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POSTER PRESENTATION Open Access

Functional characterization of human variants of *NFKBIA*: a key regulator of immune responsiveness implicated in susceptibility to infectious and inflammatory disease

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Objective/purpose

Genetic association studies have identified several polymorphisms in genes of the innate immunity cascade that appear to influence susceptibility to asthma and other inflammatory diseases. However, most candidate genes have not been functionally characterized for their impact on human immune responsiveness. An excellent candidate for functional investigation is *NFKBIA* which encodes IκBα–the major negative regulator of NFκB. Single nucleotide polymorphisms (SNPs) in the promoter region of *NFKBIA* have been implicated in various infectious and inflammatory diseases. Specifically, the linked promoter SNPs rs2233406, rs3138053 and rs2233409 have been implicated in sarcoidosis, trachoma, acute respiratory distress syndrome, invasive pneumococcal disease, Graves' disease and respiratory syncytial virus. We investigated the mechanistic and functional impact of the promoter variants of *NFKBIA* on human immune responsiveness.

Methods

Using a coding SNP that was in high linkage with *NFKBIA* SNPs rs3138053/rs2233406/rs2233409, we designed and validated an allele-specific PCR assay that could detect subtle differences in allele ratios between the major (ACC) and minor (GTT) promoter variants of SNPs rs3138053/rs2233406/rs2233409. Peripheral blood mononuclear cells of homozygous (ACC/ACC) and heterozygous (ACC/GTT) individuals were stimulated with LPS and live cultures of *Streptococcus pneumoniae* (serotype 14) for 3 and 4 hours. PBMCs of *NFKBIA* homozygotes and heterozygotes were stimulated with various Toll-like-receptor (TLR) ligands of the innate immune cascade to assay for differences in the innate immune response.

Findings

NFKBIA heterozygotes (ACC/GTT) displayed 1.21 (1.14-1.27 95% CI) - 1.26 (1.18-1.34 95% CI) fold higher expression of the major allele transcript (ACC) relative to the minor allele transcript (GTT). At 3 hours post stimulation, *NFKBIA* homozygotes (ACC/ACC) produced higher level of *NFKBIA* mRNA than heterozygotes (ACC/GTT) following stimulation with LPS (1.4 fold, $p = 0.0095$) or *S. pneumoniae* (1.51 fold, $p = 0.024$). Higher TNF-α secretion was seen from the peripheral blood mononuclear cells (PBMCs) of heterozygotes (ACC/GTT) as compared to homozygotes (ACC/ACC) when stimulated with Pam3CSK4 (2.29-fold increase; $p < 0.01$) and 3M-002 (3.30-fold increase; $p < 0.001$).

Deliverables

We have shown that the observed association of *NFKBIA* variants with infectious and inflammatory conditions has functional consequences. Individuals heterozygous for SNPs rs3138053 /rs2233406/rs1050851 display allelic imbalance, reduced levels of *NFKBIA* expression, as well as a hyper inflammatory innate immune response.

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Relevance

Functional genomic studies such as this will help realize AllerGen's goal of '*discovery of the causes of, and ways to prevent, control or eliminate allergic and related immune diseases*' by:

- Generating convincing evidence that the genetic variant is functionally relevant and likely to contribute to the development of the clinical phenotype.
- Providing insight into the mechanism underlying the genetic association and, therefore, greatly enhancing our knowledge of the disease pathogenesis.
- Identifying molecular pathways that can be targeted to prevent or treat allergic disease.

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