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Genetic influence of HLA-DR on longevity in Okinawan people

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ABSTRACT

Okinawa is well-known as one of the longevity areas in the world and the population rate of centenarians is higher by far than that of the whole of Japan. However, only few reports have used genetic approaches to explain Okinawan long lifespan. In this study, we analyzed human luekocyte antigen (HLA) both in phenotyping and in genotyping for the same subjects of Okinawan centenarians and normal adults for the purpose of clarifying one of the primary genetic factors in major histo-compatibility complex (MHC) region genes associated with longevity. Eighty-six healthy centenarians and 142 normal adults in Okinawa were studied. For HLA phenotyping, 14 antigen specificities on DR locus were typed according to the standard microdroplet lymphocyte cytotoxicity test, and for genotyping 27 alleles on DRB1 were typed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method based on the digestion of PCR-amplified DNAs with allele specific enzymes. In the present study, DR1 was found to be high in centenarians in phenotyping ($P=0.029$, $RR=5.779$), which was similar in genotypeing (DRB1*0101: $P=0.030$, $RR=5.779$), but both results were not significantly different at Pc level. However, DRB1*1401 ($P=0.00103$, $Pc=0.028$, $RR=3.456$) was significantly high. These data suggest that several alleles of HLA-DRB1, such as DRB1*1401, favourably relate to longevity. *Ryukyu Med. J., 17(2)85~88, 1997*

Key words: centenarian, HLA-DR, longevity, genetics, Okinawa

INTRODUCTION

Okinawa prefecture is well-known as one of the longevity areas in the world. The average life expectancies in Japan were 76.3 years for male and 83.0 years for female in 1992 which were the highest in the world[1]. Moreover, the average life expectancies were 76.7 years for male and 84.5 years for female in Okinawa prefecture, which were the highest for female and fifth highest for male in Japan[2]. The population rate of centenarians was 22.14 per 100,000, which was about 3.8 times higher than that of all of Japan (5.87) in 1996, according to Ministry of Health and Welfare data[3]. Although its ethnicity is Japanese, Okinawa was an independent nation from China and Japan until 1879, called the Ryukyu Kingdom. It was almost completely isolated from other nations, which might also be interesting from the view point of population genetics.

Although biological contributions to human longevity have become the object of medical and public interest[4,5] and several studies[6-12] about various view points have been carried out on Okinawan centenarians, none has examined the molecular basis of the genes of the Okinawan centenarians. In this report, we analyzed human leukocyte antigen (HLA) of DR locus both in phenotyping and in genotyping, because it is regarded as one of the main loci associated with various diseases and well studied so far[13], for the purpose of clarifying the presence of primary genetic factors associated with longevity.

SUBJECTS AND METHODS

Subjects

HLA-DR patterns of phenotypes and genotypes were investigated in 86 centenarians and in 142 normal adults (controls) in Okinawa prefecture. All of them were found to be healthy after clinical examinations. The centenarians consisted of 14 males and 72 females, aged 100 to 106 years (Mean±SD: 101.6±1.63 years) and the controls were 60 males and 82 females, aged 20 to 75 years (66.2±6.42 years). All of them were born and raised in Okinawa. Physical examination and collection of peripheral venous blood were carried out at their residence with informed consent. Controls were selected in the same vicinity as the centenarians.

Table 1 HLA-DR antigen frequencies in Okinawan people

HLA-DR antigen	centenarians (N=86) n	PF(%)	controls (N=142) n	PF(%)	P	Pc	RR
DR1	7	8.1	2	1.4	0.029	0.435	5.779
DR2	33	38.4	54	38.0	1.00		
DR3	1	1.2	2	1.4	1.00		
DR4	43	50.0	74	52.1	0.79		
DR5	3	3.5	5	3.5	1.00		
DR6	0	0	4	2.8	0.17		
DR7	0	0	1	0.7	1.00		
DR8	8	9.3	27	19.0	0.058		
DR9	22	25.6	38	26.8	0.88		
DR10	1	1.2	2	1.4	1.00		
DR11(5)	1	1.2	7	4.9	0.16		
DR12(5)	14	16.3	16	11.3	0.31		
DR13(6)	1	1.2	10	7.0	0.056		
DR14(6)	11	12.8	12	8.5	0.36		
DR-blank	27	31.4	30	21.1	0.11		

PF(%): phenotype frequency P: P-level by Fisher's exact probability test
Pc: corrected P RR: relative risk

Table 2 HLA-DRB1 gene frequencies in Okinawan people

DRB1 genes	centenarians (N=86) n	GF(%)	controls (N=142) n	GF(%)	P	Pc	RR
0101	7	4.1	2	0.7	0.030	0.81	5.779
1501	15	8.7	40	14.1	0.10		
1502	12	7.0	16	5.6	0.69		
1602	0	0	2	0.7	0.53		
0302	2	1.2	2	0.7	1.00		
0401	6	3.5	10	3.5	1.00		
0403	7	4.1	22	7.7	0.16		
0405	39	22.7	72	25.4	0.57		
0406	7	4.1	7	2.5	0.40		
0407	2	1.2	4	1.4	1.00		
0410	7	4.1	8	2.8	0.59		
1101	9	5.2	7	2.5	0.19		
1102	2	1.2	1	0.4	0.56		
1201	3	1.7	2	0.7	0.37		
1202	0	0	4	1.4	0.17		
1301	0	0	4	1.4	0.17		
1302	1	0.6	15	5.3	0.0072	0.194	0.110
1401	19	11.0	9	3.2	0.00103	0.028	3.456
1402	2	1.2	1	0.4	0.56		
1403	1	0.6	0	0	1.00		
1405	2	1.2	1	0.4	0.56		
1406	2	1.2	1	0.4	0.56		
0701	0	0	1	0.4	1.00		
0801	0	0	1	0.4	1.00		
0802	5	2.9	3	1.1	0.27		
0803	4	2.3	12	4.2	0.31		
0901	18	10.5	37	13.0	0.46		

GF(%): gene frequency P: P-level by Fisher's exact probability test
Pc: corrected P RR: relative risk

Laboratory techniques of HLA typing

Fourteen specificities on HLA-DR locus were typed according to the standard microdroplet lymphocyte cytotoxicity test[14] by using sera provided at the 9th International Histocompatibility Workshop.

In HLA-DRB1 alleles genotyping, we used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method[15, 16]. Genomic DNA (200 to 300 ng) was amplified, focusing on the HLA-class II DRB1 loci by the PCR procedure with 2 units of the Taq DNA polymerase. The reaction mixture which contained genomic DNA: 50 mM KCl; 2.5 mM MgCl2; 10 mM Tris-HCl, pH 8.4; 0.01% gelatin; 0.02% NP-40; 200 μM each of dATP, dCTP, dTTP, and dGTP; 1 mM of each of the 5' and 3' primers in a total volume of the 100 or 50 μl, was covered with 35 μl of mineral oil and subjected to 30 cycles of 1 min for denaturing, 1 min for annealing, and 2 min for extension by automatic PCR thermal cycler. After amplification, aliquots (5 to 7 μl) of reaction mixture were digested with 2 units of restriction endonucleases for 3 hours at 37℃ after addition of appropriate incubation buffer. Enzyme solution-treated reaction mixtures were classified by cleavage a nd/or no cleavage patterns of amplified fragments by electrophoresis in 12% polyacrylamide gel. DNA fragments on the gels were detected by ethidium bromide staining. HLA-DRB1 genes were defined on the basis of the generated RFLP patterns[17].

The Chi-square method with the continuity correction and Fisher's exact probability test were used for data analysis. Corrected P value (Pc) was calculated by multiplying P value by the number of alleles tested for DRB1 locus.

RESULTS AND DISCUSSION

When the frequencies of HLA-DR phenotypes were compared between centenarians and controls, DR1 was high in centenarians (P=0.029, RR=5.779) (Table 1). As for genotypes, high values of DRB1*0101 (P=0.030, RR=5.779) and DRB1*1401 (P=0.00103, RR=3.456) were recorded. On the other hand, a low rate was recognized in DRB1*1302 (P=0.0072, RR=0.110). When the correction with the number of all DR markers for each marker was done, only the deviations for DRB1*1401 remained significant (Pc=0.028). Rate of DRB1*0901 allele, which belongs to the DR9 antigen group, was not low in this study (P=0.88 in phenotyping; P=0.46 in genotyping) (Table 2).

Although there are few reports concerning HLA and longevity[6, 18], the screenings were antigenic rather than genetic. As for Okinawan-Japanese subjects, it was reported that DR1 was significantly high in centenarians, in contrast to the low rate of DR9[6]. However, low frequency of DR9 was not observed in the present study and the number of antigens for blank was rather high in the results of HLA serological typing, which suggested that antigens for blank in phenotype might include DRB1*14 group and DRB1*0101 in centenarians, and/or DRB1*1302 and DRB1*0901 in controls. Besides HLA loci, very few gene loci, such as the gene encoding apolipoprotein E and angiotensin converting enzyme,[19] have been examined in the quest to understand the biological basis of longevity[5]. However, it was more interesting and direct for us to state the molecular basis in relation to longevity by investigating HLA genes from an immunological point of view.

As in the previous study[6], similar results that DR1 was rather high in centenarians both in serological typing (P=0.029, RR=5.779) and in genotyping (DRB1*0101: P=0.030, RR=5.779) were observed in the present study. Although there was a more significant difference in phenotype between the two groups (P=0.0042, RR=12.3) in the previous study[6] than in the present study at P level, there was no significant difference between centenarians and controls at Pc level in both studies. Degree of responsiveness to Candida allergen was related to HLA-DR1 in human[20], and the low frequency or absence of DR1 might be associated with various Japanese autoimmune diseases[6].

On the other hand, DRB1*1401 was significantly high (P=0.00103, Pc=0.0028, RR=3.456) in centenarians in this study, which might be most noteworthy. In the Japanese, the most frequent allele in the DRB1*14 group is DRB1*1401 (6.2%)[21], which is lower than that of the centenarians (11.0%) and higher than that of the controls (3.2%) in the present study. For the Japanese, the increasing frequency of DRB1*1401 (P=0.03, RR=3.4) and the decreasing tendency of DRB1*1302 are regarded as some of the possible alleles contributing to the susceptibility to sarcoidosis[22], which is a chronic granulomatous disorder. Although its detailed ethiology and pathogenesis are still unknown, especially in Okinawa prefecture, increasing expression of DR antigens on macrophages suggest that their functions in class II-mediated antigen presentation to the $CD4^+$ T cells contribute to the development of sarcoidosis. However, from the examinations of amino acid residues of the DRB1 alleles, DRB 1*0101 may play a protective role in the development of sarcoidosis[22]. Thus, there could be essentially similar functions which contribute to T-cell activation both in DRB1*0101 and DRB1*1401. The frequencies of other alleles except those mentioned above were similar in the Japanese[23].

Although the possibility of a direct involvement of the non-HLA gene located within, and tightly linked to the class II antigen could not be excluded from the influence on the acheivement of longevity in centenarians, it is suggested that some alleles of HLA-DRB1, such as DRB1*1401, may be related to longevity.

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