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Environmental and Genetic Correlations Between the Metabolic Syndrome (MS) and System Variables Representing Adaptive Immunity, Innate Immunity and Hemostasis

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Environmental and genetic correlations between the metabolic syndrome (MS) and system variables representing adaptive immunity, innate immunity, and hemostasis

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Background: The MS is prevalent in Mexican Americans (MAs) and is projected to increase. It is important therefore to better understand its genetic and environmental determinants. We hypothesized that the MS reflects dysfunction in important biological systems. To evaluate this concept, we analyzed gene expression data for the adaptive-immune, innate-immune, and hemostasis systems in relation to the MS from a study on MAs of San Antonio and the Rio Grande Valley.

Methods: We analyzed gene expression data for m=456, 595, and 328 genes found at the "Reactome" website for the stated systems. SVs are defined as composite variables from principal component factor analyses (PCFA). PCFA yielded in the order given 118, 109, and 98 SVs explaining at least 80% of the total variance in each system. For a preliminary analysis, we used a bivariate model for the liability to MS and each SV one at a time, which allows estimation of genetic and environmental correlations for the trait pair. To control for multiple hypothesis testing, we controlled the false discovery rate (FDR) to FDR$\leq$0.1.

Results: The SVs from each system will be referred to by the first 4 letters of the system followed by its number. There were no significant genetic correlations with MS. However, significant environmental correlations (where r_e denotes the environmental correlation coefficient) with MS were observed for ADAP6 (r_e=-0.46), ADAP18 (r_e=0.49), INNA15 (r_e =-0.43), and HEMO11 (r_e =-0.43).

Conclusions: We found that the MS is environmentally correlated with SVs of the adaptive-immune, innate-immune, and hemostasis systems.

Introduction/Background:

Metabolic Syndrome (MS) is defined as a condition in which a number of different disorders are present concurrently in an individual and work to increase the risk of diseases such as cardiovascular disease, diabetes and cancer. The disorders linked to MS include but are not limited to, hypertension, dyslipidemia and abdominal obesity. Recent studies show that individuals affected by MS may have up to a “fivefold increased risk of Type 2 Diabetes and a twofold increased risk of CVD)” (Lusis et. al, 2008). These findings have opened doors to a whole new field of research which works to combat the devasting effects that metabolic diseases have on millions of people worldwide.

For years, researchers used traditional genetics techniques to try and understand the underlying mechanisms and pathways that are responsible for the progression of MS. These traditional methods selected clinical traits and then identified single gene variations and mutations that were responsible for the expression of the selected trait. Unfortunately, researchers soon realized that this method of research did not take a number of important interactions into consideration. The genes known to be responsible for the expression of the disorders present in MS are known to have complex interactions with other genes as well as the environment. Systems genetics methods are better suited for understanding the interplay of genes in MS because they “allow for the identification of contributions of small effects from genetic variations in many genes on traits” (Lusis et. al, 2008). Through these methods, the interactions between genes are easily seen and understood. For this reason, the introduction of the systems genetics methods and techniques are promising to bring clarity to the gene interactions present in MS.

In MS, there is dysfunction in a number of biological systems which causes the adverse effects seen in the condition. In this study, we work to study the dysfunction in the innate immune system in individuals with MS. The innate immune system includes cells such as “macrophages, which are emphasized to play key roles in the pathological process of insulin resistance” (Zhou et. al, 2018). This is of great importance because we have come to learn that insulin resistance is a strong player in MS. In order for us to understand the environmental and genetic correlations between MS and Innate Immunity, we will use a rendition of the systems biology approach in which we have a combination of a bioinformatics approach and transcriptomic data for the innate immune system.

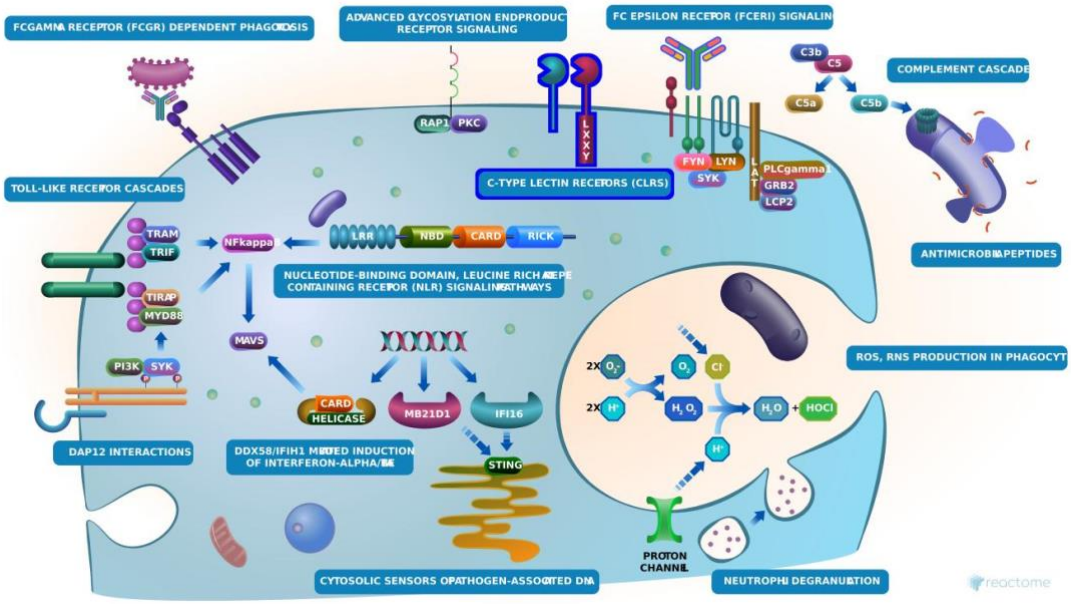

Figure 1. Genetic Pathways of the Innate Immune System from Reactome
https://reactome.org/PathwayBrowser/#/R-HSA-168249&SEL=R-HSA-5621481&PATH=R-HSA-168256

Methods:

The San Antonio Family Heart Study (SAFHS) was begun in 1992 as a study on the genetic epidemiology of cardiovascular disease (CVD) and related metabolic disorders such as obesity and type 2 diabetes (T2D) (Mitchell et al., 1996). 1236 individuals of Mexican American descent from 42 extended families residing in San Antonio were recruited for the SAFHS, where the proband was an individual with at least 6 first-degree relatives willing to participate in the study. As reported in Mitchell et al. (1996), anthropometric (such as waist circumference (WC)) and systolic and diastolic blood pressure (SBP and DBP) measurements were obtained following standard protocols at each participant's clinic visit, and their blood sample drawn, and appropriately stored. Laboratory work at the Texas Biomedical Research Institute (TBRI; then called the Southwest Foundation for Biomedical Research) were performed on the blood samples following standard protocols to obtain fasting glucose (FG), serum triglycerides (TG), and high density lipoprotein cholesterol (HDLC) (Mitchell et al., 1996).

Using Illumina® technologies—Illumina Sentrix Human Whole Genome (WG-6) Series I BeadChips, Illumina BeadArray 500GX Reader using Illumina BeadScan image data acquisition software (version 2.3.0.13), Illumina BeadStudio software (version 1.5.0.34)—in conjunction with standard protocols for the isolation of total RNA from lymphocytes, gene expression data

were obtained, quality-controlled, and normalized for 20,413 gene transcripts at the appropriate TBRI laboratories (Göring et al., 2017).

The definition of the metabolic syndrome (MS) developed by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (NCEP, 2001) was adopted. As defined therein, individuals with three or more of the following are considered to have the MS: central obesity (WC > 102 cm for males or > 88 cm for females), hypertriglyceridemia (TG > 150 mg/dL), low-HDL (HDLC < 40 mg/dL for males and < 50 mg/dL for females), hypertension (SBP/DBP ≥ 130/85 mmHg or blood pressure medication), and FG ≥ 110 mg/dL.

Using the bioinformatics resources and tools at the public website "Bioreactome", the set of genes known to be a part of the adaptive immune, innate immune, and hemostasis systems were obtained. Respectively in the order just given, 807, 1059, and 660 genes from these systems were found. For the SAFHS samples, there is adequate gene expression data for the adaptive immunity, innate immunity, and hemostasis systems for 456, 595, and 328 of the loci from these three gene sets, respectively. Principal components factor analysis (PCFA)—a standard tool of systems biology—was used to reduce the dimensionality of these systems. On applying PCFA, 109, 118, and 98 composite factors respectively for the adaptive immunity, innate immunity, and hemostasis systems, were found to explain at least 80% of the total variance in the given system. Denote each composite factor so obtained by SV for "system variable". For proof of principle, statistical genetic analysis was performed on the first 20 SVs of each system.

The bivariate variance components model (Williams et al., 1999) was used to estimate genetic and environmental correlations between the MS dichotomous trait (defined as 1 for MS, 0 otherwise) and each of the first 20 SVs for each system using the statistical genetics software SOLAR (Almasy and Blangero, 1998). To control for multiple hypothesis testing, the Benjamini-Hochberg false discovery rate was controlled to a significance level of 0.05.

Results and Discussion:

There were no significant genetic correlations with MS. However, significant environmental correlations (where r_e denotes the environmental correlation coefficient) with MS were observed for ADAP6 ($r_e = -0.46$), ADAP18 ($r_e = 0.49$), INNA15 ($r_e = -0.43$), and HEMO11 ($r_e = -0.43$).

The observation of no significant genetic correlations between traits representative of important biological systems with the MS is very unexpected. This does not preclude the possibility that the naturally occurring genetic variation existing in one or more individual genes

in these systems influences the interindividual variability in risk of MS but rather that, if so, they are not detectable at the system-wide functional level.

We found that the MS is environmentally correlated with SVs of the adaptive-immune, innate-immune, and hemostasis systems. This is notable as it indicates that non-permanent modifiable environmental variables and/or triggers play an important role in the pathogenesis of MS and that existing behavioral modification paradigms and pharmacological agents may be able to impact positively on one or more clinical manifestations of this common chronic disorder. Our findings are consistent with current thinking on the etiology of the MS positing a major role for the complex interaction between the dietary and gut-microbiome environments (Martinez et al., 2017; Belizário et al., 2018; Santos-Marcos et al., 2019). In the near future, we hope to pursue potential links between the SVs reported herein, dietary data from the SAFHS, and microbiome data for SAFHS that is currently being generated in the laboratory of Professor Harald Göring.

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References:

Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet. 1998 May;62(5):1198-211. PubMed PMID: 9545414; PubMed Central PMCID: PMC1377101.

Belizário JE, Faintuch J, Garay-Malpartida M. Gut Microbiome Dysbiosis and Immunometabolism: New Frontiers for Treatment of Metabolic Diseases. Mediators Inflamm. 2018 Dec 9;2018:2037838. doi: 10.1155/2018/2037838. eCollection 2018. Review. PubMed PMID: 30622429; PubMed Central PMCID: PMC6304917.

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001 May 16;285(19):2486-97. PubMed PMID: 11368702.

Göring HH, Curran JE, Johnson MP, Dyer TD, Charlesworth J, Cole SA, Jowett JB, Abraham LJ, Rainwater DL, Comuzzie AG, Mahaney MC, Almasy L, MacCluer JW, Kissebah AH, Collier GR, Moses EK, Blangero J. Discovery of expression QTLs using large-scale transcriptional

profiling in human lymphocytes. Nat Genet. 2007 Oct;39(10):1208-16. Epub 2007 Sep 16. PubMed PMID: 17873875.

Grandl G, Wolfrum C. Hemostasis, endothelial stress, inflammation, and the metabolic syndrome. Semin Immunopathol. 2018 Feb;40(2):215-224. doi: 10.1007/s00281-017-0666-5. Epub 2017 Dec 5. Review. PubMed PMID: 29209827; PubMed Central PMCID: PMC5809518.

Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet. 2008 Nov;9(11):819-30. doi: 10.1038/nrg2468. Review. PubMed PMID: 18852695; PubMed Central PMCID: PMC2829312.

Maron BA, Leopold JA. Systems biology: An emerging strategy for discovering novel pathogenetic mechanisms that promote cardiovascular disease. Glob Cardiol Sci Pract. 2016 Sep 30;2016(3):e201627. doi: 10.21542/gcsp.2016.27. Review. PubMed PMID: 29043273; PubMed Central PMCID: PMC5642838.

Martinez KB, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: Are they linked? Gut Microbes. 2017 Mar 4;8(2):130-142. doi 10.1080/19490976.2016.1270811. Epub 2017 Jan 6. Review. PubMed PMID: 28059614; PubMed Central PMCID: PMC5390820.

Mitchell BD, Kammerer CM, Blangero J, Mahaney MC, Rainwater DL, Dyke B, Hixson JE, Henkel RD, Sharp RM, Comuzzie AG, VandeBerg JL, Stern MP, MacCluer JW. Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study. Circulation. 1996 Nov 1;94(9):2159-70. PubMed PMID: 8901667.

Moore JX, Chaudhary N, Akinyemiju T. Metabolic Syndrome Prevalence by Race/Ethnicity and Sex in the United States, National Health and Nutrition Examination Survey, 1988-2012. Prev Chronic Dis. 2017 Mar 16;14:E24. doi: 10.5888/pcd14.160287. PubMed PMID: 28301314; PubMed Central PMCID: PMC5364735.

Oresic M, Vidal-Puig A (Eds.). A Systems Biology Approach to Study Metabolic Syndrome. New York, Springer, 2014.

Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep. 2018 Feb 26;20(2):12. doi: 10.1007/s11906-018-0812-z. Review. PubMed PMID: 29480368; PubMed Central PMCID: PMC5866840.

Santos-Marcos JA, Perez-Jimenez F, Camargo A. The role of diet and intestinal microbiota in the development of metabolic syndrome. J Nutr Biochem. 2019 Aug;70:1-27. doi: 10.1016/j.jnutbio.2019.03.017. Epub 2019 Apr 8. Review. PubMed PMID: 31082615.

Williams JT, Van Eerdewegh P, Almasy L, Blangero J. Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. Am J Hum Genet. 1999 Oct;65(4):1134-47. PubMed PMID: 10486333; PubMed Central PMCID: PMC1288247.

Zhou T, Hu Z, Yang S, Sun L, Yu Z, Wang G. Role of Adaptive and Innate Immunity in Type 2 Diabetes Mellitus. J Diabetes Res. 2018 Nov 8;2018:7457269. doi: 10.1155/2018/7457269. eCollection 2018. Review. PubMed PMID: 30533447; PubMed Central PMCID: PMC6250017.