exome sequencing with a patient-parent-parent trio design to enable the identification of de novo mutations. This approach enriches for pathogenic events, including in the understudied Alaska Native population. In addition, we use long-read Nanopore sequencing to identify complex structural de novo events in these families.

**Methods** Patients with a diagnosis of lupus prior to age 18 and their parents were enrolled in our study, which was approved by the institutional review boards of Seattle Children's Hospital and the University of Washington. Exome sequencing was performed using DNA isolated from whole blood or saliva. Long-read whole-genome sequencing (ONT PromethION) was performed using DNA from whole blood or freshly expanded lymphoblasts. In-house pipelines were used to identify de novo events. Candidate mutations were prioritized based on in silico analyses of pathogenicity, population rarity, and known biologic functions. Prioritized candidate mutations were evaluated for expression by qPCR and by western blot in transfected cells; activity of transcription factors was evaluated by luciferase assay.

**Results** Fifty-one families, ascertained on a patient with childhood-onset lupus, were sequenced to identify de novo mutations. In preliminary analysis 18 of the 51 patients were found to have promising de novo single-nucleotide variant genetic mutations. Preliminary in vitro analysis of one of these candidates, a mutation in the transcriptional repressor BACH1, suggests that the identified mutation alters protein activity: expression of HMOXI, a BACH1-regulated gene, was significantly higher in the patient compared to her parents or to unrelated controls, as measured by qPCR of whole blood; and in a luciferase assay with putative BACH1 binding sites from the HMOXI promoter, the repressor activity of mutant BACH1 was decreased in comparison with wild-type BACH1.

In addition, four of the 51 patients were found to carry previously uncharacterized de novo structural variants, including deletions of length 500kb and 1547kb, and duplications of length 786 kb and 900kb.

**Conclusions** We have established a pipeline for sensitive detection of de novo genetic variants in childhood-onset lupus, integrating short-read and long-read sequencing of patient-parent-parent trios. Preliminary analysis of these trios yields a high rate of promising candidate mutations, including a high percentage of patients carrying large structural mutations.

**Genetics**

| 097 | ANA ASSOCIATED REGULATORY POLYMORPHISMS IN HLA CLASS III REGION DOWNREGULATE COMPLEMENT 4 (C4) GENE EXPRESSION |

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**Background** Loss of self-tolerance and generation of numerous autoantibodies is a hallmark of systemic lupus erythematosus (SLE). Historically, testing for anti-nuclear antibodies (ANA) has been used as a screen for the autoimmunity underlying SLE as it can test for many reactivities in a quantitative manner. While the presence of ANA is sensitive for classifying people at risk for lupus, it is far from specific as it is commonly found in most other autoimmune diseases as well as up to 25% of healthy individuals. Longitudinal studies have demonstrated the presence of ANA 5-10 yr. prior to the development of clinical features of SLE. This has led to a model of lupus progression whereby certain risk factors (genetic, environmental, lifestyle) predispose to a breach in tolerance measured by the ANA while other factors or events then cause progression to disease. Knowledge of the risk factors for these different stages of autoimmunity will provide better information to individuals about their risk of developing lupus.

**Methods** ANA were determined in 2,903 healthy individuals, 117 first-degree relatives of SLE patients, and 883 patients with different autoimmune diseases using a solid phase test for ANA (Inova). The presence of IgG and IgM antibodies to 100 known autoantigens was determined using a solid phase array. A subset of 1,583 individuals of European ancestry were genotyped using the ImmunoChip v.1 to identify associations with a positive ANA. Linkage Disequilibrium (LD) and haplotype analysis was done on the top 19 ANA associated, potentially regulatory SNPs in the HLA-Class III region. The top 12 haplotypes and their association with ANA positive healthy group was analyzed. Median-joining (MJ) network method was used to illustrate genetic distance between risk and protective haplotypes. The functional impact of ANA associated single nucleotide variants was predicted based on functional annotation in ENCODE database. Plasma C4A protein was measured by ELISA.

**Results** Analysis of ANA as a quantitative trait identified major signals mapping to the HLA class II and class III regions. Stepwise conditional analysis identified several independent association signals. A 557 kb region in LD with SNP rs3117103 maps to the regulatory region of complement 4 (C4). This haplotype was comprised of 19 potentially functional SNPs. Association analysis showed that haplotype 4 (HAP4) was associated (OR=1.9; p=4.1 x 10^-4) with high ANA titers in the healthy population. This haplotype was a strong risk for SLE disease as well (OR=2.1; p=1.0 x 10^-10). On the other hand, haplotype 1 (HAP1) was found more frequently in healthy individuals without ANA (OR=0.8; p=9.0 x 10^-02). MJ network analysis showed that HAP4 associated with high ANA titers differs from HAP1 associated with low ANA titers by multiple potentially functional variants, which independently or in a cumulative fashion drive risk for ANA. Functional annotation of ANA associated markers showed that SNP rs3117103 has a strong eQTL effect in the Genotype-Tissue Expression database leading to downregulation C4 expression in multiple tissues and immune cell lineages. This effect was confirmed by demonstration of a significantly decreased concentration of C4A in the plasma of healthy individuals homozygous for ANA risk haplotypes.

**Conclusions** The genetic basis for risk of development of SLE can be partially explained by the risk for development of serological autoimmunity as measured by the ANA. Several genes in the HLA region contribute to this risk, including MHC class II (HLA-DR and DQ) and MHC class III, including C4. While genetic variation in C4 structure and function has long been associated with SLE, there is a quantitative association with the development of ANA that supports the hypothesis that reduced complement activation on self-proteins may predispose to their availability to induce autoantibodies.