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Genetic alterations in necrotizing enterocolitis

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ABSTRACT

An underlying genetic predisposition to necrotizing enterocolitis (NEC) is increasingly being recognized. Candidate gene or pathway approaches as well as genome-wide approaches are beginning to identify potential pathogenic variants for NEC in premature infants. However, a majority of these studies have not yielded definitive results because of limited sample size and lack of validation. Despite these challenges, understanding the contribution of genetic variation to NEC is important for providing new insights into the pathogenesis of NEC as well as allowing for targeted care of infants with inherent susceptibility. In this review we provide a summary of published genetic association studies in NEC along with defining the challenges and possible future approaches.

Introduction

Necrotizing enterocolitis (NEC) is a common and devastating inflammatory disease of the intestinal tract that affects 5–10% of premature infants weighing less than 1500 g at birth.[1] Despite considerable advancements in the care of premature infants over the past decades, outcomes for NEC have remained virtually unchanged and may even be worsening.[2] It is estimated that up to 10–30% of infants who develop NEC die, and those who survive are at significant risk for serious morbidities, including intestinal failure and neurodevelopmental disability.[3] Risk factors that predispose to altered intestinal colonization, ischemia, and inflammation in the setting of immature gut immunity have been implicated in NEC pathogenesis. However, these risk factors cannot fully account for the significant variability in the incidence and severity of disease in premature infants. In light of this, there has been increasing recognition that genetic factors modulate susceptibility or severity of NEC. Recent studies that explored the relationships between variants in innate immune, cytokine, and growth factor genes in NEC have yielded some interesting results.[4–6] In this article we (1) provide an overview of the role of genetic variation in human disease with a focus on NEC, (2) summarize and evaluate published gene-association studies in NEC, (3) discuss various challenges and limitations of these studies, and (4) discuss future approaches for identifying pathogenic loci for NEC.

The role of genetic variation in disease

Genetic factors can explain variation in liability or severity of disease phenotypes among individuals sharing similar risk factors and environmental exposures.[7] The most common and most studied type of genetic variation is the *single nucleotide polymorphism* or SNP. SNPs are naturally occurring variation in the genetic code involving a single nucleotide base. There are roughly 10 million SNPs in the human genome, which averages to about one SNP every 300 nucleotides.[8] Most SNPs in the coding or regulatory elements of a gene do not alter function or expression of the gene product.

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Some SNPs in the exonic or regulatory regions of a gene can impact function by altering the structure or expression of protein, respectively. These are more likely to be associated with a disease process or phenotype.

To evaluate the role of SNPs in disease, investigators perform *genetic association studies*, which in general are case–control studies that compare the prevalence of SNPs between those with and without the disease.[9] A significantly higher or lower prevalence of a particular SNP in cases compared to controls provides probable evidence that the SNP is associated with an increased or decreased risk for the disease, respectively. SNPs associated with disease may be functional SNPs themselves or may be in genetic linkage with SNPs that alter function. Two commonly used approaches in genetic association studies are the candidate gene/pathway approach and the genome-wide approach.[10] In *targeted approaches*, investigators evaluate SNPs of a few, pre-selected genes based on a priori hypotheses of relevance to the disease of interest. In contrast, *genome-wide approaches* utilizes next-generation sequencing to simultaneously evaluate millions of SNPs in an unbiased manner, allowing for discovery of previously unknown associations between genes and the disease. Each approach has its own advantages and disadvantages, as summarized in Table 1.

Current genetic association studies in NEC

Reviewing the literature on genetic research in NEC reveals that virtually all genetic association studies to date have thus far utilized the candidate gene approach. Although less powerful than genome-wide studies, the candidate gene approach requires less number of patients and has less stringent statistical requirements, making them ideal *preliminary studies* for evaluating the contribution of genetic factors in NEC. Based on current understanding of how NEC develops, we have depicted genetic studies in relationship with the pathogenic process (Fig.). A majority of studies have focused on genes that regulate intestinal immunity and inflammation, while others have examined SNPs in growth factors, cell-cycle regulation and oxidant/anti-oxidant genes (Table 2). In the discussion that follows, we will review the candidate genes that have thus far been evaluated in NEC, with an emphasis on understanding the biological relevance of the gene or pathway to NEC development.

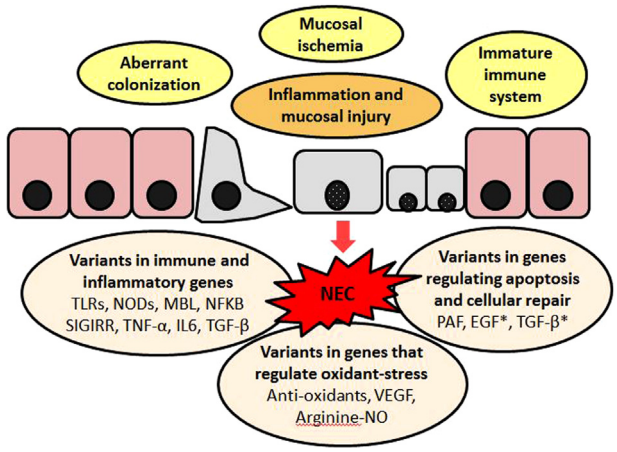

Fig – Genetic pathways in NEC susceptibility: NEC develops in the context of immature intestinal immunity, aberrant colonization and mucosal ischemia. Various genetic pathways (discussed herein) that regulate inflammation and mucosal injury can contribute to individual variation in liability to NEC. TLRs, Toll-like receptors; NODs, nuclear oligomerization domain containing receptors; MBL, mannose binding lectin; NFKB; nuclear factor-kappa B; SIGIRR, single immunoglobulin interleukin-1 like receptor; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6; TGF-β, transforming growth factor-beta; VEGF, vascular endothelial growth factor; NO, nitric oxide; PAF, platelet activating factor; EGF, epidermal growth factor. *Genes that have not yet been evaluated.

Genes that regulate intestinal immunity and inflammation

Pattern recognition receptors (PRR)

The intestinal epithelium has to accomplish the dual role of immune tolerance to commensal organisms and immune activation against invading pathogens. Central to achieving this role are the *pattern recognition receptors* (PRR); innate immune receptors that recognize conserved structural motifs in pathogens and activate the intestinal immune response. Because of the crucial role of PRRs in being the first-line regulators of inflammation in the intestines, several investigators have evaluated the contribution of variants in PRR genes in NEC. Among the PRR pathways that have been

Table 1 – Candidate gene vs. genome-wide association studies: The major differences and pros/cons inherent to these approaches are summarized in the table below.

Candidate gene approach	Genome-wide approach
Hypothesis testing	Hypothesis generating
Tests for only a few variants	Tests for millions of genetic variants
Evaluates potential contribution of genes already implicated in the disease process	Allows discovery of previously unknown association between novel genes and disease
Can be done with smaller sample size (*hundreds*)	Needs large sample size (*thousands*)
Less rigorous criteria for statistical significance ($p < 0.05$)	More rigorous criteria for statistical significance ($p < 10^{-8}$)
Less expensive	More expensive but decreasing costs