locus has powerful common variant associations with SLE risk with odds ratio = 1.5 and $p < 10^{-20}$ in all human ancestries and also has an association, though not necessarily identical to SLE, with rheumatoid arthritis, primary biliary cirrhosis, Behcet's Disease, Sjögren's syndrome, progressive systemic sclerosis, and type I diabetes.

Methods Genome wide association studies of SLE, Bayesian and frequentist fine mapping methods, DNA affinity purification assays, and electrophoretic mobility shift assays.

Results We are attempting to identify the causal variants and determine the mechanism for SLE disease risk at this locus. Our data suggest that the risk haplotype alters the expression of mRNA from both *STAT1* and *STAT4*. Application of frequentist and Bayesian methods restrict the plausibly causal variants to four possibilities in introns 4 and 5 of *STAT4* under the assumption that the association observed across human ancestries is being driven by the same causal variants. Three of these four polymorphisms are predicted to alter the binding of a specific transcription factor, leading to the hypothesis that the same transcription factor is operating at multiple sites in a risk haplotype. We have data suggesting differential and allele preferential binding of the transcription factor at one variant with evaluation of the others in process. This may possibly be the first discovered example of the phenomenon of multiple transcription factor binding on multiple variants of a risk haplotype.

Conclusion In general, genome wide association studies (GWASs) provide powerful evidence of the presence of a genetic variation altering phenotype risk without revealing what the specific responsible variant is among those in a statistical dead heat for causation, in which cell type(s) or stage(s) of differentiation in which the risk difference is relevant, or what the molecular mechanism might be. We have work underway to reveal these details for lupus loci, initially concentrating on *IRF5*, *STAT1-STAT4*, and *ETS1*. The STAT1-STAT4 association with SLE can be isolated to involve only a few variants, which are predicted to have curiously similar transcription factor binding behaviour.

GG-04 A MISSENSE MUTATION IN NEUTROPHIL CYTOSOLIC FACTOR 1 (*NCF1*) IS ASSOCIATED WITH SUSCEPTIBILITY TO MULTIPLE AUTOIMMUNE DISEASES

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Background Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with a strong genetic component. Dozens of SLE-associated loci have been identified by genome-wide associated studies (GWAS) and included in the ImmunoChip for fine-mapping.

Materials and methods Using ImmunoChip, we assessed case-control subjects including Chinese, European Americans (EA) and African Americans (AA) for association with SLE. Subsequently, we carried out trans-ancestral mapping and resequenced the complex *GTF2IRD1-GTF2I-NCF1* region on 7q11.23 to identity underlying causal variant.

Results The strongest association signal in Chinese was unexpectedly detected at rs73366469 (OR = 2.88, $P = 3.6\times10^{-29}$) within the *GTF2IRD1-GTF2I* intergenic region on 7q11.23 rather than SLE-associated GWAS loci. This association was confirmed in EA (OR = 1.37, $P = 2.5\times10^{-3}$) but not in AA. By trans-ancestral mapping and sequencing, we identified R90H of *NCF1*, a neighbouring gene of *GTF2I* encoding the p47phox subunit of NADPH oxidase, as a highly plausible causal variant. R90H was associated with SLE in East Asians (OR = 3.47, $P_{meta} = 3.0\times10^{-105}$), EA (OR = 2.11, $P_{meta} = 7.0\times10^{-8}$) and AA (OR = 1.91, $P = 7.2\times10^{-3}$), and in conditional test R90H eliminated SLE-associated signals within the *GTF2IRD1-GTF2I* region including rs73366469. Furthermore, R90H was dose-dependently associated with early age of onset in Korean ($P = 0.011$) and EA ($P = 0.012$) patients with SLE. In addition to SLE, R90H was associated with seropositive rheumatoid arthritis (RA) in Koreans (OR = 1.66, $P = 1.2\times10^{-8}$) and primary Sjögren's syndrome (SS) in EA (OR = 1.72, $P = 5.8\times10^{-3}$). The conserved arginine 90 to histidine substitution located in the PX-binding domain of p47phox is predicted deleterious, which is supported by a report showing R90H results in reduced reactive oxygen species (ROS) production.

Conclusions We identified R90H of *NCF1* as a novel risk variant for multiple autoimmune diseases, highlighting the pathogenic role of reduced ROS production in developing autoimmune diseases.

GG-05 ATAC-SEQ PROFILING REVEALS CELL-TYPE SPECIFIC EPIGENETIC FEATURES OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Background Genetics studies have now identified over 80 SLE risk loci that influence predisposition to SLE with the majority of risk variants altering regulatory elements that govern gene expression. Precise understanding of how risk variants in regulatory elements influence gene expression in different cell types and cell states is critical for defining the molecular networks leading to autoimmunity. To begin to address this issue, we profiled the chromatin accessibility landscape of three distinct, albeit heterogeneous, compartments of the immune system across three clinical states.

Materials and methods Primary B and T lymphocytes and monocytes from 5 SLE subjects with high disease activity (SLEDAI $\geq$3) and 4 SLE subjects with low disease activity (SLEDAI $\leq$2) and 5 healthy controls were collected and processed for high-throughput open chromatin profiling by ATAC-seq. Reads were aligned to the hg19 genome and regions of enriched

chromatin accessibility "peaks" were identified with MACS2. For each cell type, we identified the consensus set of epigenetically active peaks across all 14 subjects. We conducted enrichment tests of identified loci using the GREAT tool and performed differential accessibility analysis using the edgeR package in R. Transcription factor binding motif enrichment and overlaps with know SLE risk haplotypes were also determined.

Results Chromatin accessibility profiles among the three cell types shared common features as well as peaks specific to each cell-type profile. The peaks unique to each profile were enriched in genomic loci specific to their cellular function as well as their known immunologic molecular signatures in SLE. Quantitative analysis of differential chromatin accessibility loci which discriminate between individuals with SLE and healthy controls patients with high versus low disease activity. Motif analysis revealed that many consensus peaks occupy binding sites of cohesion complex subunits, suggesting that long-range chromatin interactions may mediate immune responses that drive SLE progression. In addition, 320 SLE risk SNPs were located within an open chromatin peak suggesting these as SNPs candidates for functional impact.

Conclusions Our analysis suggests that chromatin profiling may have power to differentiate patients from controls as well varying extremes of disease activity and can pinpoint putative functional SNPs. Additional insight will be gained from further refinement of immune cell compartments. Future studies will focus on long-range interactions driving differences in chromatin accessibility and integrating these data with transcriptome data. We expect this approach to exapnd our knowledge of how regulatory networks in specific cells and cell states drive SLE progression.

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GG-06 SINGLE CELL GENE EXPRESSION STUDIES IN LUPUS PATIENT MONOCYTES REVEAL NOVEL PATTERNS REFLECTING DISEASE ACTIVITY, INTERFERON, AND MEDICAL TREATMENT

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Background Our previous studies have shown that different cell types from the same sample demonstrate diverse gene expression, and important findings can be masked in mixed cell populations. In this study, we examine single cell gene expression in SLE patient monocytes and determine correlations with clinical features.

Materials and methods $CD14^{++}CD16^{-}$ classical monocytes (CLs) and $CD14^{dim}CD16^{+}$ non-classical monocytes (NCLs) from SLE patients were purified by magnetic separation. The Fluidigm single cell capture and Rt-PCR system was used to quantify expression of 87 monocyte-related genes.

Results Both CLs and NCLs demonstrated a wide range of expression of IFN-induced genes, and NCL monocytes had higher IFN scores than CL monocytes. Unsupervised hierarchical clustering of the entire data set demonstrated two unique clusters found only in SLE patients, one related to high disease activity and one related to prednisone use. Independent clusters in the SLE patients were related to disease activity (SLEDAI 10 or greater), interferon signature, and medication use, indicating that each of these factors exerted a different impact on monocyte gene expression that could be separately identified. A subset of anti-inflammatory gene set expressing NCLs was inversely correlated with anti-dsDNA titers (rho = −0.77, p = 0.0051) and positively correlated with C3 complement (rho = 0.68, p = 0.030) in the SLE patient group.

Conclusions Using single cell gene expression, we have identified a unique gene expression patterns that reflect the major clinical and immunologic characteristics of the SLE patients which are not evident in bulk cell data, supporting the critical importance of the single cell technique.

GG-07 SLE RISK HAPLOTYPES ARE ASSOCIATED WITH DEVELOPMENT OF SEROLOGIC AUTOIMMUNITY IN HEALTHY INDIVIDUALS

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Background Approximately 60 loci are associated with SLE in genotyping studies. These loci impact several pathways in the immune response. ANA are one of the earliest features of lupus, preceding the onset of clinical symptoms by many years. The genetic risk factors that underlie the development of serological autoimmunity are unknown. A genome-wide association study was undertaken to understand the genetics of ANA development

Materials and methods Serum and DNA were collected from 2,635 healthy individuals with no personal history of autoimmunity. Antinuclear antibodies were detected using an ELISA to human nuclear extract (INOVA). Sera from 724 individuals (ANA-, ANA+, and SLE) were assayed by protein microarray quantifying IgM and IgG responses to 96 human autoantigens. A nested cohort of 1,969 subjects consisting of all the ANA+ Caucasian individuals and age/gender matched ANA- controls were genotyped using the ImmunoChip SNP array.

Results In 2,635 healthy individuals, 16.2% had moderate and 8.0% had high levels of IgG antinuclear antibodies. High titer ANA was almost exclusively seen in female subjects (OR (CI): = 1.6 (1.1–2.1), p = 0.003). Age was not associated with the presence or titer of ANA. On the autoantigen microarray, ANA+ healthy individuals had a high prevalence of antibodies to non-nuclear and cytoplasmic antigens, while subjects with SLE predictably produced antibodies to a variety of nuclear antigens. A quantitative genetic association test with ANA identified genomic loci associated with high ANA phenotype. *HLA* was second strongest signal ($p = 6.2 \times 10^{-6}$). The frequencies of the SLE risk haplotypes at *STAT4*, *TNFAIP3*, *BLK*, *BANK1*, *NCF2*, *and NMNAT2* were also significantly ($p<0.05$) increased in the ANA high positive group compared to ANA negative healthy subjects. On the other hand, SLE risk haplotypes in *ITGAM*, *UBE2L3*, *IRF5-TNPO3* loci were only high in the SLE group, suggesting their main role in a transition to clinical disease.