Molecular intermediate phenotype mapping of IL-6 and TNFα levels reveals genes critical for chronic systemic inflammation

S.J. Schrodi[1,6], D. David[2], J.K. Meece[2,6], R. Strenn[3], J.J. Mazza[4], M.H. Brilliant[1,6], J.A. Smith[5,6]

[1]Center for Human Genetics, Marshfield Clinic Research Foundation
[2]Core Laboratory, Marshfield Clinic Research Foundation
[3]Biomedical Informatics Research Center, Marshfield Clinic Research Foundation
[4]Clinical Research Center, Marshfield Clinic Research Foundation
[5]Department of Pediatrics, University of Wisconsin-Madison
[6]Institute for Clinical and Translational Research, University of Wisconsin-Madison

Marshfield Clinic Research Foundation

Personalized Medicine Research Project

UW Institute for Clinical and Translational Research
UW ICTR
UNIVERSITY OF WISCONSIN-MADISON
MARSHFIELD CLINIC

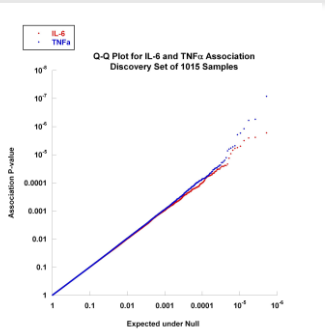
WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

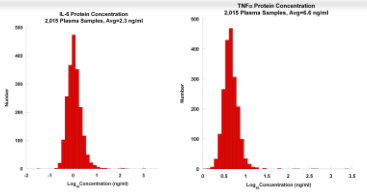
Background

Numerous common diseases exhibit an underlying component of chronic systemic inflammation. Discovery of genetic variants that produce susceptibility to these diseases has many pitfalls, not the least of which lies in the clinical definition of disease which may or may not reflect pathological mechanisms at the molecular level. Hence, redefining phenotypes based on molecular characteristics may elicit more robust and lucid genetic association signals. This study uses a composite metric of the circulating levels of two important inflammatory cytokines, TNFα and IL-6, in the general population as a molecular intermediate phenotype. Both cytokines are important in the immunobiology of proinflammatory processes and have been directly targeted by biological therapeutics to remediate autoimmune conditions. To identify novel genes involved in regulation of TNFα/IL-6-mediated chronic inflammation, we performed a quantitative trait GWAS on TNFα/IL-6 levels in a genetically homogeneous population in Central WI, largely derived from Bavarian immigrants in the late 1800s.

Materials and Methods

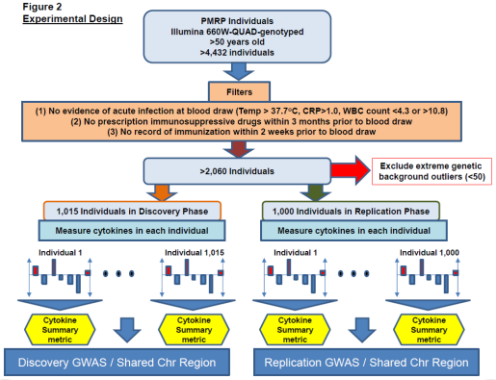
Using the Meso-Scale Discovery platform, we measured levels of TNFα and IL-6 in plasma from 2015 individuals not taking immune-modulating therapies or having evidence of acute infection. A small number of genetic background outliers were removed from the sample set. The Illumina 660W-QUAD array was applied to all samples and a linear correlation test was applied for the genotypes at each of >500,000 SNPs to transformed TNFα/IL-6 concentration data. PLINK was run on the individual SNP data. The VEGAS program was applied to individual SNP data to obtain gene-based association P-values.





Distribution of $\log_{10}$ calculated concentration for IL-6 and TNFα as based on a spatially-arrayed multiplex ELISA using electro-chemiluminescence detection and 8-point standard curve.
Mean IL-6 =2.31 ng/ml
Mean TNFα = 6.59 ng/ml

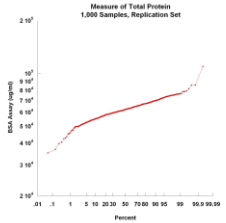
Figure 2
Experimental Design



The metric for the i^{th} individual is defined as

$$S_i = -\sum_{j=1}^{k} \ln(1 - Q_j)$$

where Q_j is the quantile for the j^{th} cytokine.



BSA used to adjust cytokine levels

Results

The GWAS on the transformed concentrations of these two critical proinflammatory cytokines yielded Q-Q plots that did not show evidence of widespread population stratification or biased sampling. Individual SNPs suggest several regions important for IL-6 and TNFα concentrations, but replication in the second set is necessary. Gene-based analysis, adjusted for LD patterns in the CEU, yielded gene-wise significant findings from the discovery sample set (detailed below). However, replication is necessary for strong statistical claims.

Gene-based analysis

From roughly 18,000 genes evaluated, two statistically compelling regions emerged from the analysis: 1) the *RXFP3-SLC45A2* region on the short arm of Chr5 (P<1E-06), and 2) the *GPR31-CCR6* region on the long arm of Chr6 (P<1E-06). *RXFP3* encodes for a relaxin/insulin-like receptor, and may wield anti-fibrotic, and anti-inflammatory actions. *SLC45A2* encodes for a melanocyte differentiation antigen and is intimately involved in melanin synthesis (OMIM). Interestingly, Sellick *et al* (2005) mapped the *GPR31-CCR6* region as conferring strong predisposition to small vessel lymphocytic vasculitis within an extended family. Additionally, *CCR6* encodes for a chemokine receptor expressed on lymphocytes. These results may provide unique insight into the molecular mechanisms behind proinflammatory processes.

Conclusions

We have commenced the first inflammatory-based protein eQTL study for the purpose of understanding immune pathophysiology at the molecular level. We are currently in the process of testing for replication using the second, independent set of samples. If the signals do indeed replicate, then we have the ability to utilize linked electronic medical records to investigate the clinical disease states correlated to these genetic and cytokine patterns.

References

1. Liu JZ et al. (2010) AJHG 87
2. http://www.mesoscale.com
3. Purcell S, et al. (2007) AJHG 81
4. Sellick et al. (2005) Hum Genet 118

Funding sources: UW Institute for Clinical and Translational Research through pilot grant/Clinical and Translational Science Award Program 9U54TR000021, Marshfield Clinic Research Foundation, The Wisconsin Genomics Initiative