Whole Genome Association Study of Quantitative CCP Titer in Rheumatoid Arthritis

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PURPOSE

To identify genes that influence anti-CCP titer in rheumatoid arthritis (RA) patients.

METHOD

- **Study Population** BRASS Longitudinal RA cohort. Enrolling patients that meet ACR RA criteria since 2003
- **Anti-CCP and HLA** Anti-CCP measured using a second generation ELISA assay. HLA-DRB1 serotypes assessed at the DNA sequence level using allele-specific polymerase chain reaction (AS-PCR).
- **Genotyping and quality control**
  - Genotyping performed at the Broad Institute using the Affymetrix 100K chip containing 116,204 SNPs.
  - Quality control filtering criteria: genotype call rate >=0.9, minor allele frequency (MAF) >=0.01, p-value for Hardy-Weinberg equilibrium test >=0.0001.

Population Stratification

Datasets screened for population stratification by evaluation of pairwise IBS sharing distance across all samples using PLINK.Standard classical multidimensional scaling was used. Results shown in Figure 1.

- **Statistical Model**
  - Simulation study was done to validate if general linear regression (GLM) is appropriate. Result supported use of GLM is valid (not shown).
  - Each SNP is tested for association with Anti-CCP titer using a GLM model, the additive effect is modeled by encoded 0, 1, 2 for the effect of the three genotypes if MAF>=0.2, otherwise the dominant model is used.
  - Additional modeling controlling for HLA-DRB1 SE locus were also performed.

RESULTS

Of 575 individuals genotyped, 531 passed population stratification. Demographics and clinic characteristics of the final analysis are summarized in Table 1.

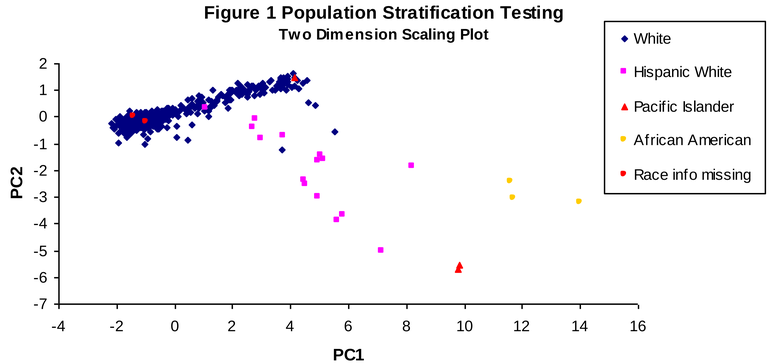
Most significant SNP is in the MHC region. There are several other regions with intermediate levels of significance that might contain potential candidate genes that affect Anti-CCP titer. The top 25 SNPs are listed in Table2. A graphical summary is in Figure 2.

Table 1 Demographics of the 100K BRASS cohort (N=531)

Mean age (SD)	58.0±13.6
Mean age at RA diagnosis (SD)	41.9±14.8
Mean years disease duration (SD)	16.1±12.5
Percent female	82.3
Percent early onset RA (new RA, <2 yrs)	13.7
Percent treated with medications at baseline	
Methotrexate alone	31.3
Anti-TNF alone	21.4
Methotrexate and anti-TNF	17.4
Percent CCP+	75.2
Mean Anti-CCP titer (SD)	142.59±121.51
Percent RF+	75.5
Percent HLA-DRB1 Shared Epitope copies	
0	31.4
1	40.7
2	27.9

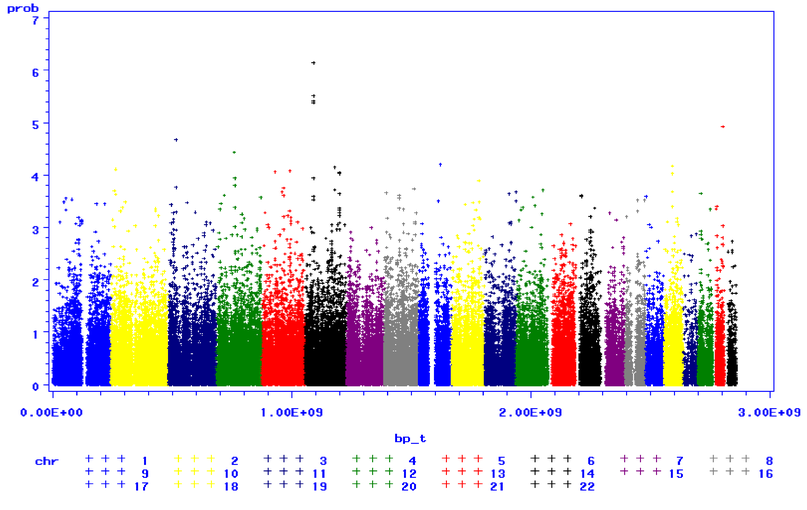
RESULTS

Figure 1 Population Stratification Testing
Two Dimension Scaling Plot



- Two-dimensional scaling plot from PLINK to visualize samples similarity.
- Result support that all self-reported non-Hispanic Caucasian could be utilized in the analysis.

Figure 2 GWAS on Anti—CCP titers
genotype rate>=.95



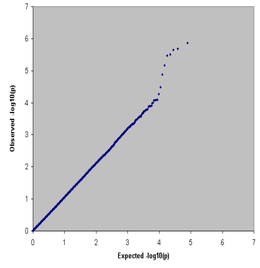
RESULTS

Table 2 Top 25 findings for Anti-CCP titer using GLM, after adjusting for SE status

Chr	RS number	Physical Location	Gene	P value	P value adjusted SE	MAF
6	rs1041885	32520787	HLA-DRA	1.41E-06	0.000105	0.133523
6	rs2395167	32496286	BTNL2	2.07E-06	0.000173	0.130975
6	rs2001097	32491836	HLA-DRA	2.32E-06	0.000117	0.132296
6	rs2213580	32496552	HLA-DRA	3.23E-06	0.000215	0.132075
6	rs2001099	32491611	BTNL2	3.53E-06	0.000235	0.134216
22	rs12781	34909742	APOL4	7.05E-06	1.13E-05	0.461145
21	rs2837108	39953480	C21orf8	1.33E-05	2.18E-05	0.473384
5	rs2112342	116261135	---	3.34E-05	0.000126	0.367675
9	rs4877406	87990359	SPIN	5.37E-05	0.00015	0.27593
8	rs2945913	8235635	---	8.23E-05	0.00036	0.150962
2	rs9287830	164097697	FIGN	8.39E-05	0.000218	0.016129
18	rs1147760	32654700	C18orf1	8.55E-05	9.59E-05	0.136364
6	rs10484713	140662577	---	8.64E-05	0.000209	0.254826
4	rs842873	73061500	GPR74	0.000103	7.88E-05	0.439623
6	rs5000563	32512113	HLA-DRA	0.000106	0.000147	0.254269
19	rs10518269	35720506	ZNF536	0.000125	2.32E-05	0.149057
5	rs2056403	52399256	ITGA2	0.00013	8.24E-05	0.257198
3	rs725382	7786984	GRM7	0.000131	8.03E-05	0.190341
18	rs10502668	32756590	KIAA132	0.000131	0.000155	0.129735
5	rs10514299	87699366	MGC3321	0.000135	0.000524	0.238679
2	rs2033873	12053428	LPIN1	0.000162	0.000184	0.358527
6	rs1357056	122405433	GJA1	0.000166	0.000332	0.063327
3	rs16824162	155741255	MME	0.00017	9.92E-05	0.01127
3	rs10510617	28989642	RBMS3	0.000175	0.00011	0.480189
6	rs6922541	135524512	HBS1L	0.000178	0.000444	0.403846

Permutation testing was done to evaluate the significance of the GWAS. Phenotype and genotype were randomly linked 100 times to get null distribution 'significant' count at each alpha level. Permutation result was compared with the observed association. If SNPs with genotype rate greater than 95% were used, 128 tests reach significance level of 0.0001; we would expect 90 by chance. A Q-Q plot also showed in Figure 3.

Figure 3 Q-Q plot



This Quantile-quantile plots is comparing expected statistics against the observed ones. In this case, the significance findings exceeded the expected, suggesting there are real SNPs influencing the CCP-titer.

CONCLUSION

This GWAS conducted on the anti-CCP titer in RA suggest that the most significant genome region associated with anti-CCP is MHC. We also identified a set of potential SNPs outside the MHC region, which may influence anti-CCP titer. These findings should be confirmed in an independent sample.