

# CRITICAL CARE ROUNDS™

March 2002  
Volume 3, Issue 3

AS PRESENTED IN THE GRAND ROUNDS OF  
PARTICIPATING HOSPITALS ACROSS CANADA

## Genetic predictors of adverse outcome from sepsis, ARDS and SIRS

BY KEITH R. WALLEY, M.D. AND JAMES A. RUSSELL, M.D.

An increasing number of articles have been appearing in the critical care literature describing associations between genetic polymorphisms and adverse outcomes in acute systemic inflammatory states, ie, sepsis and septic shock, acute respiratory distress syndrome (ARDS), and the associated systemic inflammatory response syndrome (SIRS). These exciting new discoveries are due in part to the completion of the human genome project and associated databases that provide the raw genetic information needed to identify genetic variation between individuals. They are also due to improvements in technology that allow easy measurement of genetic polymorphisms, enabling the search for associations between genetic variations and important biologic effects.

This issue of *Critical Care Rounds* will discuss:

- why genetics may play a role in sepsis, ARDS, and SIRS
- what a genetic polymorphism is
- why a number of investigators have chosen association study designs when investigating the role of genetics in sepsis, ARDS, and SIRS
- some of the weaknesses of the association study design and how to address them
- how one might choose candidate genes to study using an association study design and which candidate genes already show promise
- why haplotypes and haplotype clades may be a useful next step.

### Role of genotype in sepsis, ARDS, and SIRS

Genotype has been shown to contribute substantially to the incidence and outcome of inflammatory and infectious diseases. In an early and very important study, Sorensen and colleagues<sup>1</sup> studied the outcome of adopted children when the cause of death of the biologic parent was known. To provide a comparison, they found that adopted children with a biologic parent dying of cancer before age 50 had a relative risk of 1.2 of dying of cancer themselves, confirming that there is a genetic contribution to cancer risk. Surprisingly, they also found that adopted children with a biologic parent dying of infectious disease before age 50 had a relative risk of 5.8 of themselves dying of an infectious disease. This genetic contribution to risk exceeded even that of cardiovascular disease. This study highlighted early on the potentially important contribution that genetic background makes to the outcome of infectious disease.

Further support for this conclusion comes from twin studies that show greater concordance in monozygotic twins than in dizygotic twins for tuberculosis, leprosy, poliomyelitis, and hepatitis B.<sup>2</sup> Since infection itself is not a genetic disease, this means that genetic variation in a patient's inflammatory response leads to significant differences in outcome. Since sepsis, ARDS, and SIRS represent expressions of intense inflammatory responses, it is reasonable to postulate that genotype is important.



### The Canadian Critical Care Society (CCCS) — Editorial Board

Paul J. E. Boiteau, M.D.,  
*President, CCCS*  
University of Calgary

Deborah Cook, MD, *President, CCCTG*  
McMaster University

John Granton, MD, *Editor*  
University of Toronto

Mark Heule, MD,  
University of Alberta

Daren Heyland, MD  
Queen's University

Jacques Lacroix, MD  
University of Montreal

Claudio Martin, MD  
University of Western Ontario

Graeme Rocker, MD  
Dalhousie University

Dean Sandham, MD, *President,*  
*Canadian Intensive Care Foundation*  
University of Calgary

Sam Shemie, MD  
University of Toronto

### The Canadian Critical Care Society

*Correspondence:*  
John Granton, MD  
The Toronto General Hospital  
10 EN-220  
200 Elizabeth Street  
Toronto, ON. M5G 2C4  
Fax: 416-340-3359

The editorial content of *Critical Care Rounds* is determined solely by the Canadian Critical Care Society.

Using genotype to predict adverse outcome in sepsis, ARDS, and SIRS may play an important role in developing new therapeutic strategies. For example, over the past 15 years, a large number of randomized controlled trials (RCT) of anti-cytokine and anti-inflammatory therapies for sepsis have failed.<sup>3</sup> However, in most of these studies, post-hoc analyses demonstrated that the patients who were most severely ill may have derived some benefit from the RCT therapeutic strategy. Thus, these therapeutic strategies (which have not gone on to enter our clinical practice), may well be beneficial if we could simply identify in advance which patients will progress to adverse outcome. Genotype provides a potential strategy for identifying patients at risk of adverse outcome.

It is not hard to imagine that future therapeutic strategies will involve rapid genotyping of patients early in the course of sepsis, ARDS, or SIRS or, better yet, when patients present with risk factors (such as major high risk surgery) before the development of these inflammatory states. A patient with a genotype that predicts an adverse outcome and a beneficial response to immunomodulator therapy could then be treated at this early stage. Thus, it is likely that therapeutic strategies will become increasingly specific, based on individual genotypes.

## Polymorphisms

Sequencing of the human genome has disclosed that there is very little difference from one person to the next in terms of their genetic code, only about 0.1%. The most common genetic variation (polymorphism) between individuals is a difference in a single nucleotide base pair. This is called a single nucleotide polymorphism (SNP). In a population, the most common nucleotide variant is called the common allele and the alternative nucleotide variant is called the rare allele. Frequently occurring SNPs (ie, those SNPs whose rare allele frequency is greater than 10%), occur approximately every 500 to 1000 base pairs.<sup>4</sup> SNPs found within protein coding regions of a gene occur with the least frequency, presumably because SNPs that alter protein sequence likely alter function and therefore would be selected against.<sup>5</sup> In contrast, SNPs within non-coding introns occur much more frequently, probably because these SNPs either have no, or very minimal, biologic effect so that they are not selected against. SNPs within promoter regions of genes occur with an intermediate frequency. Promoter SNPs do not alter the protein structure, but they may alter the rate of transcription of mRNA and therefore, the degree of protein expression and biologic effect. These SNPs may be selected against.

Many studies investigating the role of genotype in inflammatory disease have focused on SNPs. Of course, other forms of genetic variation have also been observed and have played similarly important roles in the discovery of relationships between specific genotypes and phenotypes. For example, the insertion or deletion of one, several, or many base pairs is another fairly common genetic variant. Multiple repeated segments of DNA that

vary in the number of repeats from one individual to another (microsatellites are 2, 3, or 4 base pair repeats, variable number of tandem repeats (VNTR) are repeats of longer base pair sequences) are also very common, particularly in inter-genomic regions. Microsatellites have been used successfully in whole genome screens to identify chromosomal regions containing disease-causing mutations. On a larger scale, alterations in the genome that are detectable using karyotyping (eg, trisomy 21, translocations of one chromosome arm to another) have marked biologic effects, but this is not the topic of this review.

## Association study design

Investigators have taken a number of approaches in searching for polymorphisms that lead to adverse outcomes. A classical approach in Mendelian disorders (disorders caused by rare recessive mutations whose effect can be predicted by classical Mendelian genetics, eg, cystic fibrosis) is linkage analysis. In this approach, genetic markers (eg, microsatellites) spaced throughout the entire genome are genotyped in patients and the families of patients with these disorders. When one of these markers is found to be closely associated with the presence of the disorder, the hunt for the causal mutation focuses on the region of the genome surrounding the associated marker. Unfortunately, this approach has not worked for complex diseases. Affected sib-pair analysis has identified loci linked to complex genetic disorders such as diabetes, asthma, and schizophrenia, yet this approach has had limited success in specifically identifying causal genes or polymorphisms. In complex diseases, this linkage analysis approach has only been successful in identifying two risk factors thus far.<sup>6,7</sup>

These traditional study designs often fail in complex disorders when there is a significant gene-environment interaction. This is particularly true in diseases such as sepsis, ARDS, and SIRS, that require an inciting environmental stimulus (such as infection). In this instance, a typical family design would fail because siblings, parents, and grandparents may not have been exposed to infection in the same way, so that the adverse-outcome phenotype may not have had a chance to be expressed.

An alternative approach to identifying polymorphisms that predict adverse outcome is the association study design. Using this study design, candidate genes and polymorphisms are chosen prior to the study based on *a priori* information that implicates the gene in the pathophysiology of the adverse disease phenotype. For example, TNA- $\alpha$  plays an important role in sepsis, ARDS, and SIRS. Therefore, some of the earliest association studies carried out in sepsis patients have focussed on polymorphisms in the TNA- $\alpha$  gene.<sup>8-14</sup>

Using an association study design, polymorphism genotype and phenotype (eg, clinical outcome or cytokine expression) are measured in large populations. Statistical analysis then looks for a significant association between the presence of a specific genetic variant and an adverse outcome. One important feature of association studies is

that they make no assumptions regarding mode of inheritance, penetrance, allele frequencies, etc. These studies are often easier to perform since they do not require the inclusion of family members, but simply look at control and affected populations. Association studies are particularly helpful in diseases that depend heavily on gene-environment interactions for adverse phenotype expression.

### Problems with association studies and ways to address them

A major limitation of association studies is that they are frequently confounded by type I error. That is, an association is identified when in fact there is no true relationship. A second problem is that association between a polymorphism and adverse outcome could occur if the polymorphism caused the adverse outcome, or it could simply be due to linkage between the polymorphism under study and the true causal polymorphism. Association studies do not identify causality.

Association studies are quite sensitive to population admixture that may lead to a spurious false-positive result. For example, both a disease and a particular SNP allele may occur more frequently in one racial group, even though there is no true relationship between the disease and the SNP. If this racial group is admixed with another population in an association study, then a spurious association may arise. One way to address this issue is to conduct a transmission disequilibrium test (TDT).<sup>15,16</sup> This test is based on the observation that heterozygous parents are equally likely to transmit either allele of a polymorphism to their children. Therefore, affected individuals who have heterozygous parents for the polymorphic site in question should be equally likely to have either allele. If these affected individuals have a preponderance of one allele, then this allele is likely associated with the disease. This study design is independent of the effects of population admixture of racial groups. Therefore, the TDT has become an important additional tool to verify the findings of association studies. The TDT has been extended substantially over the past few years.<sup>17-21</sup> For example, when parents are not available for study, genotyping of siblings is used to constrain the probability of the parental genotype so that a TDT-based test statistic can still be derived.<sup>17,19,20</sup>

There are several important approaches for addressing potential errors of association studies. Large population sizes are important. Long and Langley<sup>22</sup> performed an extensive simulation study to determine the power of association-based studies when a dense, but not exhaustive, set of SNPs is available over a candidate gene region. A hidden polymorphism was assumed to contribute to the variance of a quantitative trait, and the number of measured nearby SNPs was varied in an estimation of the number of subjects necessary to detect the causative locus. Using assumptions based on these models, it is evident that a sample size of 500 or more is required to detect association with 80% power when 10% or less of the variance in the trait is explained by the locus.

Cardon and Bell<sup>23</sup> have reviewed association study designs for complex diseases. The common problems with association studies that they identified are summarized in Table 1. All of these issues can contribute to type I error, identifying spurious associations. Their first very important recommendation for eliminating potential spurious associations is replication of the results.<sup>23</sup>

### Candidate genes that already show promise

A number of polymorphisms have been reported to contribute to the inflammatory response and SIRS.

#### *Pro-inflammatory mediator genes*

**TNF- $\alpha$ :** A TNF- $\alpha$  single nucleotide polymorphism (SNP) exists at position -308 in the promoter region of the TNF- $\alpha$  gene (G is the common allele and A is the uncommon allele). TNF- $\alpha$ -308A increases gene expression<sup>24,25</sup> in vitro<sup>26</sup> and in vivo.<sup>27</sup> The TNF- $\alpha$ -308A allele is associated with adverse outcome in a variety of infectious and inflammatory diseases, including cerebral malaria,<sup>28</sup> meningococcal disease,<sup>8</sup> celiac disease, and septic shock.<sup>9</sup> This polymorphism may also play a role in acute lung injury<sup>14</sup> leading to ARDS. However, the relevance of the TNF- $\alpha$ -308A allele for exaggerated TNF- $\alpha$  gene expression has not been uniformly supported.<sup>11,29</sup>

**TNF- $\beta$  (lymphotoxin):** The A allele of a SNP within the first intron of the TNF- $\beta$  (lymphotoxin) gene<sup>30</sup> is associated with higher circulating TNF- $\alpha$  concentrations, increased organ failure scores, and increased mortality in patients with severe sepsis<sup>10,11</sup> and in initially uninfected trauma patients.<sup>12</sup> This polymorphism or one in linkage disequilibrium may contribute to adverse outcome in acute lung injury.<sup>14</sup> The TNF- $\beta$ -252A allele is common, with a frequency of 65%-68% in an adult ICU patient population.<sup>10,31</sup>

**IL-6:** A G/C polymorphism at -174 in the promoter region of the IL-6 gene has been reported.<sup>32</sup> The frequency of the IL-6-174C allele was 40% in the general population and was decreased in patients with the inflammatory

**Table 1: Common problems with association studies**  
(Modified from reference 23)

- Small sample size
- Population admixture and lack of Hardy Weinberg equilibrium
- Multiple testing of subgroups
- Poorly matched control group
- No study replication
- Failure to detect linkage disequilibrium with adjacent loci
- Data over-interpretation and publication bias
- Post-hoc and unwarranted "candidate gene" declaration after identifying association in an arbitrary genetic region

disease, juvenile rheumatoid arthritis.<sup>32</sup> Transfection of the IL-6-174C allele into HeLa cells in vitro resulted in reduced production of IL-6. In healthy subjects, those with the IL-6-174C allele had significantly lower plasma concentrations of IL-6.<sup>32</sup> Other polymorphisms in the promoter region of the IL-6 gene may influence IL-6 transcription, with complex interactions determined by the haplotype.<sup>33</sup>

**Anti-inflammatory mediator genes**

**IL-1ra:** A polymorphic region within intron 2 of the IL-1ra gene contains a variable number of 86 bp tandem repeats (6 alleles). The IL-1ra A2 allele is associated with increased IL-1ra production and

reduced IL-1 $\alpha$  production by monocytes.<sup>34</sup> The IL-1ra A2 allele occurs with increased frequency in patients who have severe sepsis.<sup>35,36</sup>

**Coagulant factor genes**

**Protein C:** Three polymorphisms have been identified in the promoter region of the protein C gene (Protein C -1654 C/T, -1641 A/G, and -1476 A/T)<sup>37,38</sup> of which the haplotype of -1654C / -1641G reduces the rate of transcription of the protein C gene.<sup>39</sup> This potentially adverse haplotype has a prevalence of 35%-39% in Caucasian populations.<sup>39</sup> Plasma protein C concentrations are reduced by 22% in patients homozygous for the -1654C /-1641G

**Figure 1: Relationships between haplotypes.** The top panel illustrates TNF- $\alpha$  haplotypes in a format similar to Patil et al.<sup>41</sup> Each row represents an observed haplotype in a mixed Caucasian and African-American population. Each column represents a SNP. For example, the TNF- $\alpha$ -308 G/A SNP is the second column. Red-colored boxes represent the common allele of that SNP, while gray-colored boxes represent the rare allele. These haplotypes were inferred from genotypic data in Nickerson's NIH GenBank submission<sup>44</sup> using the program PHASE. It can be seen, for example, that TNF- $\alpha$ -308A is an older mutation because it has subsequently undergone further mutation to result in a total of 4 observed haplotypes (haplotype #s 7-10). In the bottom panel, the evolutionary relationships between these haplotypes was then inferred using the program MEGA2 to produce an evolutionary tree structure. The number of times that this haplotype was observed (total of 36 individuals with sufficient data in this analysis) is shown under (n) to the right of this figure. The TNF- $\alpha$ -308A mutation is identified in this evolutionary tree for illustrative purposes, and identifies a related family of haplotypes (a clade). For example, in future studies of haplotypes of the TNF- $\alpha$  gene, if all members of this clade are associated with a specific phenotype (eg, adverse outcome), while all other haplotypes are not associated with this specific phenotype, then TNF- $\alpha$ -308A may be a causal allele. If the relationship is not this clear then an alternative explanation is that TNF- $\alpha$ -308A is linked (in linkage disequilibrium) to the causal allele.



haplotype. The haplotype occurs more frequently in patients with venous thrombosis.<sup>37,39</sup>

**PAI-1:** An insertion/deletion polymorphism of a single base pair within the promoter region of the PAI-1 gene has been linked to outcome in trauma patients.<sup>40</sup> The PAI-1 4G allele was associated with an increase in PAI-1 concentrations and a greater increase in TNF- $\alpha$  and IL-1 following severe trauma, as well as a reduction in survival by half in heterozygotes and a further reduction by half in homozygotes. This high-risk allele has a frequency of 63% in a Caucasian population.

### Haplotypes and haplotype clades may be useful

Each gene and each contiguous segment of genomic DNA contains multiple SNPs. Within a gene (which is typically in the range of 10 kilo bases long), there is generally a close correlation between the alleles at one SNP site and alleles at another nearby SNP site. Thus, SNPs are not transmitted through the population one at a time, independent of their neighbours, but rather, are transmitted as sets of SNP alleles called haplotypes. Patil et al recently demonstrated that linked sets of SNP alleles often extend about 10 kilo bases,<sup>41</sup> or about the length of a typical gene. Therefore, one approach is to consider haplotypes as the fundamental polymorphic unit when examining genetic associations with adverse outcome. There is generally more information in haplotypes than in SNP analysis because haplotypes can be surrogates or markers for potentially important unidentified polymorphisms. Furthermore, there are generally only two alleles for each SNP, and yet there are multiple different haplotypes for each gene. Thus, it is much more likely that an individual will be heterozygous for haplotypes than heterozygous for SNP. This is extremely helpful when conducting a TDT analysis because almost all parents will be heterozygous for the gene haplotype so that the statistical test of association between haplotype and adverse outcome can be determined with greater power from a smaller population.

In addition, by examining the haplotypes present in a population, clear relationships between haplotypes can be seen to exist (Figure 1). In fact, the haplotypes can be positioned in an evolutionary tree. This process is called cladistic analysis.<sup>42,43</sup> Older, more prevalent SNPs are located at main branch points in the evolutionary tree, while younger mutations that are rare are located at the tips of the tree. Cladistic analysis may help in identifying causal SNPs. For example, if an adverse phenotype is associated with all haplotypes in a clade, the SNP located at the main branch point for the entire clade is a good candidate for the causal SNP.

### Summary

There is recent and ongoing enthusiasm for identifying genetic polymorphisms associated with adverse outcome in sepsis, ARDS, and SIRS. Identifying polymorphisms that portend adverse outcome is likely to be useful in identifying sub-populations of patients who will benefit from individualized immunomodulation and other therapy. It is likely that in the near future we will hear more about inflammatory gene haplotypes and haplotype clades associated with adverse outcome in sepsis, ARDS, and SIRS. This will not only help in individualizing patient therapy, but when causal SNPs are identified, will also help in identifying new targets for therapeutic intervention.

---

**Keith R. Walley, M.D. and James A. Russell, M.D.** are affiliated with the University of British Columbia McDonald Research Laboratories/The iCAPTURE Centre, supported by the Canadian Institutes of Health Research, Vancouver, British Columbia.

---

### References

1. Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. *N Engl J Med* 1988;318(12):727-732.
2. Cooke GS, Hill AVS. Genetics of susceptibility to human infectious disease. *Nat Rev Gen* 2001;2:967-977.
3. Marshall JC. Clinical trials of mediator-directed therapy in sepsis: what have we learned? *Intensive Care Med* 2000;26(Suppl 1):S75-S83.
4. Kruglyak L, Nickerson DA. Variation is the spice of life. *Nat Genet* 2001; 27(3):234-236.
5. Stephens JC, Schneider JA, Tanguay DA, et al. Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 2001;293(5529):489-493.
6. Horikawa Y, Oda N, Cox NJ, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 2000; 26(2):163-175.
7. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; 411(6837):599-603.
8. Nadel S, Newport MJ, Booy R, Levin M. Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. *J Infect Dis* 1996;174(4):878-880.
9. Mira JP, Cariou A, Grall F, et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study [see comments]. *JAMA* 1999;282(6):561-568.
10. Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-alpha concentrations and outcome of patients with severe sepsis [see comments]. *Crit Care Med* 1996;24(3):381-384.
11. Stuber F, Udalova IA, Book M, et al. -308 Tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. *J Inflamm* 1996;46(1):42-50.
12. Majetschak M, Flohe S, Obertacke U, et al. Relation of a TNF gene polymorphism to severe sepsis in trauma patients. *Ann Surg* 1999; 230(2):207-214.
13. Tang GJ, Huang SL, Yien HW, et al. Tumor necrosis factor gene polymorphism and septic shock in surgical infection. *Crit Care Med* 2000; 28(8):2733-2736.
14. Waterer GW, Quasney MW, Cantor RM, Wunderink RG. Septic shock and respiratory failure in community-acquired pneumonia have different TNF polymorphism associations. *Am J Respir Crit Care Med* 2001;163(7): 1599-1604.
15. Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet* 1996;59(5): 983-989.
16. Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype-phenotype associations. *Eur J Hum Genet* 2001;9(4):301-306.
17. Allison DB, Heo M, Kaplan N, Martin ER. Sibling-based tests of linkage and association for quantitative traits. *Am J Hum Genet* 1999; 64(6):1754-1764.
18. Allison DB. Transmission-disequilibrium tests for quantitative traits. *Am J Hum Genet* 1997;60(3):676-690.
19. Horvath S, Laird NM. A discordant-sibship test for disequilibrium and linkage: no need for parental data. *Am J Hum Genet* 1998;63(6):1886-1897.
20. Spielman RS, Ewens WJ. A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. *Am J Hum Genet* 1998;62(2): 450-458.

21. Zhu X, Elston RC. Transmission/disequilibrium tests for quantitative traits. *Genet Epidemiol* 2001;20(1):57-74.
22. Long AD, Langley CH. The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits. *Genome Res* 1999;9(8):720-731.
23. Cardon LR, Bell JL. Association study designs for complex diseases. *Nat Rev Genet* 2001;2(2):91-99.
24. Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LB, Duff GW. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. *J Exp Med* 1993;177(2):557-560.
25. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A* 1997;94(7):3195-3199.
26. Louis E, Franchimont D, Piron A, et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998;113(3):401-406.
27. Warzocha K, Ribeiro P, Bienvenu J, et al. Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. *Blood* 1998; 91(10):3574-3581.
28. McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature* 1994;371(6497):508-510.
29. Brinkman BM, Zuiddeest D, Kaijzel EL, Breedveld FC, Verweij CL. Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation [see comments]. *J Inflamm* 1995;46(1):32-41.
30. Webb GC, Chaplin DD. Genetic variability at the human tumor necrosis factor loci. *J Immunol* 1990;145(4):1278-1285.
31. Weitekamp JH, Stuber F, Bartmann P. Pilot study assessing TNF gene polymorphism as a prognostic marker for disease progression in neonates with sepsis [In Process Citation]. *Infection* 2000;28(2):92-96.
32. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998; 102(7):1369-1376.
33. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem* 2000; 275(24):18138-18144.
34. Danis VA, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1RA) gene polymorphism. *Clin Exp Immunol* 1995; 99(2):303-310.
35. Fang XM, Schroder S, Hoefl A, Stuber F. Comparison of two polymorphisms of the interleukin-1 gene family: interleukin-1 receptor antagonist polymorphism contributes to susceptibility to severe sepsis [see comments]. *Crit Care Med* 1999; 27(7):1330-1334.
36. Zheng C, Huang D, Bergenbrant S, et al. Interleukin 6, tumour necrosis factor alpha, interleukin 1beta and interleukin 1 receptor antagonist promoter or coding gene polymorphisms in multiple myeloma. *Br J Haematol* 2000;109(1):39-45.
37. Spek CA, Greengard JS, Griffin JH, Bertina RM, Reitsma PH. Two mutations in the promoter region of the human protein C gene both cause type I protein C deficiency by disruption of two HNF-3 binding sites. *J Biol Chem* 1995;270(41): 24216-24221.
38. Spek CA, Koster T, Rosendaal FR, Bertina RM, Reitsma PH. Genotypic variation in the promoter region of the protein C gene is associated with plasma protein C levels and thrombotic risk. *Arterioscler Thromb Vasc Biol* 1995;15(2):214-218.
39. Aiach M, Nicaud V, Alhenc-Gelas M, et al. Complex association of protein C gene promoter polymorphism with circulating protein C levels and thrombotic risk. *Arterioscler Thromb Vasc Biol* 1999;19(6):1573-1576.
40. Menges T, Hermans PW, Little SG, et al. Plasminogen-activator-inhibitor -1 4G/5G promoter polymorphism and prognosis of severely injured patients. *Lancet* 2001;357(9262):1096-1097.
41. Patil N, Berno AJ, Hinds DA. Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21. *Science* 2001;294:1719-1723.
42. Templeton AR, Crandall KA, Sing CF. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 1992;132(2): 619-633.
43. Templeton AR, Weiss KM, Nickerson DA, Boerwinkle E, Sing CF. Cladistic structure within the human Lipoprotein lipase gene and its implications for phenotypic association studies. *Genetics* 2000;156(3):1259-1275.
44. Rieder MJ, Carrington DP, Chung M-W, et al. SeattleSNPs. *NHLBI Program for Genomic Applications*. Seattle, WA: UW-FHCRC; GenBank AY066019. 2001.

Change of address notices and requests for subscriptions are to be sent by mail to P.O. Box 310, Station H, Montreal, Quebec H3G 2K8 or by fax to (514) 932-5114 or by e-mail to [info@snellmedical.com](mailto:info@snellmedical.com). Undeliverable copies are to be sent to the address above.

## Abstract of Interest

### Septic shock and respiratory failure in community-acquired pneumonia have different TNF polymorphism associations.

WATERER GW, QUASNET MW, CANTOR RM, WUNDERINK RG, PERTH, AUSTRALIA.

Genetic factors are likely to contribute to the variable presentation of community-acquired pneumonia (CAP). The purpose of this prospective cohort study was to determine whether the LTalpha+250 (TNFbeta+250) and TNFalpha-308 gene polymorphisms are associated with different presentations of CAP. Septic shock (SS) was defined using American College of Chest Physicians/Society of Critical Care Medicine (ACCP-SCCM) criteria. Type I respiratory failure (TIRF) was defined as an O(2) saturation on room air of < 90% with a normal PCO(2). A total of 280 patients were genotyped; 31 had SS, 80 had TIRF. Genotype proportions are given in the order of AA/GA/ GG. The proportion of patients in each genotype developing SS was as follows: LTalpha+250 0.19/0.07/0.09 (p = 0.01 AA versus non-AA); TNFalpha-308 0.16/0.06/0.12 (p = NS). Carrying at least one AA (tumor necrosis factor [TNF] high secretor) genotype had an 18.0% risk of SS versus 6.8% (p = 0.006). GG homozygotes (TNF low secretors) at both loci had only a 2.9% risk of SS. Septic shock was associated with the LTalpha+250:TNFalpha-308 A:G haplotype but not the A:A haplotype, suggesting that LTalpha+250 is a marker, rather than a causative polymorphism. Carriage of the G:G haplotype had a significant protective effect against the development of septic shock (p = 0.011). TIRF was not associated with LTalpha+250 AA genotype. In the absence of septic shock, there was a significant trend to greater TIRF in patients with LTalpha+250 GG (TNFalpha hyposecretor) genotype (p = 0.03). Our finding of different genotype associations for SS and TIRF has important implications for immunotherapy in both CAP and sepsis, as well as for the definition of the systemic inflammatory response syndrome (SIRS).

*Am J Respir Crit Care Med* 2001;163(7):1599-1604.

## Upcoming meetings

17-22 May, 2002

### 2002 American Thoracic Society Meeting 98<sup>th</sup> International Conference

Atlanta, Georgia

CONTACT: [www.thoracic.org](http://www.thoracic.org)

31 October-2 November, 2002

### Toronto Critical Care Medicine Symposium

Metro Toronto Convention Centre

Calling for Abstracts

CONTACT: <http://www.tccms.com>

## Websites of interest

Canadian Critical Care Society  
[www.canadiancriticalcare.org](http://www.canadiancriticalcare.org)

Canadian Association of Critical Care Nurses  
[www.caccn.ca](http://www.caccn.ca)

Society of Critical Care Medicine  
[sccm.org](http://www.sccm.org)

This publication is made possible by an educational grant from

**Eli Lilly Canada Inc.**