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The promise of immunogenomics at the bedside: Genetic risk of intra-abdominal candidiasis

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Invasive fungal infections are predominantly due to *Candida* and *Aspergillus* and occur in those predisposed due to mucocutaneous barrier disruption or immune defects. According to data from the U.S. National Hospital Discharge Survey from 1996-2003, invasive candidiasis incidence rose to 29 per 100,000 population per year – a burden of 63,000 infections per year (1). In the surgical critical care setting, intra-abdominal candidiasis may develop without fungemia following gut perforation, making early diagnosis challenging. Investigators have explored the use of pre-emptive therapy based on fungal biomarkers such as serum beta-D-glucan. However, such biomarkers can lead to false positives – particularly in the setting of intra-abdominal candidiasis -- despite combinations with clinical scores and colonization indices (2, 3).

Addressing this problem, host genetic surrogates offer the promise to improve the predictive values of current diagnostics. In this issue (4), Wójtowicz *et al* analyzed 18 single nucleotide polymorphisms (SNPs) in 16 genes that had previously been implicated in infectious outcomes, studied in 89 surgical intensive care unit patients at high risk for intra-abdominal candidiasis due to recurrent gastrointestinal perforation and acute necrotizing pancreatitis. The study design was powerful; targeted small sample gene studies have a power advantage over whole genome-wide (GWAS) ‘discovery’ studies in that false positive correlations are reduced (5). In addition, consideration of common variant SNPs (rather than rare alleles) matched the commonality of intra-abdominal candidiasis in this patient population at high risk. All variants were in Hardy-Weinberg equilibrium, suggesting independence of each locus in risk prediction. Principally, the authors found that tumor necrosis factor- α , *TNF- α* (*rs1800629* [AA/GA]), and β -defensin1, *DEFB1* (*rs1800972* [GG/CG];) were each associated with a 2.5 times higher frequency than controls, portending a potentiated hazard for intra-abdominal candidiasis. Despite wide confidence intervals, the magnitude of effect is striking and significance remained with a Bonferroni corrected significance of $p < 0.003$. A Bonferroni-type correction for multiple comparisons is essential, even when analyzing polymorphisms of known relevancy in other conditions, since the odds of a random association are not related to biology. An additional consideration in such studies is the genetic model used to analyze the results. The dominant model used in the present study

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assumes that any of the allelic replacements, GG → GA or AA will yield the outcome studied, in this case intra-abdominal candidiasis. In contrast, a recessive model implies both alleles are required for risk, in this case, only GG → AA, and an additive model would assume that the risk of two alleles (i.e. GG → AA) would be greater than having either alone (GG → GA or AA). An additive model is plausible for the TNFα polymorphism where previous studies have implicated progressively higher levels of TNFα with each promoter allelic replacement and such a model would be interesting to pursue in future studies. Confirmatory studies assessing TNFα expression levels during human infection could also help to confirm the genetic results, since expression levels in normal volunteers were found to be cell and stimuli dependent (6, 7). Combining SNPs increased the positive predictive value for intra-abdominal candidiasis to 83.3% from 62%, offering the promise of increased predictive robustness, while the negative predictive value was not significantly changed. This suggests that combining sets of associated SNPs could one day provide valid risk assessment for *Candida* infection control and prophylactic strategies—an exciting development.

In addition, the authors performed subgroup analyses to explore underlying co-morbidities such as neoplasia that could help explain genetic mechanisms of *Candida* acquisition but were limited as a consequence of small numbers. Interestingly, the *TNF-α* SNP -- rs1800629 [AA/GA] – has been associated with pancreatitis, which could implicate a pathobiological mechanisms for this genetic association (8). Other co-variables such as pathogen identify were also difficult to control for, given the small numbers of patients, but could be important. For example, peripheral blood mononuclear cells stimulated with *C. parapsilosis* produce a skewed T-helper lymphocyte response with more IL-10 (anti-inflammatory) and less IL-1β, IFN-γ, IL-17, and IL-22 (pro-inflammatory) than *C. albicans* (9). Th17 cells produce IL-17 and IL-22 that trigger β-defensin – a gene implicated in the present study – and their defects have been associated with chronic mucocutaneous candidiasis (10, 11). Given the current epidemiologic shift towards more non-*albicans* species causing IC with different antifungal susceptibilities and virulence, species identification may be important to consider in future trials studying genetic risk stratification in intra-abdominal candidiasis. (1). An additional consideration is the inclusion of genetic admixture controls (12). Population admixture confounding can occur when individuals of a reported ethnic group contain unexpected alleles due to mixed ancestry. Inadvertent genetic associations can then arise if an associated mutation occurs at greater frequency in an ethnic group that is at risk for the disease. Conveniently, SNP markers for common ancestries are readily available and are a useful control to prevent such spurious associations. A second part of the study sought to associate genetic polymorphisms to *Candida* colonization measured by surveillance cultures, but identified a different set of genes (TLR4 and SP-A2), and utilized a univariate comparison. This apparent discrepancy could be due to a weak biological association between the two conditions or the statistical power of the limited sample.

Several previously identified polymorphisms associated with invasive candidiasis were not identified in the present study and could represent differences in intra-abdominal candidiasis pathobiology or a lack of statistical power inherent to small sample sizes. For example, the dectin-1 signal adaptor protein, CARD9 (caspase recruitment domain-9), has been associated with both IC and mucocutaneous disease but was not found to be associated with either in the present study (11). In addition, while insertion/deletion polymorphisms in the promoter regions of IL-10 and IL-12B that produce less inflammation have been associated with persistent fungemia in IC, these allelic variations were not identified in the present study (13). Since only a minority of gene mutations represent robust risk alleles, multiple studies are still required to facilitate the development of robust multi-gene risk stratification tools that identify groups for preventive strategies or pre-emptive therapy. Fortunately, development of real-time PCR modalities that can detect a wide range of host and pathogen

informative sequences has moved into the clinical laboratory and has been critical for pre-emptive therapy of other diseases such as cytomegalovirus disease in hematopoietic stem cell transplants that expedite therapy, conserve resources and minimize toxicity (14). Multi-sequence panels can also incorporate analysis of key pathogen sequences that could predict therapeutic failure such as the recently FDA-approved Xpert MTB/RIF assay that detects the *Mycobacterium* and resistance in one step (www.dfa.gov). In addition, new potential risk alleles such as polymorphisms in the chemokine CX3CR1 recently reported are daily adding to our 'bank' of genetic 'deposits' that will facilitate development of robust genetic tools for the clinician's armamentarium (15). Thus, while additional development is likely required for routine host genetics to impact day-to-day clinical practice for invasive candidiasis, the work by Wójtowicz *et al* puts us further down the road.

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