IDENTIFYING GENETIC ASSOCIATIONS WITH VARIABILITY IN METABOLIC HEALTH AND BLOOD COUNT LABORATORY VALUES: DIVING INTO THE QUANTITATIVE TRAITS BY LEVERAGING LONGITUDINAL DATA FROM AN EHR*

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A wide range of patient health data is recorded in Electronic Health Records (EHR). This data includes diagnosis, surgical procedures, clinical laboratory measurements, and medication information. Together this information reflects the patient's medical history. Many studies have efficiently used this data from the EHR to find associations that are clinically relevant, either by utilizing International Classification of Diseases, version 9 (ICD-9) codes or laboratory measurements, or by designing phenotype algorithms to extract case and control status with accuracy from the EHR. Here we developed a strategy to utilize longitudinal quantitative trait data from the EHR at Geisinger Health System focusing on outpatient metabolic and complete blood panel data as a starting point. Comprehensive Metabolic Panel (CMP) as well as Complete Blood Counts (CBC) are parts of routine care and provide a comprehensive picture from high level screening of patients' overall health and disease. We randomly split our data into two datasets to allow for discovery and replication. We first conducted a genome-wide association study (GWAS) with median values of 25 different clinical laboratory measurements to identify variants from Human Omni Express Exome beadchip data that are associated with these measurements. We identified 687 variants that associated and replicated with the tested clinical measurements at $p<5x10^{-08}$. Since longitudinal data from the EHR provides a record of a patient's medical history, we utilized this information to further investigate the ICD-9 codes that might be associated with differences in variability of the measurements in the longitudinal dataset. We identified low and high variance patients by looking at changes within their individual longitudinal EHR laboratory results for each of the 25 clinical lab values (thus creating 50 groups – a high variance and a low variance for each lab variable). We then performed a PheWAS analysis with ICD-9 diagnosis codes, separately in the high variance group and the low variance group for each lab variable. We found 717 PheWAS associations that replicated at a p-value less than 0.001. Next, we evaluated the results of this study by comparing the association results between the high and low variance groups. For example, we found 39 SNPs (in multiple genes) associated with ICD-9 250.01 (Type-I diabetes) in patients with high variance of plasma glucose levels, but not in patients with low variance in plasma glucose levels. Another example is the association of 4 SNPs in *UMOD* with chronic kidney disease in patients with high variance for aspartate aminotransferase (discovery p-value: $8.71x10^{-09}$ and replication p-value: $2.03x10^{-06}$). In general, we see a pattern of many more statistically significant associations from patients with high variance in the quantitative lab variables, in comparison with the low variance group across all of the 25 laboratory measurements. This study is one of the first of its kind to utilize quantitative trait variance from longitudinal laboratory data to find associations among genetic variants and clinical phenotypes obtained from an EHR, integrating laboratory values and diagnosis codes to understand the genetic complexities of common diseases.

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1. Introduction

In this era of personalized medicine, emphasis is on preventive care facilitated by integration of a patient's medical and genomic information. De-identified electronic health records (EHR) and bio-repositories represent significant resources of information that have been widely used for association studies in past decade[1]. Electronic health record (EHR) data is primarily designed for clinical care and is represented in both structured (such as ICD-9 codes, medication information, clinical laboratory values) as well as unstructured (physician notes) forms. Many association studies have utilized ICD-9 codes as well as clinical lab variables (structured forms of EHR data) to identify variants associated with EHR-derived phenotypes that might be of clinical relevance[2–4]. The number of association studies using EHR-derived phenotypes (both structured and unstructured data) has been increasing rapidly[5].

The complete blood count (CBC) panel and comprehensive/basic metabolic panel (CMP/BMP) are part of routine medical care for all medical practices. These panels are comprised of tests that help clinical practitioners identify underlying causes for conditions like weakness and fatigue, as well as to identify chronic illnesses (e.g., kidney failure, heart disease). These tests are generally conducted on patients that show some signs of illness, but these routine measurements are conducted from time to time on healthy individuals as well. Thus, utilizing these panels can help us understand overall health of patients by comparing these measurements across all patients in an EHR. These tests are recorded as quantitative variables for which units of measurements can be standardized across multiple clinical practices. ICD-9 codes and clinical measurements go hand in hand for a patient's medical record as a diagnosis code may either initiate the lab test which confirms the code or the code may be entered as a result of the test. Thus, integrating both clinical laboratory measurements and diagnosis codes present powerful approaches for understanding genetic variants that show similar associations with both data types obtained from an EHR[3]. The majority of association studies that use quantitative traits derived from an EHR as phenotypes use either mean/median values[3,6] or most recent measurements[7]. While this approach has been successful, utilizing only mean/median values limits the understanding of these traits by neglecting the variability over time that may be present in an individual patient's clinical history. This can be captured for analysis by using unique longitudinal information from EHR. Longitudinal data provides a better picture of the patient's health by actually pinpointing the time of disease onset, or time in which the quantitative trait became out of the normal range, which is especially important for the diseases that are more heterogeneous in nature and progress over time/age. A strategy such as this has been applied to family-based studies, using a mixed effects model to find associations among candidate genes and longitudinal data[8]. Utilizing the longitudinal data in some way other than considering one value also provides the opportunity to consider not just the average, but also the variability in these traits over time. In this study, our goal was to develop a strategy to embrace the longitudinal data in a population-based dataset, using trait variance, rather than a measure of central tendency approach such as median values, by binning patients in high and low variance groups separately to then test for associations. This strategy allows for the integration of clinical lab measurements as quantitative traits, embracing the variability in the traits, along with ICD-9 code PheWAS associations as well as SNPs.

2. Materials and Methods

2.1 Genotype Data

The MyCode® Community Health Initiative is a research initiative to engage Geisinger Health System patients in research and integrate their clinical EHR data along with genetic information to make discoveries in health and disease[9]. Over 109,000 Geisinger patients have consented to participate in MyCode and approximately 50,000 participants have whole exome sequencing and genome-wide genotype data generated. For this study, we used participants that have been genotyped using the Illumina Human Omni Express plus Exome beadchip. This dataset contains 45,899 samples and ~600K variants after some initial quality control procedures. For this analysis, after sample QC (removing one sample from pairs of highly related samples up to 1st cousins and removing any samples that did not pass a sample call rate filter of 90%), we divided the total dataset into two random sets to perform discovery and replication analyses. We included only European American samples with age >18 years. Our discovery dataset consisted of 17,347 samples and our replication dataset consisted of 17,348 samples (see **Supplementary Table** 1 for demographic information on these samples). We also filtered the variants that did not pass a genotype call rate filter of 99% to keep only high quality SNP data. To test common variants only, we applied a minor allele frequency (MAF) filter of 1%. This resulted in a total of 629,274 variants that were considered for association testing in the discovery dataset and 629,016 variants tested in the replication dataset.

2.2 Phenotype Data

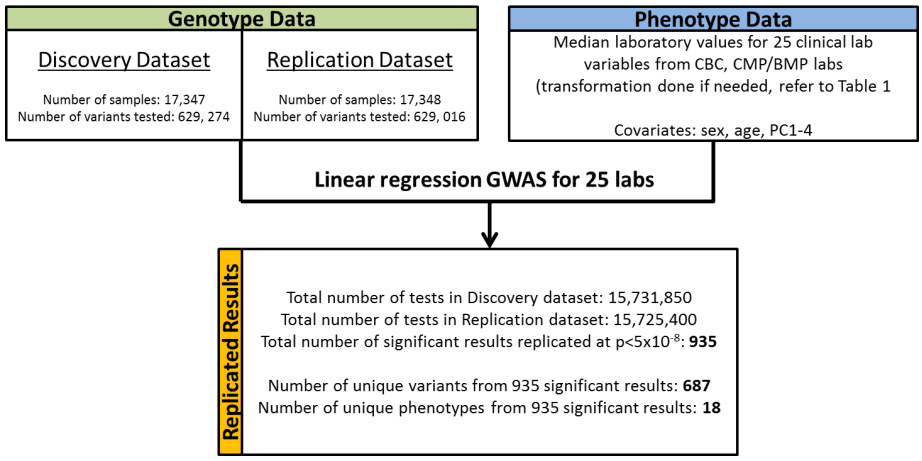
Twenty-five clinical laboratory variables were extracted from EHR outpatient data and checked for consistency of unit measurements. A list of all 25 variables is provided in Table 1, along with information on the panel from which they were obtained. The phenotype data is extracted from the EHR as longitudinal data for all patients across their clinical history. Thus, each sample has multiple entries for each variable. The first step in conducting our GWAS analysis was to obtain median values for all 25 variables across patients' longitudinal data. We wanted to be able to compare the GWAS on median values with the analyses in the high-variance and low-variance groups. We visually inspected the clinical lab variable distributions to determine which variables needed a natural log transformation. We also removed all outliers that were more than 2.5 standard deviations from the mean. While this could lose some very interesting data points, for this pilot analysis, we wanted to be sure to remove gross errors in lab variable coding/data entry. Supplementary Figure 1 and 2 show the distribution of discovery and replication datasets, respectively, after removing outliers and performing natural log transformation wherever necessary. Table 1 lists the name of the variable, how the sample is collected (i.e. Blood or Serum/Plasma), which panel the variable is obtained from (i.e. Complete Blood Count (CBC) or Comprehensive Metabolic Panel (CMP) or Basic Metabolic Panel (BMP)), the total sample size for each phenotype in both discovery and replication datasets, and whether or not the data were transformed.

Table 1. List of 25 clinical laboratory measurements that are used in the analysis.

Clinical Laboratory Measurement	Panel type	Discovery Sample Size	Replication Sample Size	Transformation
ALANINE AMINOTRANSFERASE (ALT) - SERUM/PLASMA	CMP	15527	15393	Yes
ALBUMIN - SERUM/PLASMA	CMP	15519	15439	Yes
ALKALINE PHOSPHATASE - SERUM/PLASMA	CMP	15189	15088	Yes
ANION GAP - SERUM/PLASMA	BMP/CMP	15954	15849	No
ASPARTATE AMINOTRANSFERASE (AST) - SERUM/PLASMA	CMP	15406	15310	Yes
BILIRUBIN - SERUM/PLASMA	CMP	15224	15141	Yes
CALCIUM (CA) - SERUM/PLASMA	BMP/CMP	16164	16098	No
CARBON DIOXIDE (CO2) - SERUM/PLASMA	BMP/CMP	16309	16203	No
CHLORIDE (CL) - SERUM/PLASMA	BMP/CMP	16235	16130	No
CREATININE - SERUM/PLASMA	BMP/CMP	16403	16323	Yes
Erythrocyte Distribution Width (RDW) - BLOOD	CBC	16032	15974	Yes
GLUCOSE - SERUM/PLASMA	BMP	16184	16137	Yes
Hematocrit (HCT) - BLOOD	CBC	16213	16184	No
HEMOGLOBIN - BLOOD	CBC	16234	16186	No
Mean Corpuscular Hemoglobin (MCH) - BLOOD	CBC	16175	16120	No
Mean Corpuscular Hemoglobin Concentration (MCHC) - BLOOD	CBC	16166	16114	No
Mean Corpuscular Volume (MCV) - BLOOD	CBC	16220	16161	No
PLATELET - BLOOD - COUNT	CBC	16122	16099	No
Platelet Mean Volume (MPV) - BLOOD	CBC	16281	16247	No
POTASSIUM (K) - SERUM/PLASMA	BMP/CMP	16255	16165	No
PROTEIN - SERUM/PLASMA	CMP	15002	14932	No
RBC-COUNT-BLOOD	CBC	16187	16142	No
SODIUM (NA) - SERUM/PLASMA	BMP/CMP	16222	16144	No
UREA NITROGEN - SERUM/PLASMA	BMP/CMP	16147	16049	No
WBC-COUNT-BLOOD	CBC	16478	16455	Yes

For the variance based analysis, we first calculated the variance for each sample across their longitudinal clinical data from EMR. For each clinical lab variable, we visually inspected scatterplots of the variance distribution and determined a threshold for discovery and replication datasets separately (**Supplementary Table 2**). Next, samples were divided into high and low variance groups. For the high-variance/low-variance PheWAS analyses, we extracted all ICD-9 codes from the EHR. Participants were defined as cases if they had 3 or more instances of a particular ICD-9 code; less than 3 instances per participant were set to missing; and for no occurrence of an ICD-9 code, participants were designated control status. This resulted in testing a total of 541 ICD-9 codes.

Figure 1. Flow chart describing the analyses for median lab variable linear regression GWAS on 25 clinical labs



2.3 Analysis Methods

We performed the analysis for this study as a two-step process. First we performed a GWAS on median values for 25 different clinical lab variables (**Figure 1**). Next, we took the SNPs associated with the median trait values and performed an ICD-9 code PheWAS after grouping the participants into high-variance and low-variance groups for each clinical lab variable (Figure 2). Each of these analyses is described in more detail in the following sections.

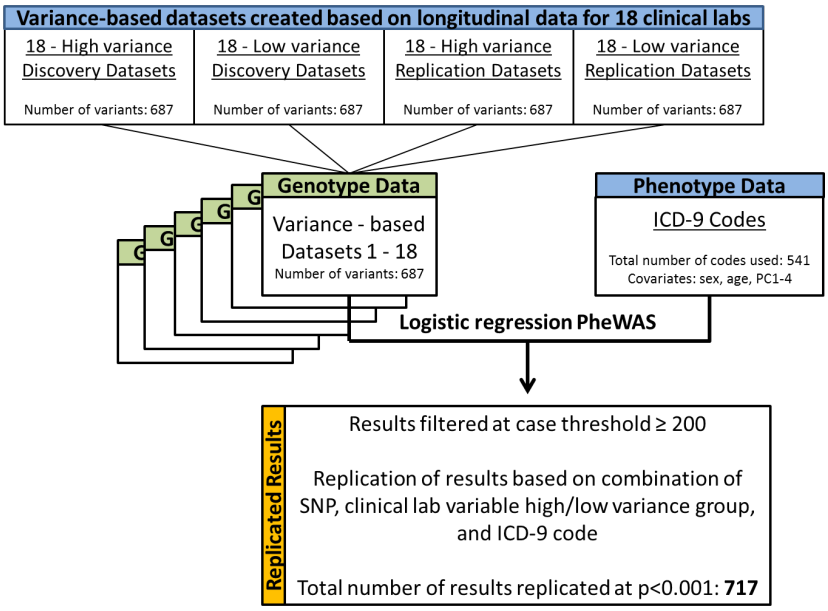
2.3.1 Genome wide association analysis for 25 median clinical laboratory measurement

We performed a genome-wide association study (GWAS) to identify associations among all variants from the data (after quality control data cleaning) with median lab values for each of the 25 phenotypes. Linear regression analysis was performed using PLATO[10] (http://ritchielab.psu.edu/software/plato-download). All models were adjusted for age, sex and first 4 principal components to control for confounding influences in the analysis. Approximately 15M (~600,000 SNPs and 25 variables) tests were performed for each patient for both discovery and replication datasets. This analysis was repeated for both discovery and replication datasets separately and then we identified p-values for all variant and clinical lab combinations that were below genome-wide significance (p-value $5x10^{-8}$) in both datasets (discovery and replication).

2.3.2 Variance-based analysis to identify associations with ICD-9 codes

For all phenotypes from the median lab GWAS that has statistically significant replicating results (18 out of the 25 clinical lab variables, see **Figure 1**), we obtained longitudinal data for each patient across the EHR and calculated the trait variance for each lab variable. Next, for each of the 18 variables, we created scatterplots of the variance to identify samples that can be categorized as high

Figure 2. Flow chart describing the PheWAS analyses for high/low variance based datasets



and low variance. Individual scatter plots for all of these variables are shown in **Supplementary Figure 3 and 4** for the discovery and replication datasets. For each variable, we created high variance and low variance groups based on a user-defined threshold to allow for PheWAS analyses separately in groups with high variability or low variability in each of the clinical lab variables. **Supplementary Table 2** lists the thresholds and samples sizes for low and high variance categories in both discovery and replication datasets. Participants below the chosen thresholds (based on looking at individual scatterplots) were categorized as low variance and above threshold were categorized as high variance.

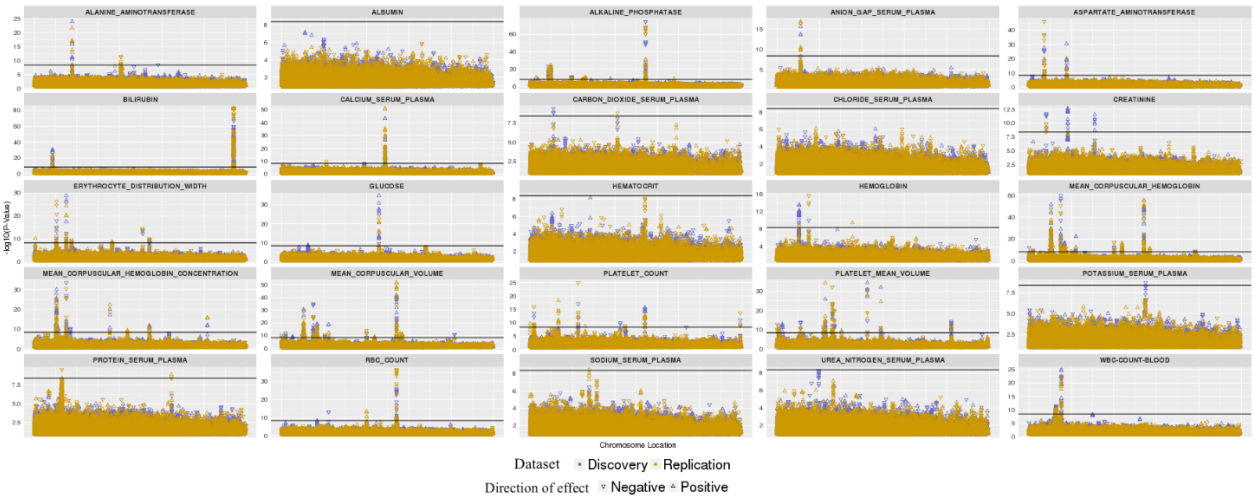
The genotype data was filtered to include only those variants (687 SNPs) that were significantly associated in both the discovery and replication datasets for one or more clinical lab variables in the GWAS of median clinical lab values. Here, we are interested in the following question: Are genetic variants that are associated with a median clinical lab variable, also associated with diagnosis codes in patients with high variability or low variability in that lab variable? In other words, are there diseases that show association with that SNP in patients who are highly variable in their lab values or perhaps have low variability in their lab values? To investigate diagnosis codes that are associated with these variants, we performed logistic regression analysis for ICD-9 codes using PLATO

(http://ritchielab.psu.edu/software/plato-download) by adjusting all models by age, sex and first 4 principal components. We only considered ICD-9 codes that had at least 200 or more cases with the code to reduce any false positive associations. Thus, for each sample 371,667 tests were erformed (687 SNPs and 541 ICD-9 codes). Lastly, we report the PheWAS results below a p-value threshold of 0.001 that replicate in low variance and/or high variance categories.

3. Results

Genome-wide association studies for median values from 25 clinical laboratory variables produced 935 SNP-phenotype associations that are present in discovery and replication sets at p-value less than $5x10^{-8}$. Association results below p-value 0.1 are shown in **Figure 3** as Manhattan plots for both discovery and replication datasets. Among the top results are multiple variants in the *UGT1A* gene family associated with serum bilirubin levels, where p-values for both discovery and replication datasets is 3.29×10^{-83}. This association has been identified and extensively reported by candidate gene and genome-wide association studies[11]. Hyperbilirubinemia results from a mutation in the *UGT1A1* gene which causes the non- or slow elimination of bilirubin from the body. We also identified variants in *SLCO1B1* associated with bilirubin levels, as suggested by previous GWAS studies [12–14] (rs4149081, Discovery p-value: $8.18x10^{-31}$ Replication p-value:$3.81x10^{-22}$).
Another association we identified is between missense variant, rs855791, on chromosome 22 in

Figure 3. Manhattan plots for GWAS performed on all 25 clinical lab variables. X-axis represents the chromosome and base pair location of each SNP and Y-axis represent the –log10 of p-value from association analysis. The two colors represent p-value for discovery and replication datasets. Direction of effect (positive or negative) is shown by the direction of arrows. Results at p-value <0.1 are shown in the plot. Black line indicates genome-wide significance (5e-08) threshold.



gene *TMPRSS6* (Discovery p-value: $2.04x10^{-60}$ (beta=-0.27); Replication p-value: $1.73x10^{-51}$ (beta=-0.25)). This association was identified by previous GWAS studies with hemoglobin levels as well as hemoglobin concentration[15,16]. It has been suggested that *TMPRSS6* is essential for maintaining iron levels in blood as it is involved in the control of iron homeostasis [16,17]. In addition, our GWAS analyses also identified many more previously reported associations, including variants

in the *ABO* gene with alkaline phosphatase[18] (rs505922, discovery p-value: 2.41×10^{-52}, replication p-value:8.48×10^{-65}), the *CASR* gene with calcium levels[19,20] (rs17251221, discovery p-value: 6.55×10^{-44}, replication p-value:2.31×10^{-51}), and the *TCF7L2* gene with glucose levels[21] (rs7903146, discovery p-value: 1.41×10^{-35}, replication p-value:6.23×10^{-24}).

To explore pleiotropic associations among variants where one SNP is associated with multiple phenotypes, we generated a phenogram plot[22] shown in **Figure 4.** This plot shows, for example, multiple associations on chromosome 10 in gene *JMJD1C* to be associated with platelet mean volume as well as alkaline phosphatase (red box on **Figure 4**). Different GWAS studies performed separately on blood and metabolic panels have identified these associations[23,24] and our study serves as confirmation for these associations when both panels are combined together and analysis is run on the same patients. In our analysis, we see opposite directions of effect for both of these associations, i.e. erythrocyte distribution width (discovery beta: -0.004 and replication beta: -0.004) and mean corpuscular hemoglobin (discovery beta: 0.09 and replication beta: 0.12) which confirms the relationship observed in anemic patients, where elevation in RDW and decrease in hemoglobin is observed.

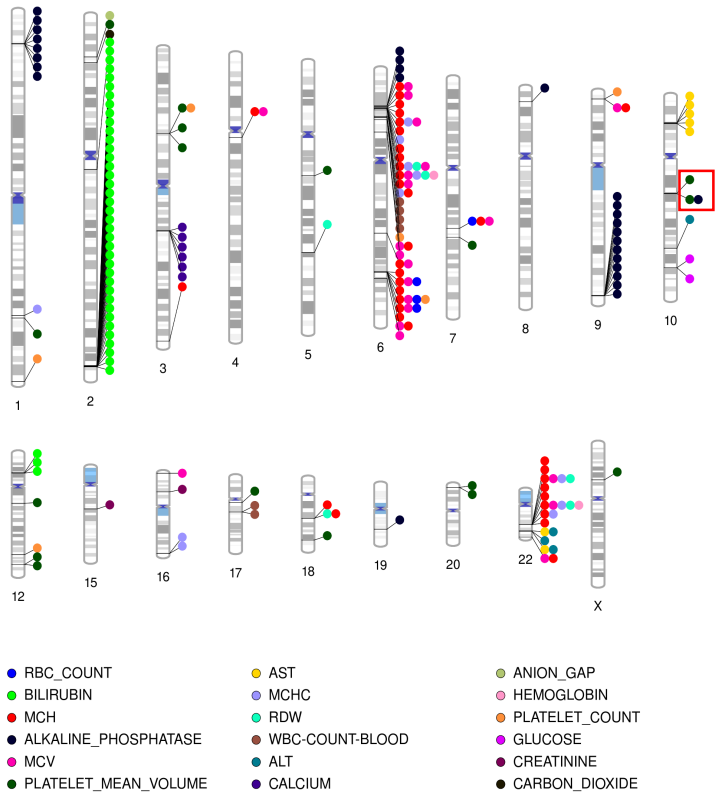


Figure 4. Phenogram plot representing pleiotropic associations. Here each colored circle is a SNP and its location is represented on the chromosome. SNPs are color coded based on the phenotype colors as shown in the legend. SNPs are also pruned to LD threshold of 0.4. Here MCH is Mean Corpuscular Hemoglobin; MCHC is MCH is Mean Corpuscular Hemoglobin Concentration; AST is Aspartate Aminotransferase; RDW is Erythrocyte Distribution Width; ALT is Alanine Aminotransferase.

Among our novel associations are intronic variant rs8095374 in gene *C18orf25* associated with erythrocyte distribution width known as RDW (discovery p-value: 8.79×10^{-10}, and replication p-value: 2.16×10^{-10}) and mean corpuscular hemoglobin (discovery p-value: 3.57×10^{-9}, and replication p-value: 1.84×10^{-13}). Both laboratory measurements are for red blood cells and could be useful in understanding the etiology of anemia.

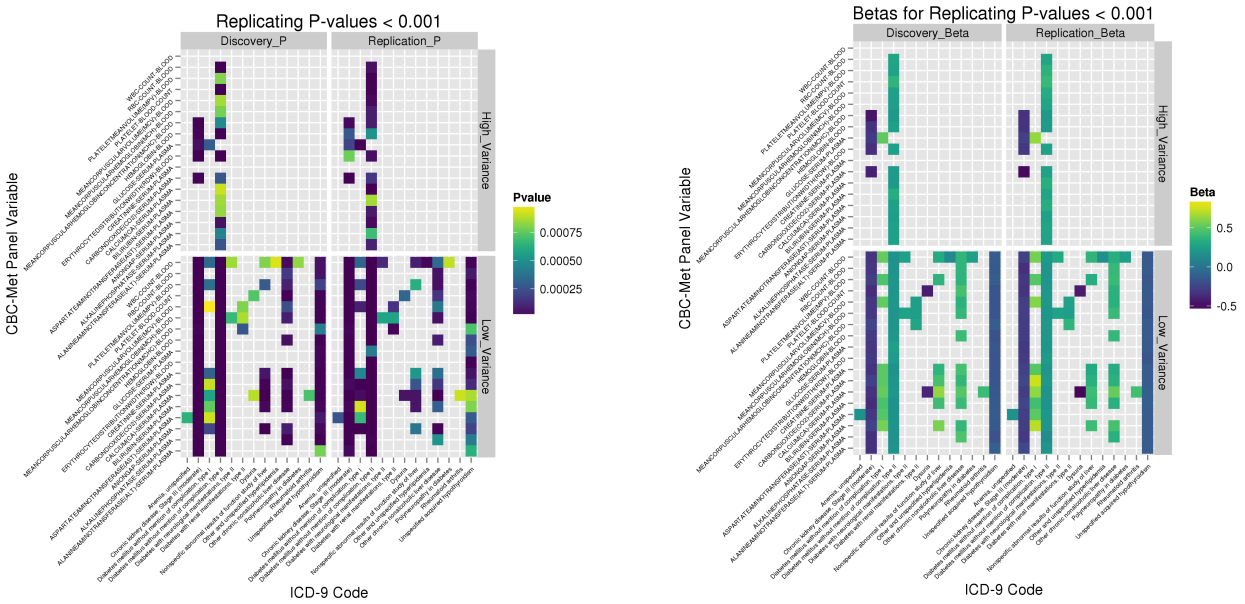


Figure 5. Heat map representing p-values (on left) and beta (on right) from variance based analysis for the combination of a SNP, ICD-9 code and clinical lab measurement in both high and low variance categories. Each point is the replicating SNP with the color gradient showing the range of p-value and beta. The results are only shown for replicating results at p-value<0.001 for both discovery and replication datasets in both high variance and low variance categories. X-axis lists all the ICD-9 codes and Y-axis lists the corresponding clinical lab variable for which replicating association is observed.

Our next approach was to integrate ICD-9 code data along with clinical lab variables to identify variants that we have found to be associated with median values of quantitative traits, and are *also* linked to diagnosis codes in the EHR. To perform this analysis, we wanted to utilize longitudinal data, rather than a measure from a single point in time. Hence, we divided patients into categories of high and low variance as described in *Methods*. Replication was observed based on the combination of SNP, clinical lab variable, ICD-9 code, and variance category (high or low). Replicated results are shown in form of a heat map in **Figure 5**. These heat maps show that in our study, the majority of our replicating associations occur in the low variance category. The primary reason for this is likely due to low sample size in the high variance groups gave us less statistical power to detect associations; although we would like to continue to explore this to determine whether there is a biological explanation for this. In total, this analysis resulted in 717 replicated associations.

We observed 39 SNPs on chromosome 6 that map to multiple genes (*C6orf10, FKBPL, BAT3, BAT2, EGFL2, RDBP, MSH5, TNXB*, C6orf27, *CSNK2B and BAT1*) are associated with Type 1 Diabetes (ICD-9 code 250.01) when the samples with high variance glucose levels were evaluated. These associations were not seen in samples in the low variance glucose category. One of the most interesting associations identified is between four SNPs in the *uromodulin (UMOD)* gene and ICD-9 code 585.3 (Chronic kidney disease) in patients with low variance for aspartate aminotransferase (discovery p-value: 8.71×10^{-9} and replication p-value: 2.03×10^{-6}). It has been observed by previous studies that patients with chronic kidney disease usually have low levels of aminotransferase in serum[25]. This association was not replicated in the high variance aspartate aminotransferase group. Association of variants in the *UMOD* gene with chronic kidney disease, kidney stones, and end

stage renal diseases has been previously established[26,27] but an association with aspartate aminotransferase levels has not been identified by previous studies. Next, to integrate both the GWAS results and variance-based grouping PheWAS results, we generated networks of all genome-wide significant results from GWAS analysis and replicated results from variance based PheWAS analysis using Cytoscape[28] as shown in **Figure 6**. We explored the integrated results for SNP-Clinical lab variable- ICD-9 code in order to identify the three-way associations that are indicative of disease diagnosis. This figure shows the three top integrated networks from our analysis where both ICD-9 codes and clinical lab variables are linked via a SNP. One thing to note here is that all these networks resulted from the low variance groups only.

From the network visualization, we determined three variants in gene *TCF7L2* are associated with Type 2 Diabetes (T2D) and glucose levels. This association is expected because these variants have been reported by many previous studies to be associated with T2D[21,29,30]. Similarly, from this network analysis we also observed variants in the *UMOD* gene associated with chronic kidney disease and creatinine levels obtained from serum which has been previously reported by GWAS[26,27,31]. Lastly, a novel network obtained from this analysis is a link between rs3132941 (mapped to gene, *EGFL8*) with WBC count and Type I Diabetes. A high WBC has been observed in a few studies in T1D patients[32,33]. The *EGFL8* gene maps near the MHC region (Major-histocompatibility complex) on chromosome 6 and thus its association with T1D can be easily

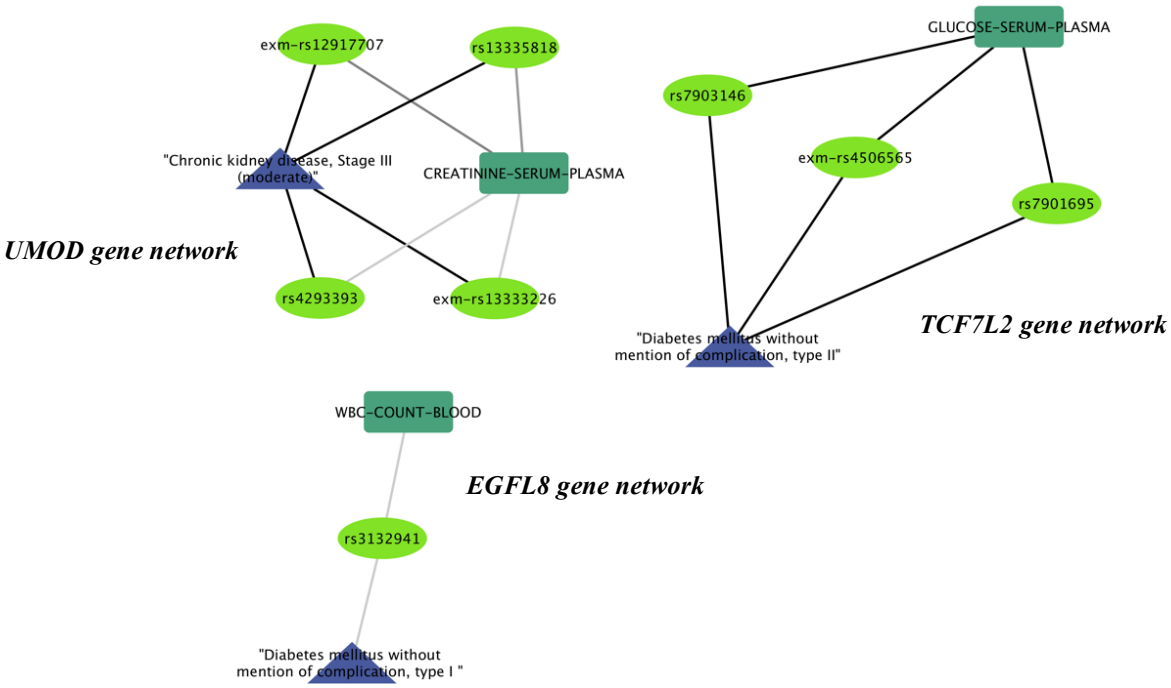


Figure 6. Network visualization generated by Cytoscape using replicated results from both GWAS and variance based analysis. Here, triangles represent ICD-9 code description, rectangles represent clinical lab variable, and ovals represent SNP. Darker edges represent more significant associations.

established[34,35] but its association with WBC has not been found in any previous studies. Our study presents this novel result which warrants further investigation.

4. Discussion

Genome-wide association studies have been tremendously successful in unravelling the etiologies of common complex diseases and the use of EHR in conducting such genome-wide and phenome-wide studies has shown resounding progress. Many researchers are now working on approaches to incorporate longitudinal information from the EHR into these studies. As a proof of concept, in this study we aimed at advancing the use of longitudinal information from laboratory values by looking at the variance for each outpatient clinical lab value rather than just mean/median or most recent value. We first conducted a GWAS for 25 clinical lab median values and then, based on variance, we divided participants into high and low variance groups. Next, we conducted a PheWAS to identify which SNPs are associated with median clinical lab variable *and* ICD-9 codes. This study represents a proof-of concept approach for utilizing trait variance and the longitudinal data as we successfully identified and confirmed many previously known associations. We also described several novel associations observed from our study. Variance, rather than mean/median may better capture the richness of the longitudinal data. In this pilot analysis, we demonstrate that this approach can be used to identify networks which reveal trends of associations among SNPs, laboratory measurements, and diagnosis codes. In the future, we plan to replicate this analysis with a larger sample size and in an independent EHR system. We also plan to use variance as the outcome for an association study in all 50,000 patients from Geisinger MyCode dataset and replicate in an independent dataset. One limitation of our approach here is that the use of longitudinal data in the way shown in this study ignores the fact that in an EHR, the duration of longitudinal information varies from patient to patient. Future approaches should also focus on developing methods which adjust for the duration of longitudinal information. Developing approaches, such as the one described in this manuscript, to explore the longitudinal nature of EHR data will provide greater opportunities for discovery and understanding of the genetic and clinical architecture of common diseases.

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