192 Interactions Between *SLC22A5, IL13* and *SMAD3* Modulate Spirometric Indices in Chinese Children

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RATIONALE: A recent large-scale genome-wide association study (GWAS) by GABRIEL Consortium identified 10 asthma susceptibility loci in multiple European populations. However, the importance of these loci for asthma subphenotypes remains unclear. This study investigated the relationship between asthma diagnosis, spirometric indices and top signals from these candidate loci in Hong Kong Chinese children.

METHODS: School-age children with asthma were recruited from both hospital and community, whereas non-allergic controls came from independent community cohorts. Single-nucleotide polymorphisms (SNPs) with the strongest associations from the above asthma GWAS were genotyped by TaqMan assays on ABI-7900HT thermocycler or iPLEX Gold assays on Sequenom MassArray. The interactions between these SNPs for asthma diagnosis and spirometric indices were analyzed by generalized multifactor dimensionality reduction (GMDR).

RESULTS: 903 asthmatics and 1205 controls were recruited. Two SNPs on GSDMA and HLA-DQ failed TaqMan design, and were genotyped by iPLEX Gold assays. Asthma diagnosis was associated with rs2305480 of *GSDMB* on 17q21 (OR 0.69, 95% CI 0.57-0.83, $P<0.001$) but not SNPs from the other loci. Rs2305480 was also associated with elevated plasma total IgE levels ($P=0.002$), FEV_1 ($P=0.034$) and FEV_1/FVC ($P=0.027$). GMDR analyses revealed significant 3-locus and 5-locus interactions for FEV_1 ($P=0.001$ and 0.003 respectively) and 2-locus, 4-locus and 5-locus interactions for FVC ($P=0.018$, 0.039 and 0.014 respectively). *SLC22A5*_rs2073643, *IL13*_rs1295686 and *SMAD3*_rs744910 were the SNPs most consistently associated with spirometric indices in our children.

CONCLUSIONS: *GSDMB* is a candidate gene for asthma diagnosis and subphenotypes in Chinese children. Epistatic interactions are also detected among *SLC22A5*, *IL13* and *SMAD3*that modulate childhood lung function.

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193 Effects of Maternal Allergy On Umbilical Cord Blood Regulatory T Cell Forkhead Box Protein 3 (FOXP3) DNA Methylation

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RATIONALE: Regulatory T Cells (Tregs) are important immunomodulatory cells thought to influence the development of allergy and asthma. The Forkhead Box Protein 3 (FOXP3) transcription factor is essential for Treg function and is regulated by epigenetic mechanisms, including DNA methylation in the proximal promoter region. We hypothesized that FOXP3 DNA methylation would be altered in Tregs isolated from umbilical cord blood of babies born to allergic mothers.

METHODS: Cord blood samples were collected at the time of delivery from 49 non-smoking women who gave informed consent. Allergy was defined as a positive self-report of allergy verified by a clinically relevant positive skin test. $CD4^+CD127^{low}CD49d^-$cells were isolated using magnetic sorting techniques and DNA was bisulfite converted. DNA methylation at the FOXP3 proximal promoter and Treg-specific demethylated region (TSDR) were assessed by pyrosequencing.

RESULTS: 13 women were classified as allergic and 36 as non-allergic. Cord blood Tregs from babies born to allergic mothers exhibited significantly lower DNA methylation at each of the 8 CpG sites examined in the FOXP3 proximal promoter region, when tested individually ($P<0.05$). The average percent methylation in the FOXP3 promoter was $27.1\%\pm6.8\%$ in cord blood of allergic mothers, significantly lower ($P<0.05$) than the average of $45.0\%\pm2.9\%$ in non-allergic mothers. Purity of the isolated Tregs was assessed through bisulfite pyrosequencing of the TSDR region.

CONCLUSIONS: Children at increased allergic risk, attributable to maternal allergy, exhibit reduced FOXP3 promoter methylation in Tregs at birth. This may be relevant to immunomodulatory function.

194 Sequencing of the ST2 Gene Reveals a Haplotype That Determines Serum Total ST2 Levels in Individuals of African Ancestry

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RATIONALE: *ST2* (*IL1RL1*), is an IL1 family receptor that mediates important effectors of Th2 functions. Its soluble form (sST2) neutralizes its ligand, IL-33, by acting as a decoy receptor. Serum sST2 has been used as a biomarker for disease severity and outcome for multiple inflammatory and lung diseases, including atopic asthma. We undertook a targeted deep resequencing of *ST2* gene in 241 samples of African ancestry to identify *ST2*variants controlling serum sST2 levels.

METHODS: Serum sST2 concentration was measured by ELISA, and resequencing of ~50kb (chr2:102922962-102973497) encompassing the *ST2*gene was performed using Illumina's HiSeq2000. Single-variant tests for all common variants (MAF≥5%) were performed using linear regression assuming an additive model on log serum total ST2 considering age, gender and the first two principal components on a pre-existing genome-wide association panel of ancestry informative markers to adjust for admixture.

RESULTS: A total of 565 *ST2* variants were identified, 192 of which had a MAF≥5% including 3 coding synonymous and 6 missense variants. In the sST2 level analysis, ten SNPs in strong linkage disequilibrium yielded p-value less than 10^{-3}; a single common haplotype (frequency=65%) across all 10 SNPs yielded an overall p-value = 0.0002 and was negatively associated with sST2 levels (β = -0.09).

CONCLUSIONS: Sequencing *ST2* gene revealed a novel haplotype influencing sST2 levels in individuals of African ancestry, including 5 variants mapping to intron 1 and 5 mapping to the 5' region of *ST2*. Further work is ongoing to fully explore this association in an additional 400 subjects of African ancestry.