The Genetics of Red Blood Cell Density in Sickle Cell Anemia Patients

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Introduction

Sickle cell disease (SCD) is an inherited recessive genetic disorder that affects hemoglobin, the protein in red blood cells that carries oxygen throughout the body. Individuals with the disease produce an abnormal hemoglobin called hemoglobin S, which causes red blood cells to assume their sickled shape. The disease is not contagious; it can only be passed on from parents to their children through genes[1].

According to the World Health Organization, the disease is observed most commonly in people with African ancestry, but it is also found in individuals of Hispanic, Mediterranean, Indian and Arab descent. Additionally, the organization estimates that as of 2011, about 5% of the global population carries the gene causing the disease.

Background

The clinical features of SCD vary greatly from patient to patient. As a result, it is very challenging to predict which patients have an increased risk for clinical complications and thus which treatment to prescribe[2]. Dehydrated red blood cell density (DRBC) is a typical attribute of sickle cell disease (SCD) patients. Recent studies[3] have linked higher percentage of red blood cell density with increased incidence of renal dysfunction, skin ulcers, and priapism. These complications are SCD-related pathophysiologies caused by the deformation of erythrocytes in blood vessels.

Objective

Based on these novel findings, we sought to identify the genetic determinants of DRBC.

Methods

Association Tests Pipeline

We conducted a genome-wide association study in 405 SCD from the GEN-MOD study. These participants are of recent African descent and were recruited in Paris, France. Genotyping was done on the Illumina Infinium HumanOmni2.5Exome-8v1.1 array and imputation was performed using 1000 Genomes Phase 3 haplotypes (version 5). Because some rare DNA sequence variants were lost during imputation, we analyzed in parallel the genotyped (2,405,472 markers) and the imputed (31,257,879 markers) datasets for associations with DRBC. We also performed gene-based tests (SKAT & VT), focusing on non-synonymous and splice site variants with minor allele frequency < 5%. DRBC was analyzed using additive linear regression model, correcting for age, sex, and the first 10 principal components. Figure 1 provides more details on the approach.

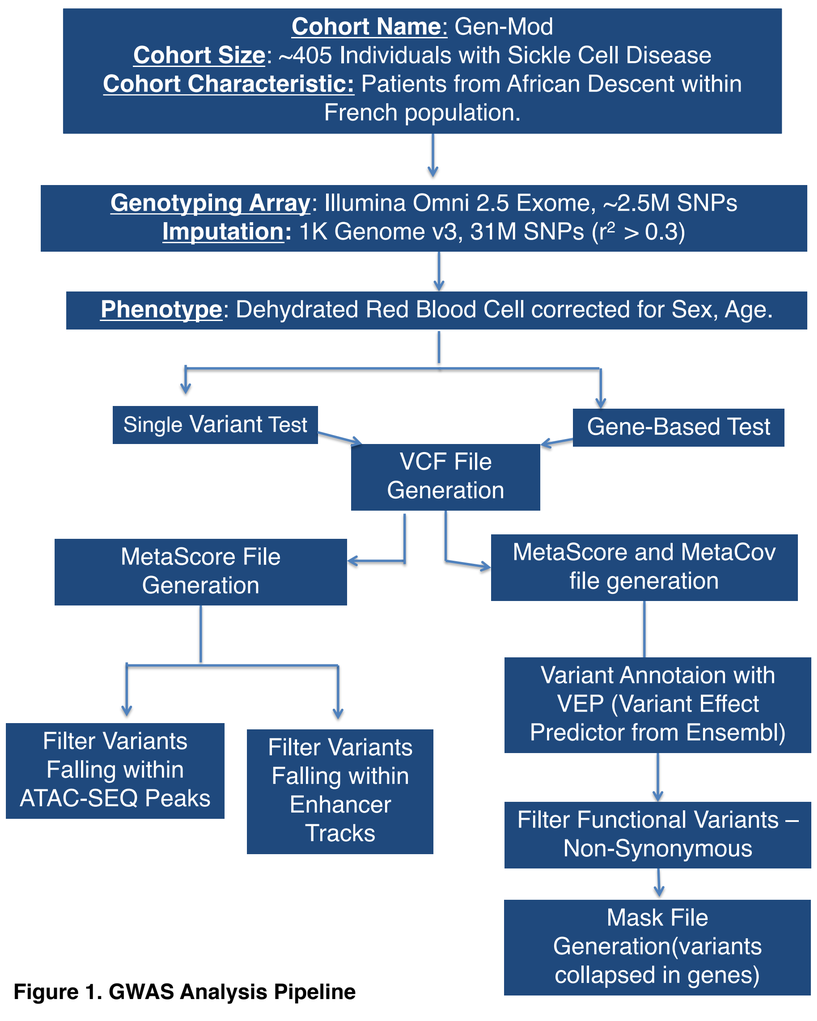

Figure 1. GWAS Analysis Pipeline

Methods (continued)

Polygenic Risk Scoring (PRS)

In the absence of significant results in the GWAS, we created a polygenic risk score based on the results from a genome-wide association study of red blood cells in 135,367 individiduals[4]. The PRS were calculated using the risk allele's effect size, using PLINK's –score function[5]. We selected the sentinel SNPs at each of the 75 loci reported. In our model, both the scores and the %DRBC were normalized, and for each association we adjusted for gender, age, and the first 10 ancestry components to account population stratification.

Results

Association Tests – 31 Millions SNPs

The association tests were inconclusive as none of the variants reached genome-wide significance ($P<5x10^{-8}$). Potentially because of our modest sample size and thus limited statistical power (See Figure 2 and Figure 3).

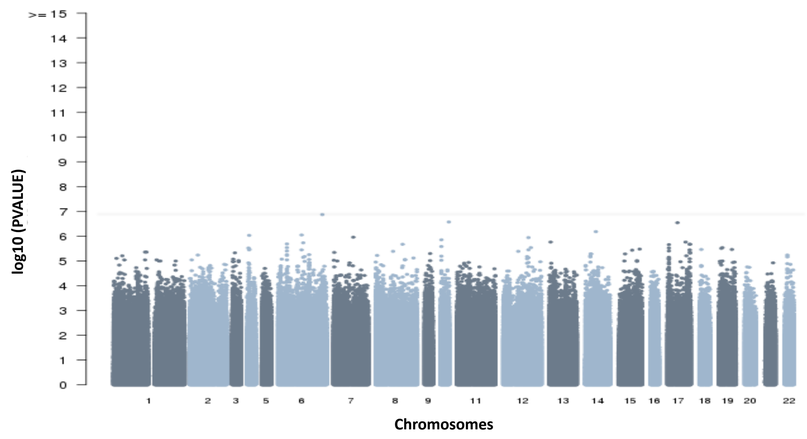

Figure 2. Manhattan Plot - Single Variants Analysis -Imputed Dataset

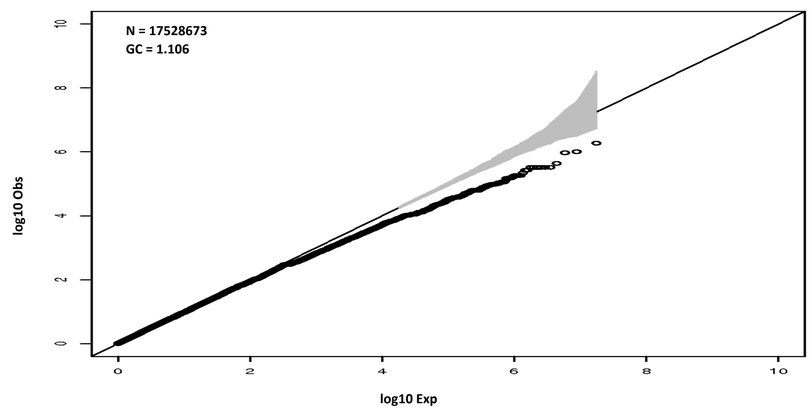

Figure 3. QQ-Plot Single Variants Analysis - Imputed Dataset

Polygenic Risk Scoring – 75 Loci

Table 1. List of variants with nominal significant results in GENMOD cohort

SNP	EA	Beta	GENMOD P value	Phenotypes
rs2867932	G	-0.021	0.013	MCHC
rs7551442	A	-0.023	0.021	MCHC
rs888424	A	0.006	0.022	MCH
rs7529925	C	0.014	0.022	RBC
rs3184504	T	0.051	0.025	HB
rs10159477	A	0.087	0.031	HB
rs1175550	G	0.008	0.032	MCHC
rs855791	G	0.012	0.033	MCH
rs13061823	T	-0.168	0.034	MCV
rs2572207	C	0.153	0.038	MCV
rs5754217	G	0.194	0.044	MCV
rs9369427	A	0.042	0.046	HB

EA: Effect Allele, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean Corpuscular Hemoglobin, MCV: Mean Cell Volume, HB: Hemoglobin, RBC: Red Blood Cell

Results (continued)

Table 2. Polygenic Risk Score (PRS) association with red blood cell traits.

DRBC ~ $PRS_{RBC\ Traits}$	Beta	P value	SE	Adj.R^2	N	SNPs (N)
DRBC ~ PRS_{All}	0.002	0.972	0.054	0.010	374	75
DRBC ~ PRS_{MCHC}	0.011	0.837	0.055	0.010	374	8
DRBC ~ PRS_{MCH}	0.051	0.331	0.053	0.013	374	19
DRBC ~ PRS_{MCV}	-0.123	0.026	0.055	0.024	374	23
DRBC ~ $PRS_{MCV\ Unweighted}$	-0.132	0.018	0.056	0.025	374	23

Beta: regression coefficient in linear regression, SE: Standard Error, Adj R^2: adjusted variance.

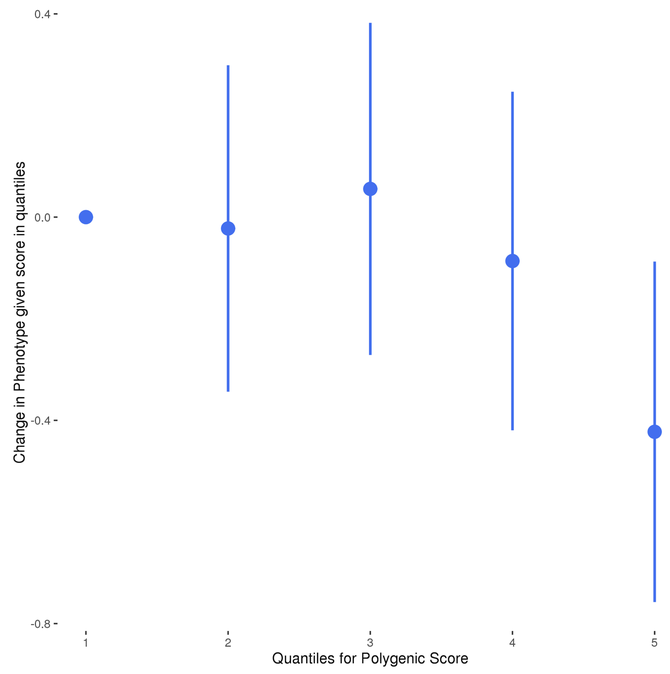

Figure 4. PRS DRBC~MCV Coefficient versus Quantiles

Conclusions

We present the first genetic study of DRBC, a biomarker and potential modifier of severity in SCD. We found no evidence of common variants with strong effect on DRBC variation. Our polygenic risk supports that SNPs associated with MCV contribute to the genetics of DRBC. Additional samples are required to replicate this result, and thus further confirm this association.

References

1. NIH. (2015, June 12). *National Heart, Lung and Blood Institute*. Retrieved January 15, 2016, from Health Information for the Public: http://www.nhlbi.nih.gov/health/health-topics/topics/sca
2. Elmariah, H.et al. Factors associated with survival in a contemporary adult sickle cell disease cohort. American journal of hematology 89, 530-˘-535, (2014).
3. Pablo Bartolucci, C. B.-P. (2012). Erythrocyte density in sickle cell syndromes is associated with specific clinical manifestations and hemolysis. *Blood Journal , 120* (15), 3136 – 3141.
4. Van der Harst et al. (2012). Seventy-five genetic loci influencing the human red blood cell. Nature, 2012: p. 369-75.
5. Purcell S et al., (2007), PLINK a toolset for whole genome wide association and population based linkage analysis. American Journal of Human Genetics, 81

Acknowledgements

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