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Brief Communication

Large Cohort Association of Single Nucleotide Polymorphism of *PLA2G4A* Gene with White Blood Cell Counts in Korean Population

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The PLA2G4A catalyzes the hydrolysis of membrane phospholipids to release arachidonic acid, which is metabolized into lipid-based cellular hormones that regulate inflammatory response. The circulating blood cell numbers can be influenced by stress, infection or inflammation. Quantitative blood cell count traits analysis for the 19 SNPs in the *PLA2G4A* gene in the Korean Association Resource (KARE) cohort (7551 subjects) was performed. The only one SNP (rs10752979) in the all blood cell count was satisfied with the Bonferroni corrected *P*-value (<0.00263). Furthermore, 6 of the 19 SNPs in the *PLA2G4A* gene showed a weak or moderate association with blood cell count (*P*-values: 0.0048~0.042), suggesting the clue of an association between the *PLA2G4A* gene and blood cell count, especially white blood cell count. This study may provide insight into the genetic basis of blood cell count related with reaction of infection.

Key Words: Single nucleotide polymorphism, Blood cell count, Association, *PLA2G4A*

The *PLA2G4A* gene [phospholipase A2, group IVA (cytosolic, calcium-dependent)] encodes a member of the cytosolic phospholipase A2 group IV family. The phospholipase enzyme catalyzes the hydrolysis of membrane phospholipids to release arachidonic acid which is subsequently metabolized into eicosanoids (Matin and Jung, 2011). Eicosanoids, including prostaglandins and leukotrienes, are lipid-based cellular hormones that regulate hemodynamics, inflammatory responses and other intracellular pathways (Dennis, 1994; Ghannoum, 2000; Istivan and Coloe, 2006). Furthermore, phospholipases are proven targets in the prevention and treatment of microbial infections. For example, targeting phospholipases using synthetic compounds successfully treated *Candida* infections (Hanel et al., 1995). It has also reported that phospholipase A2 be associated with *Acanthamoeba* infections (Mortazavi et al., 2011; Matin and Jung, 2011).

Proliferation of hematopoietic stem cells and their differentiation into mature white blood cells (WBC) in the bone marrow, followed by release into the circulation of mature WBC, is a highly regulated process (Metcalf, 2008). WBC comprises several subtypes including neutrophils, lymphocytes, monocytes, eosinophils and basophils. These cells play an essential role in innate and adaptive immunity against invading microorganisms. The circulating numbers of leukocytes can be influenced by stress, infection or inflammation.

The objective of this study was to identify polymorphisms associated with *PLA2G4A* gene and inflammation through WBC counts, a large cohort association study of typed SNPs in Korean population was performed.

The subjects of the Korean Association Resource (KARE) study which was applied in this study have been described in the previous report (Cho et al., 2009). Briefly, a total of 8842 participants aged from 40 to 69 years was recruited from two community-based epidemiological cohorts, the rural community of Ansung and the urban community of

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Ansan cities. Both cohorts were studied in 2001 as part of the Korean Genome Epidemiology Study (KoGES). To analyze accurate blood cell count traits, 1291 subjects who had been treated with drugs were excluded, and the remaining 7551 subjects were finally investigated in this study. The basic characteristics of the subjects are shown in Table 1. The parameters that were measured were height, weight and body mass index [BMI: weight (kg)/square of height (m^2)]. Blood samples were drawn for blood cell count: white blood cell, red blood cell, and platelet, respectively. This study was approved by the Institutional Review Board Committees of the Korean National Institute of Health. Written informed consent was obtained from all the subjects.

Table 1. Basic characteristics of the subjects in the KARE study cohort

Characteristics	Mean ± SD
Number of subjects	7551
Gender [men (%)/women (%)]	3747 (49.6) / 3804 (50.4)
Age (years)	51.44 ± 8.78
Body mass index, BMI (kg/m^2)	24.42 ± 3.07
White blood cell ($10^3/\mu L$)	6.54 ± 1.82
Red blood cell ($10^3/\mu L$)	4.42 ± 0.47
Platelet ($10^3/\mu L$)	264.67 ± 64.43

Abbreviation: SD, standard deviation.

The genotype data were graciously provided by the Center for Genome Science, the Korea National Institute of Health. The detailed genotyping and quality control processes have been described in the previous report (Cho et al., 2009). Briefly, most DNA samples were isolated from the peripheral blood of participants and genotyped using the Affymetix Genome-Wide Human SNP array 5.0 (Affymetrix, Santa Clara, CA, USA). The SNPs studied in the *PLA2G4A* gene were selected based on their locations within the gene boundary (20 kb upstream and downstream of the first and last exons, respectively) according to NCBI human genome build 36 (Table 2). The location of the SNPs was validated with the Ensemble BioMart database (http://www.ensembl.org/biomart).

Most statistical analyses were performed using PLINK

Table 2. Information on the SNPs analyzed in the *PLA2G4A* gene

No.	SNP	Location (bp)	Minor allele	MAF	Genotyping rate	HWE *p*	Function
1	rs4651330	185083273	G	0.245	98.85	0.2853	Intron
2	rs10752979	185102618	T	0.235	99.97	0.8825	Intron
3	rs17591814	185113221	A	0.087	99.81	0.2263	Intron
4	rs12720526	185117329	A	0.012	97.01	0.6409	Intron
5	rs10911944	185117543	G	0.025	99.88	0.1875	Intron
6	rs2049963	185117839	A	0.492	99.36	0.0092	Intron
7	rs16826049	185119264	A	0.027	99.99	0.3184	Intron
8	rs12042344	185124758	C	0.367	98.12	0.3555	Intron
9	rs1569479	185140327	A	0.446	99.25	0.0696	Intron
10	rs10798068	185141631	G	0.445	99.92	0.0330	Intron
11	rs10798069	185142082	A	0.445	99.99	0.0430	Intron
12	rs4336803	185161025	A	0.393	99.47	0.0095	Intron
13	rs10911963	185178610	G	0.396	100.00	0.0558	Intron
14	rs7555140	185184143	C	0.161	99.93	0.9371	Intron
15	rs12720662	185200715	A	0.163	99.93	0.9688	Intron
16	rs35782442	185204926	C	0.137	99.98	0.3222	Intron
17	rs6690278	185212633	T	0.028	99.98	0.1073	Intron
18	rs10737278	185214650	G	0.450	99.97	0.2128	Intron
19	rs10489410	185220818	C	0.189	100.00	0.1754	Intron

Abbreviation: HWE *p*, Hardy-Weinberg equilibrium *P*-value; MAF, minor allele frequency.

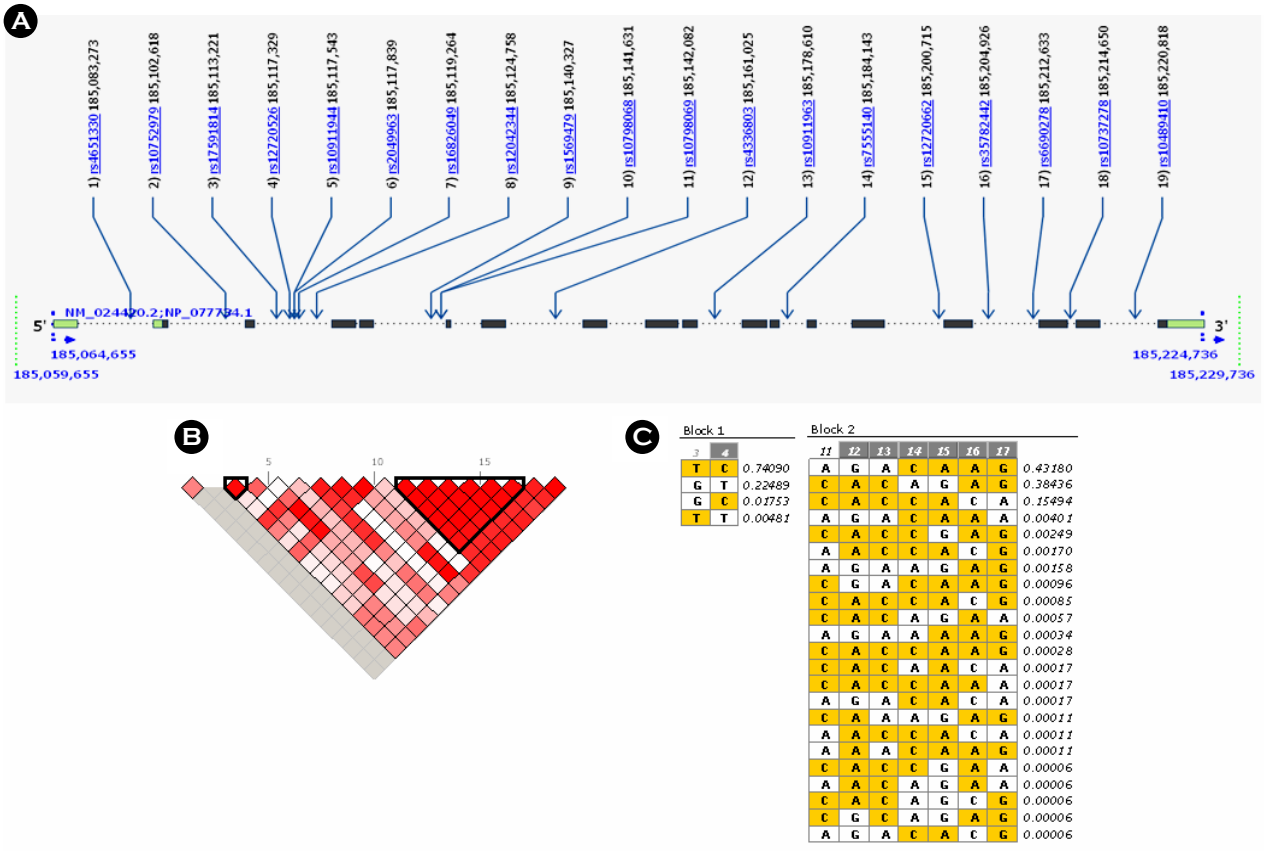

Fig. 1. The structure and SNPs of *PLA2G4A* gene (A), LD Map (B) and LD-Block and Haplotype (C)

version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink) and PASW Statistics version 17.0 (SPSS Inc., Chicago, IL, USA). Linear regression was used to analyze the three kinds of blood cell count as quantitative traits. All association tests were based on the additive, dominant and recessive model, and *P*-values were not adjusted for multiple tests. Age, gender, body mass index (BMI) and cohorts were included as covariates in all the models. Statistical significance was determined by the two-tailed Student's *t*-test at a *P* value of <0.05. The SNPStudio program (ISTECH Inc., Seoul, Korea) was used to examine the structure of the linkage disequilibrium (LD) block using the KARE genotype data.

Basic characteristics of the total 7551 subjects in the KARE study cohort are shown in Table 1. In the quantitative analysis of the 7551 subjects, sex ratio was approximately equal and the mean age was 51.44 ± 8.78 years. Mean body mass index was 24.42 ± 3.07 kg/m^2. The mean value of white blood cell count was 6.54 × 10^3/μL, that of red blood cell count was 4.42 × 10^3/μL, and the that of platelet was 264.67 × 10^3/μL.

Information about the SNPs analyzed in the *PLA2G4A* gene is shown in Table 2. The LD blocks of the gene were generated by the SNPStudio program using the KARE data of Korean population and are shown in the representative Fig. 1. The total 19 SNPs in the *PLA2G4A* gene were partitioned into major 2 LD blocks.

The quantitative blood cell count traits analysis for the 19 SNPs in the *PLA2G4A* gene in the total KARE study cohort (7551 subjects) was performed. The only one SNP (rs10752979) in the all blood cell count was satisfied with the Bonferroni corrected *P*-value (<0.00263 calculated by 0.05/19 SNPs) (Table 3). Furthermore, 6 of the 19 SNPs in the *PLA2G4A* gene showed a weak or moderate association with blood cell count (*P*-values: 0.0048~0.042) (Table 3), suggesting the clue of an association between the *PLA2G4A* gene and blood cell count, especially white blood cell count.

In the HuGe Navigator database (http://hugenavigator.net), 32 research articles demonstrating the association results of

Table 3. The associations with genetic variations of *PLA2G4A* gene and blood cell count, controlling for cohort, age, sex and BMI

SNP	Minor allele	MAF	White blood cell				Red blood cell				Platelet			
			beta ± s.e.m.	Add*p*	Dom*p*	Rec*p*	beta ± s.e.m.	Add*p*	Dom*p*	Rec*p*	beta ± s.e.m.	Add*p*	Dom*p*	Rec*p*
rs4651330	G	0.245	-0.069 ± 0.034	0.042	4.8×10^{-3}	0.539	-0.009 ± 0.007	0.192	0.239	0.371	0.272 ± 1.210	0.822	0.836	0.322
rs10752979	T	0.235	-0.084 ± 0.034	0.015	5.5×10^{-4}	0.288	-0.010 ± 0.007	0.143	0.207	0.262	-0.083 ± 1.226	0.946	0.705	0.524
rs17591814	A	0.087	-0.023 ± 0.053	0.664	0.683	0.809	0.000 ± 0.011	0.988	0.984	0.982	1.645 ± 1.879	0.381	0.369	0.908
rs12720526	A	0.012	-0.374 ± 0.140	7.8×10^{-3}	7.8×10^{-3}	NA	-0.033 ± 0.028	0.240	0.240	NA	-5.239 ± 4.998	0.295	0.295	NA
rs10911944	G	0.025	-0.005 ± 0.092	0.959	0.870	0.386	-0.024 ± 0.019	0.191	0.186	0.899	-0.723 ± 3.286	0.826	0.612	0.029
rs2049963	A	0.492	0.034 ± 0.030	0.258	0.511	0.236	-0.009 ± 0.006	0.117	8.8×10^{-3}	0.909	0.678 ± 1.060	0.523	0.209	0.814
rs16826049	A	0.027	-0.010 ± 0.088	0.913	0.656	0.020	0.020 ± 0.018	0.252	0.245	0.918	-0.961 ± 3.145	0.760	0.579	0.093
rs12042344	C	0.367	0.039 ± 0.030	0.199	0.120	0.733	-0.006 ± 0.006	0.368	0.075	0.462	0.377 ± 1.081	0.728	0.561	0.893
rs1569479	A	0.446	-0.043 ± 0.029	0.139	0.326	0.140	0.000 ± 0.006	0.951	0.821	0.879	-0.815 ± 1.037	0.432	0.589	0.445
rs10798068	G	0.445	-0.043 ± 0.029	0.137	0.350	0.121	-0.001 ± 0.006	0.892	0.785	0.940	-0.832 ± 1.031	0.420	0.581	0.430
rs10798069	A	0.445	-0.044 ± 0.029	0.127	0.342	0.109	-0.001 ± 0.006	0.892	0.820	0.984	-0.873 ± 1.031	0.397	0.572	0.399
rs4336803	A	0.393	0.045 ± 0.030	0.126	0.179	0.250	0.003 ± 0.006	0.559	0.475	0.864	0.228 ± 1.050	0.828	0.526	0.671
rs10911963	G	0.396	0.050 ± 0.030	0.088	0.196	0.123	0.002 ± 0.006	0.697	0.707	0.805	0.477 ± 1.052	0.651	0.405	0.813
rs7555140	C	0.161	-0.003 ± 0.040	0.934	0.386	0.030	-0.004 ± 0.008	0.627	0.396	0.412	0.888 ± 1.407	0.528	0.746	0.262
rs12720662	A	0.163	0.003 ± 0.039	0.946	0.566	0.068	-0.005 ± 0.008	0.553	0.359	0.505	0.873 ± 1.402	0.533	0.649	0.469
rs35782442	C	0.137	0.001 ± 0.042	0.991	0.691	0.206	-0.006 ± 0.008	0.469	0.343	0.681	1.137 ± 1.498	0.448	0.700	0.149
rs6690278	T	0.028	-0.141 ± 0.088	0.111	0.149	0.195	0.001 ± 0.018	0.967	0.917	0.700	1.523 ± 3.143	0.628	0.661	0.691
rs10737278	G	0.450	0.012 ± 0.029	0.683	0.518	0.984	-0.004 ± 0.006	0.460	0.391	0.749	-0.405 ± 1.037	0.696	0.900	0.589
rs10489410	C	0.189	-0.012 ± 0.037	0.751	0.785	0.112	0.003 ± 0.007	0.668	0.706	0.751	0.802 ± 1.313	0.541	0.384	0.713

Statistical significance ($P<0.05$) are indicated in bold and underline. Abbreviation: MAF, Minor allele frequency; S.E.M, standard error of the mean; NA, not applicable; Add*p*, *P* value of additive genetic model; Dom*p*, *P* value of dominant genetic model; Rec*p*, *P* value of recessive genetic model.

the *PLA2G4A* gene with various phenotypes, including schizophrenia, premature birth, inflammation, asthma, cardiovascular disease, and so on, have been listed.

In this study, we aimed to confirm this association result and have found that genetic variation of the *PLA2G4A* gene is significantly associated with blood cell count in the Korean study cohorts that recruited participants from rural or urban areas. Especially, the *P*-values of the significant SNP, rs10752979 for WBC were lower in satisfying the Bonferroni corrected *P*-value (<0.00263) (Table 3). These

results imply that microbial infections be associated with the action of PLA2G4A.

In summary, quantitative blood cell count traits association analysis for the genetic variation of the *PLA2G4A* gene in the Korean cohorts (total 7751 unrelated subjects) revealed a significant association between the SNP and blood cell count traits. In addition to, our study provides additional evidence in support of the finding that the *PLA2G4A* gene may be related to infection of microbes. This study may provide insight into the genetic basis of blood cell count related to reaction of infection.

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