic shock and ARDS likelihood compared with heterozygote and wild allele homozygote. The association with ARDS was specially marked on septic shock patients. In ARDS patients, the genotype 54BB was associated with increased number of organ dysfunctions and mortality.

Among the macrophage produced mediators, the macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine with important role on endotoxemic response. In 506 sepsis subjects DNA samples, both with sepsis-induced ALI and healthy controls, there was no correlation between individual SNPs and sepsis or ALI. An association was found between the haplotypes located in the MIF gene 3’ region and sepsis and ALI susceptibility.

The pre-B-cell colony-enhancing factor (PBEF), also known as visfatin, is an originally isolated from lymphocytes isolated cytokine. PBEF expression is increased in human ALI animal models. The genetics role for ARDS development was studied in 787 at risk and 375 established syndrome patients. The PBEF gene -1001T>G polymorphic variation was associated with increased susceptibility and mortality risk, while the -1543C>T polymorphism was associated with better outcomes.

The κ-B nuclear factor (NF-κB) has an important role in inflammatory response, regulating several genes and inflammation mediators’ expression, as adhesion molecules and cytokines. In non-stimulated cells, NF-κ-B is found in its inactive form in cytoplasm, I-κB (NFKBIA). When stimulated, NFKBIA is rapidly degraded and translocated to the nucleus, aiming to activate the target genes expression. The GTC haplotype of NFKBIA gene is associated with increased ARDS risk in Caucasians, particularly in male and primary lung injury patients. An insertion/deletion polymorphism (ID) in the NFKB1 gene affects ARDS severity without influencing mortality.

Oxygen free radicals (O₂FR) are produced in ischemia-reperfusion conditions. For protecting from the harmful effects these molecules, cells produce antioxidants which, by interacting with O₂FR generate less active products. Superoxide dismutase (SOD) is one of the cell protecting antioxidant enzymes. Extra-cellular (EC) SOD is largely expressed in the lungs, playing an important role in inflammation and oxidative stress regulation. In humans, the SOD gene is at the chromosome 4, and a SNP occurs in an encoder region resulting from an Arginine replacement for a Glycine. The end-product is a 10-fold increase in EC-SOD levels.

In addition to this change, at least three other were already identified as having important effects on ALI patients phenotype: in a 157 Caucasian patients population, those with GCCT haplotype had reduced lung inflammation, MV time and mortality.

Urokinase is a serine protease acting on plasminogen activation to form plasmin, a potent fibrinolytic mediator. The urokinase gene is at the chromosome 10. In an association study, 98% of this gene haplotype were analyzed. The CGCCCC haplotype showed association with 60 days mortality and MV-free days, being a risk factor for unfavorable outcomes.

The C protein pathway prevents exaggerated blood coagulation cascade activation, protecting against inadvertent clot formation by Va and VIIIa factors inactivation. Leiden factor V SNP changes this natural anticoagulation pathway normal activity to produce factor Va resistant to activated C protein inactivation. In ARDS patients, heterozygote subjects (Arg/Gln; with the Gln residue encoding allele) for the Leiden factor V genotype have improved 30 days survival than homozygotes (Arg/Arg).

GENES RELATED TO VASCULAR PERMEABILITY AND TONUS, FIBROBLASTS ACTIVITY AND SURFACE TENSION

Since ALI/ARDS involve alterations in permeability, vascular tonus, lung injury and repair and, in the ability to keep alveoli open, characterizing a severe respiratory failure picture, the genes involved in this process have an important role. The angiotensin converting enzyme (ACE) is a zinc dependent metalo-peptidase which main role is converting angiotensin-I to angiotensin-II, and bradykinin inactivation. The ACE gene is at the 17q23 locus and has 26 exons. This gene
best known polymorphism consists in insertion (allele I) or deletion (allele D) of an Alu sequence of 287 pairs of bases near to intron 16 extremity 3'. This polymorphic variation produces three possible genotypes: II, ID and DD.\(^{(40)}\) Three studies showed an association between these polymorphisms and ARDS mortality, two in Caucasian patients\(^{(41,42)}\) and another in Oriental patients.\(^{(43)}\) Recently a study in Spain failed to show an association between these polymorphisms and ARDS susceptibility or mortality,\(^{(44)}\) a finding which was confirmed by our group.\(^{(40)}\) Another polymorphism in the angiotensinogen gene promoter region (AGT) (-6) A/G, which is directly related to ACE action, did not show an association with susceptibility or mortality.\(^{(42)}\)

The vascular endothelial growth factor (VEGF) is a potent vasodilator.\(^{(40)}\) VEGF has a 936C>T polymorphism which was studied to determine its participation in genetic ARDS susceptibility. An association study prospectively evaluated 137 normal subjects and 220 MV patients. MV patients were grouped as: ARDS risk (n=103) and ARDS (n=112), and five were excluded from the analysis. The genotypes 936CT and 936TT were significantly more frequent in ARDS patients than in the normal group (P=0.02) and ARDS risk (P=0.03). The 936T allele was more frequent in the ARDS group (P=0.04) compared to the other groups. There was no mortality difference between the groups ARDS risk and ARDS established, however ARDS patients with genotype 936CT and 936TT had a higher Acute Physiologic Chronic Health Evaluation III (APACHE III) than 936CC homozygote patients (P<0.05). These findings suggest that exist an association between 936T allele with ARDS and physiologic disorders, as evidenced by the APACHE III score.\(^{(45)}\)

The surfactant protein B (SP-B) is a hydrophobic protein with properties which are crucial for surfactant function. The SP-B encoding gene is at the chromosome 2 short arm, and has about 9,500 base pairs (bp).\(^{(46)}\) A polymorphism in this gene intron 4 is associated with ARDS susceptibility.\(^{(47)}\) In an ARDS risk population after stratification for gender and adjustments for confounding factors, the SP-B polymorphic variation has shown an association with primary ARDS susceptibility in women, but not in men.\(^{(46)}\)

**CHEMOTAXIS AND CELL MOTILITY RELATED GENES**

Clara cell protein 16 (CC16) is a potent neutrophil chemotaxis and phospholipase A\(_2\) activity inhibitor. An association study with 117 Caucasian ARDS patients and 373 healthy controls failed to show a relationship between the -26G>A polymorphism and ARDS susceptibility.\(^{(48)}\)

The Myosin Light Chain Kinase (MYLK) gene importance is related to its participation in the leucocytes apoptosis and diapedesis, two ALI/ARDS essential events.\(^{(49)}\) In an association study with a Caucasian subjects group, and another group with Afro-Americans, Gao et al.\(^{(50)}\) identified risk for sepsis-induced ALI/ARDS in specific region 5’ haplotypes patients for both groups, and on region 3’ only for Afro-Americans. ALI following trauma is also influenced by polymorphisms in this gene, particularly among Afro-Americans.\(^{(49)}\)

**THE ROLE OF GENOMICS IN CLINICAL PRACTICE**

The use of association studies information, as every new method, should be careful. For the physician, the initial results of these studies should be confirmed in similar populations by different investigators.\(^{(51)}\) Sometimes a given SNP may not show an association, but this should not preclude its disease involvement, as the haplotype based approach with multiple genetic markers may identify an association.\(^{(52)}\) The ALI/ARDS genetic epidemiology objectives are to identify genes influencing susceptibility, lung inflammation intensity, need of specific treatments, prognosis and mortality.

To value genetic association studies, a fundamentally important aspect is their quality. This can be evaluated considering: (1) sample size; (2) a suitable control group; (3) Hardy-Weinberg equilibrium; (4) well defined cases for study disease; (5) the sequencing primer should be reproducible; (6) the genetic analysis investigator should not know the subject clinical status (blinding); (7) the study should have statistical power; (8) genotyping should be replicated.\(^{(53)}\)

**FINAL COMMENTS**

The use of genetics for identifying ALI/ARDS risk populations in a different way opens a new field in medicine. The search for improvement of care in the critically ill patients initially involves the identification of candidate genes, which can be done either in experimental or clinic scenarios. The second step is the development of robust and high quality and reproducible association studies.
Care strategies can be established based on such information, since early identification of risk populations until choosing of individualized therapies.

**RESUMO**

A lesão pulmonar aguda e sua forma mais grave, a síndrome da angústia respiratória aguda, são o denominador comum de várias doenças que podem provocar uma inflamação exagerada nos pulmões. Nos últimos anos, essa variabilidade tem sido atribuída, pelo menos em parte, a fatores genéticos. O presente estudo tem por objetivos revisar o papel dos principais genes envolvidos na suscetibilidade, morbidade e mortalidade na lesão pulmonar aguda e na síndrome da angústia respiratória aguda. Através de pesquisa nas bases de dados PubMed e LiLACS, empregando-se os unitermos lesão pulmonar aguda, síndrome da angústia respiratória aguda e síndrome da angústia respiratória aguda/síndrome da angústia respiratória aguda, os polimorfismos genéticos são variantes gênicas presentes em pelo menos 1% da população. A presença destas variantes genéticas pode influenciar a expressão de mediadores da resposta inflamatória, afetando diretamente a suscetibilidade à lesão pulmonar aguda, a intensidade da inflamação no parênquima pulmonar, a evolução e o desfecho destes pacientes. Estudos de associação com grandes populações e passíveis de reprodução permitirão de modo definitivo a inclusão da genômica no arsenal diagnóstico, prognóstico e terapêutico de pacientes com lesão pulmonar aguda/síndrome da angústia respiratória aguda.

**Descritores:** Lesão pulmonar; Síndrome da angústia respiratória do adulto; Polimorfismo genético

**REFERENCES**


