

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 known 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 expand 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<sup>++</sup>CD16<sup>-</sup> classical monocytes (CLs) and CD14<sup>dim</sup>CD16<sup>+</sup> 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.

**Conclusions** As has been seen in previous cohorts, a quarter of healthy individuals in this study made antinuclear antibodies, often at high titers. ANA testing, however, underestimates the repertoire of autoantibodies in these individuals. Healthy individuals who react in ANA testing produce antibodies against both non-nuclear and cytoplasmic antigens while SLE patients react to the classical RNA and DNA associated proteins. There is genetic risk for the development of ANA that includes many of the previously documented SLE risk haplotypes. However, other genetic associations are specific for SLE, suggesting distinct risk factors for ANA and for lupus.

#### GG-08 TRANSANCESTRAL MAPPING AND GENETIC LOAD IN SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background** Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects multiple organs, and disproportionately affects women and individuals of non-European ancestry. Here, we report the results of genotyping individuals of European Ancestry (EA), African American (AA), and Hispanic (Amerindian) American ancestry (HA) on the ImmunoChip (196,524 polymorphisms: 718 small insertion deletions, 195,806 SNPs).

**Methods** Genotype calling was completed in multi-ancestral batches (AA: 2,970 cases, 2,452 controls; EA: 6,748 cases, 11,516 controls; HA: 1,872 cases and 2,016 controls). Admixture estimates were computed using the program ADMIXTURE. To test for an association between a SNP and case/control status within an ancestry, a logistic regression analysis was computed adjusting for admixture factors as covariates. Transancestral meta-analysis was computed using the inverse normal method, weighted by sample size. The EA SLE-risk allele genetic load was computed as the weighted (log of the odds ratio (OR)) and unweighted sum of the number of EA risk alleles. The genetic load was computed in an independent set of EA 2000 cases and 2000 controls, and AA and HA samples. Individuals whose genetic load (risk allele count) was in the lower 10% of the count distribution were the reference group.

**Results** In total, 9, 58, and 6 distinct non-HLA regions had  $P < 1 \times 10^{-6}$  (Bonferroni threshold) for the AA, EA, and HA cohorts, respectively. The three-ancestry meta-analysis was particularly informative for 22 additional SLE-associated regions that met  $P < 5 \times 10^{-8}$ : 11 novel regions, 3 published regions now genome-significant, a complex multigenic region identified by adjusting for HLA alleles, and 7 established regions more sharply localised by transancestral mapping or novel to these ancestries

Genetic load was strongly predictive of SLE status in the 2000 EA cases/controls that were independent from the discovery set ( $OR_{unweighted} > 30$  and  $OR_{weighted} > 100$ ). There was a greater than additive effect in the log(OR) (i.e.,  $\beta$  parameter denoting slope) for the highest quarter of the genetic load range, suggesting the cumulative effect is greater than the sum of the individual effects (cumulative hit hypothesis). HA and AA showed markedly smaller ORs (between 3 and 10), reflecting a reduced predictive

ability of EA-identified SLE risk loci in non-EA populations and the lack of capturing non-EA SLE risk loci on the ImmunoChip.

**Conclusion** The multi-ancestral analysis of the ImmunoChip data identified numerous novel SNP associations. The genetic load leads us to posit a cumulative hit hypothesis, where the cumulative effect is greater than the sum of the individual alleles' effects.

#### GG-09 COMPARATIVE ANALYSIS OF GENE EXPRESSION IN LUPUS-AFFECTED TISSUES REVEALS COMMON AND DISPARATE PATHWAYS OF INFLAMMATION

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**Background** Immunologic mechanisms causing tissue damage in autoimmune diseases such as SLE are not fully understood. The hypothesis to be tested is that gene expression analysis of lupus-affected tissues will generate novel insights into targets of immunological intervention.

**Materials and methods** To gain additional insight, gene expression profiles obtained from lupus affected skin, synovium and kidney were obtained, compared to meta-analysed data obtained from active lupus B, T and myeloid cells, and cross-referenced to various pathway analytic tools including Molecular Signature (MS<sup>®</sup>)-Scoring, Ingenuity Pathway Analysis<sup>®</sup> Upstream Regulator (IPA<sup>®</sup>-UR) analysis, and Library of Integrated Network of Cellular Signatures (LINCS).

**Results** More than 300 arrays from lupus patients and appropriate controls were analysed to determine differentially expressed (DE) genes [8279 discoid lupus skin, 5465 synovial lupus arthritis, 6381 glomerulus (G) lupus nephritis, 5587 tubulointerstitium (TI) lupus nephritis]. Notably, the majority of lupus affected tissue DE genes were detected in more than one tissue and 439 were differentially expressed in all tissues. Tissue lymphocyte infiltration was documented by cell markers as well as by published unique gene expression signatures (BIG-C<sup>®</sup>). Common up-regulated transcripts in affected tissues displayed a variety of functions including pattern recognition receptors, p38/MAPK14 activation, endothelial endocytosis, and TLR activation. Unique targets of intervention were discovered when up-regulated transcripts in all lupus tissues were cross-referenced to molecular pathway and drug interaction databases. Canonical signalling pathways, published to be important for lupus pathogenesis, such as CD40L-CD40, IL-6, and IL-12/23 were visualised in IPA. Both MS<sup>®</sup>-scoring and IPA<sup>®</sup>-UR analysis predicted that signalling mediated by CD40 and IL12R occur in lupus skin, synovium and kidney glomeruli. LINCS connectivity analysed the effect of *in vitro* knockdown of ligand-receptor pairs and compared the genes affected with lupus tissue DE lists. Lupus nephritis (LN) kidney glomeruli received a LINCS connectivity score of -77 for CD40, implying that DE genes in this tissue have a high likelihood of being regulated by CD40-induced signalling. Skin and lupus nephritis kidney glomeruli received LINCS connectivity scores of -73 and -97, respectively, for the key signalling molecule required for IL6 signalling, IL6ST/gp130. All lupus-affected tissues had negative connectivity scores (skin, -98; synovium, -89; LN glomeruli, -91 and LN TI, -87) for IL12 $\alpha$ . Examination of curated functional groups from the STRING output of